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QTL Mapping of Mineral Element Contents in Rice Using Introgression Lines Derived from an Interspecific Cross

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Abstract: Developing rice varieties with increased mineral element content is the most cost-effective and efficient approach for alleviating human malnutrition and nutrient deficiencies. In this study, quantitative trait loci (QTLs) were mapped for mineral element content in 96 introgression lines derived from a cross between the elite Korean *Oryza sativa japonica* cultivar "Hwaseong" and the wild rice *Oryza rufipogon* (IRGC105491). The population was grown in two locations, and Fe, Zn, Mn, and Ca contents of the brown rice were measured. Six QTLs were identified on chromosomes 6, 8, and 10, and all *O. rufipogon* alleles increased trait values. The positions of *qFe10* and *qZn10* were further defined; higher Fe and Zn contents are related to the 375-kb *O. rufipogon* segment between the markers RM1873 and RM25612. The combined analysis of the whole-genome sequencing data, spatiotemporal expression profile, and gene expression suggested that a transcription factor gene, namely the rice homeobox gene 9 (LOC_Os10g33960) marks as the high potential candidate associated with Fe and/or Zn regulation. This study provides valuable information on candidate genes *qFe10* and *qZn10* from *O. rufipogon*, which may be vital in developing rice varieties with increased Fe and/or Zn content without any penalty in traits of agronomic importance.

Keywords: introgression lines; mineral elements; quantitative trait locus; rice

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1. Introduction

Mineral elements play a crucial role in human health. For proper growth and development, humans require at least 49 nutrients [1,2]. Among cereal crops, rice (*Oryza sativa*) is one of the most essential, and it is commonly consumed because it supplies proteins, starch, carbohydrates, and nutrients the human body requires. In Asia, rice provides approximately 35–59% of the energy consumed by three billion people [3]. Therefore, the nutritional value of rice is vital for human health. Compared to other staple crops such as wheat, corn, tubers, and legumes, rice grains have comparatively small amounts of key nutrients, such as iron (Fe), zinc (Zn), and calcium (Ca) [4,5]. Deficiencies or insufficient nutrient intake can lead to human diseases and malnutrition. Malnutrition, or hidden hunger, is an existing health problem affecting a multitude of people, particularly in developing countries where rice is the staple food. Approximately 60% of the global population suffers from Fe deficiency, while more than 30% are Zn deficient [6,7]. Various approaches to prevent malnutrition have already been put into practice, including food supplementation, food fortification, and dietary diversification. Fortification programs have already been implemented in various developed countries, but similar approaches are impractical in developing countries. Therefore, a more sustainable and cost-effective approach should be

implemented globally. Breeders usually focus on high-yield rice and pay less attention to the nutritional quality of rice; thus, modern rice varieties commonly have lower mineral element content in their grains. Developing rice varieties with enhanced grain mineral element content is becoming a target of breeding programs to address and overcome disease-related malnutrition.

The accumulation of minerals is a complex quantitative trait controlled by several genes and is largely affected by genetic × environmental interactions. Thus, quantitative trait locus (QTL) mapping provides a chance to identify the natural variation associated with the accumulation of mineral elements in rice grains, which can be used in breeding programs [8]. The utilization of molecular markers closely or tightly linked to QTL will allow rice breeders to consider the positive selection of essential elements and the negative selection of potentially toxic elements in rice grain using marker-assisted selection. In addition, with recent advances in genome sequencing technologies, single nucleotide polymorphisms (SNPs) have been used as DNA markers. Kompetitive allele-specific polymerase chain reaction (KASP), a method for detecting important allelic variations among cultivars by typing SNPs, has been used to map QTLs and identify genes associated with target traits [9–11].

Wild rice relatives serve as an important gene pool and can be putative resources for diversifying the genetic base of cultivated rice to improve various agronomic traits, grain quality, and grain nutrient traits [12–15]. Several studies have already exploited the use of *Oryza rufipogon*, which is widely recognized as the direct ancestor of cultivated *O. sativa* [16]. Previously, 31 putative QTLs associated with Fe, Zn, manganese (Mn), copper, Ca, magnesium, phosphorus (P), and potassium contents were detected in a cross between the *indica* cultivar Teqing and the wild rice *O. rufipogon* [2]. In addition to *O. rufipogon*, other wild rice species such as *Oryza meridionalis*, *Oryza nivara*, and *Oryza longistaminata* have become potential genetic resources for QTL mapping and for developing lines with enhanced nutrient content. Ishikawa et al., 2017 [17] detected four QTLs responsible for high grain Zn content using backcrossed recombinant inbred lines derived from *O. sativa* "Nipponbare" and *O. meridionalis* W1627. Swamy et al., 2018 [18] identified 30 QTLs responsible for grain Fe and Zn contents using two BC_2F_3 mapping populations derived from the crosses of *O. sativa* Swarna with two different accessions of *O. nivara* (IRGC81848 and IRGC81832). The Fe and Zn levels in the two *O. nivara* accessions were 2–3 times higher than those in *O. sativa* Swarna. A total of 33 QTLs responsible for Fe, Zn, selenium, cadmium, mercury, and arsenic contents were identified using backcross inbred lines derived from *O. longistaminata* [19]. These results indicate that the mineral element content in rice is enhanced by the transfer of useful genes from wild rice into cultivars. However, little effort has been made to exploit and identify candidate genes or QTLs on chromosome 10 in wild rice species [2,14,17,20].

In this study, 96 introgression lines (ILs) derived from a cross between the elite Korean *O. sativa japonica* cultivar "Hwaseong" and *O. rufipogon* (IRGC105491) were used to identify QTLs associated with mineral element content in brown rice. The mineral element contents (Fe, Zn, Mn, and Ca) of brown rice were evaluated at the Chungnam National University (CNU) and Chungcheongnam-do Agricultural Research and Extension Services (CNARES). Here, we also characterized, further defined, and validated the locations of *qFe10* and *qZn10* through hydroponic experiments. The co-localization of QTLs associated with grain Fe and Zn contents was also evaluated in this study. We also characterized candidate genes on chromosome 10, covering the *qFe10* and *qZn10* regions.

2. Materials and Methods

2.1. Plant Materials and Field Trials

A population consisting of 96 ILs was developed from a cross between the Korean *O. sativa japonica* cultivar "Hwaseong" and the wild rice *O. rufipogon* (IRGC105491) [21]. The 96 ILs and the parental lines were grown in the experimental fields at CNU in Daejeon, South Korea, for two years (summer of 2016 and 2019) and at CNARES for one year. In both locations, the germinated seeds were sown in the middle of April, and 30-day-old seedlings were transplanted into the paddy field. In CNU, each IL and the parental line was grown in a single row of 25 plants at 30 × 15 cm intervals, while in CNARES, each IL along with parental lines was grown in a single row of 33 plants with 30 × 15 cm spacing. The experiment was laid out in a completely randomized block design with two replications.

To narrow down the locations of *qFe10* and *qZn10*, four ILs (CR2, CR5, CR7, and CR24) were selected (Figures S1 and S2) for substitution mapping. To validate *qFe10* and *qZn10*, two separate hydroponic experiments were conducted under greenhouse and growth chamber conditions using *O. rufipogon*, Hwaseong, and two ILs (CR2 and CR5). Plant materials were used to compare the agro-morphological traits and mineral content under Fe-deficient, Fe-sufficient, Zn-deficient, and Zn-sufficient conditions. More detailed information is provided in Section 2.6. "Validation and evaluation of *qFe10* and *qZn10*."

2.2. DNA Extraction and Genotyping

Total genomic DNA was extracted from fresh leaves of each plant line in bulk using a chloroform-based DNA extraction protocol, as described by Causse et al. (1994) [22]. Simple sequence repeat (SSR) analysis was performed as described by Panaud et al. (1996) [23] to analyze the genotypes. Primer sequences for 138 rice microsatellite (RM) markers, which are SSRs located within the target region, were collected from the Gramene marker database (http://www.gramene.org (accessed on 24 November 2016); Table S1) [24].

In addition to SSR analysis, KASP analysis of 96 ILs and parental lines was performed according to the method described by Yang et al. (2019) [10] at the Seed Industry Promotion Center, Foundation of Agriculture Tech. Commercialization and transfer in Korea. A total of 69 KASP markers were used in this study (Table S2). To further define the location of *qFe10* and *qZn10*, additional SSR, KASP, and three insertion-deletion (InDel) markers were used (Table S3) [25,26].

2.3. Mineral Element Content Analysis

The seeds of 96 ILs and parental lines grown at the CNU and CNARES were used for the analysis. A total of 120 brown rice grains per line grown at CNU were dehulled, pulverized, and sent to Chungnam National University Joint Laboratory in Daejeon, South Korea, to measure the Fe, Zn, Mn, and Ca contents of 96 ILs and parental lines using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) following the protocol as described by Kwon et al., (2014) [27]. The 96 ILs and parental lines grown at CNARES were analyzed in the laboratory following the same protocol.

2.4. QTL Mapping and Statistical Analysis

The mean trait values for each IL in each location were used for QTL analysis. The linkage map was constructed using QTL IciMapping 4.1 software [28] with the Kosambi mapping function. In grouping, the by anchor only was used. The order of the markers in the linkage group was determined using the 'by anchor order' algorithm, and the criterion for rippling was the sum of adjacent recombination fractions. QTL analysis was carried out by single marker analysis (SMA), interval mapping, and composite interval mapping (CIM). The likelihood of odds (LOD) value of $\geq$2.0 was used as the threshold for declaring a QTL (both for SMA and CIM), which was based on a 1000-permutation test with a 95% confidence level. The existence of a QTL was declared if it was detected in both locations or at least in two years.

Statistical analyses, including mean, range, coefficient of variation (CV), skewness, kurtosis, and trait correlations among mineral element contents in brown rice grains, were performed using MINITAB 16.2.4. The phenotypic means of each genotype class were compared using Tukey's test. The frequency distributions of each mineral element content were plotted to observe the nature of the variation in each trait within the IL population.

2.5. Whole Genome Sequencing of the Parental Lines

Seedlings of each parental line were sent to Macrogen Inc. (Beotkkot-ro, Geumcheon-gu, Seoul, South Korea) for whole genome sequencing. Shotgun DNA libraries were prepared from high molecular weight genomic DNA of the parental lines using the TruSeq Nano DNA Kit (San Diego, CA, USA).

2.6. Validation and Evaluation of qFe10 and qZn10

Seeds of *O. rufipogon*, Hwaseong, and two ILs (CR2 and CR5) were surface sterilized with 70% ethyl alcohol and 5% sodium hypochlorite and then germinated on filter paper soaked with water for two weeks under controlled conditions at 28 °C with a 12-h photoperiod. After two weeks, the seedlings were transplanted into a hydroponic setup consisting of a 7-L plastic container filled with half-strength Yoshida's rice nutrient solution [29,30] for one week and then grown in a full-strength Yoshida's rice nutrient solution for another two weeks. The full-strength Yoshida's nutrient solution contained 40 mg N L^{-1} (as NH_4NO_3), 10 mg L^{-1} (as $NaH_2PO_4 \bullet 2H_2O$), 40 mg K L^{-1} (as K_2SO_4), 40 mg Ca L^{-1} (as $CaCl_2$), 40 mg Mg L^{-1} (as $MgSO_4 \bullet 7H_2O$), 0.5 mg Mn L^{-1} (as $MnCl_2 \bullet 4H_2O$), 0.05 mg Mo L^{-1} [as $(NH_4)_6\ MO_7O_{24} \bullet 4H_2O$], 0.54 mg B L^{-1} (as H_3BO_3), 0.01 Zn mg L^{-1} (as $ZnSO_4 \bullet 7H_2O$), 0.01 mg Cu L^{-1} (as $CuSO_4 \bullet 5H_2O$), and 2 mg Fe L^{-1} [as $FeCl_3 \bullet 6H_2O$ (in monohydrate citric acid)]. Then, plants were grown under various treatments (Fe-deficient (0), hereinafter referred to as "-Fe"; Fe-sufficient (2 mg L^{-1}), hereinafter referred to as "+Fe"; Zn-deficient (0), hereinafter referred to as "-Zn"; and Zn-sufficient (0.01 mg L^{-1}), hereinafter referred to as "+Zn") in triplicate for two weeks in a greenhouse with natural light and under growth chamber conditions with 60/80% day/night relative humidity, and 28/24 °C day/night temperature [31]. Twenty-one seedlings were grown for each replicate. The pH was adjusted to 5.2 ± 0.2 with 5N NaOH and/or concentrated HCl to maintain identical conditions. The culture solution was changed every two days [30]. Two weeks after treatment, the shoots and roots were harvested for RNA extraction. All samples were frozen in liquid nitrogen and stored at −80 °C. Seven agro-morphological traits were evaluated, namely root length, shoot length, root dry weight, culm dry weight, leaf dry weight, shoot dry weight, and root-to-shoot ratio. Plant tissue analysis was performed two weeks after treatment. Five plants per line were randomly selected per replicate to evaluate the agro-morphological traits and mineral element content. The plant tissues were separated into roots, culms, and leaves for elemental analysis. Plant materials were oven-dried at 60 °C for 72 h and ground into a fine powder [31]. Elemental concentrations in root and shoot tissues were determined via ICP-AES at the Chungnam National University Joint Laboratory in Daejeon, South Korea.

2.7. Analysis of Candidate Genes Underlying the qFe10 and qZn10 Region

To predict possible candidate genes, all genes on chromosome 10 within the *qFe10* and *qZn10* region were downloaded from the Rice Annotation Project Database (https://rapdb.dna.affrc.go.jp/ (accessed on 21 March 2022)). We compared the sequence of each gene using the whole genome sequencing data of the parental lines. The InDels in the promoter regions or the nonsynonymous SNPs and/or Indels in the DNA coding regions were considered potential functional polymorphisms. The tissue-specific expression pattern of each candidate gene was obtained and analyzed from the RiceXpro database (https://ricexpro.dna.affrc.go.jp/ (accessed on 20 May 2022)). In addition, we analyzed the candidate gene expression data obtained from the Rice Expression Database (http://expression.ic4r.org/index (accessed on 1 June 2022)). We compared the function of

the candidate genes in the current study to those from earlier studies on Fe and/or Zn uptake and transport. Finally, the selected candidate genes were subjected to quantitative real-time polymerase chain reaction (qRT-PCR) analysis. Total RNA was extracted from the root and shoot tissues of *O. rufipogon*, Hwaseong, CR2, and CR5 that were subjected to different Fe and Zn treatments under greenhouse conditions. cDNA was synthesized from the total RNA samples. RT-PCR of housekeeping and target genes was conducted in triplicate for each sample using a kit (SMART GENE, Daejeon, Republic of Korea), following the manufacturer's instructions; the primer sequences are listed in Table S4. qRT-PCR was performed using a CFX real-time PCR system with SYBR Green master mix (SMARTGENE). The rice Ubiquitin5 (*UBQ5*) gene was used as an internal control [32]. Relative gene expression was calculated using the $\Delta\Delta C_t$ method [33].

3. Results

3.1. Phenotypic Characterization of the Parental Lines and Introgression Lines

Seed morphology and mineral element content in the brown rice grain of the parental lines were compared (Table 1 and Figure S1). The brown rice grains of *O. rufipogon* had significantly higher Fe, Zn, Mn, and Ca contents than those of Hwaseong across locations and years. The Fe, Zn, Mn, and Ca contents of *O. rufipogon* varied from 10.8 to 23.3 ppm, 20.2 to 25.7 ppm, 23.3 to 32.9 ppm, and 146.2 to 211.4 ppm, respectively, while the Fe, Zn, Mn, and Ca contents of Hwaseong varied from 8.5 to 20.2 ppm, 14.6 to 20.4 ppm, 15.2 to 21.0 ppm, and 100.8 to 165.0 ppm, respectively. The overall mean, range, CV, skewness, and kurtosis values of the IL population were also evaluated for Fe, Zn, Mn, and Ca contents (Table 1). The frequency distribution of the grain mineral element content of the 96 ILs and parental lines is presented in Figure 1. The coefficient of variation for each mineral element content in the IL population was >10%, except for Ca content (7.7%) in CNARES. Fe and Ca contents of the ILs in CNARES were higher than those in CNU, possibly due to variations in environmental conditions and agricultural practices (i.e., soil condition and fertilizer application).

Table 1. Descriptive statistics of the mineral element contents in brown rice in 96 ILs and parental lines.

Location	Trait (ppm) [a]	Parents			Introgression Lines				
		Hwaseong	*O. rufipogon*	Difference [b]	Mean ± SD	Range	CV (%)	Skewness	Kurtosis
2016 CNU	Fe	8.5 ± 0.1	11.3 ± 0.2	***	8.8 ± 1.4	6.1–14.5	15.9	1.03	1.82
	Zn	20.1 ± 0.4	25.7 ± 0.2	***	17.8 ± 3.0	12.7–27.7	16.9	0.91	0.60
	Mn	21.0 ± 0.3	23.3 ± 0.2	***	18.3 ± 3.1	12.9–28.3	16.9	1.27	1.81
	Ca	103.0 ± 0.5	184.8 ± 1.3	***	97.5 ± 11.3	97.5–136.5	11.6	0.24	1.07
2019 CNU	Fe	9.6 ± 0.1	10.8 ± 0.1	***	8.7 ± 1.5	5.6–14.22	17.2	1.11	2.54
	Zn	20.4 ± 0.3	24.8 ± 0.1	***	21.2 ± 3.6	13.8–33.0	16.83	0.95	1.52
	Mn	17.9 ± 0.4	32.9 ± 0.4	***	22.9 ± 4.1	13.0–35.8	17.9	0.06	0.67
	Ca	100.8 ± 0.6	146.2 ± 2.0	***	79.1 ± 10.7	54.0–117.5	13.5	0.57	1.02
CNARES	Fe	20.2 ± 0.0	23.3 ± 2.2	***	16.8 ± 2.7	10.7–23.6	16.1	0.37	−0.04
	Zn	14.6 ± 0.0	20.2 ± 4.1	***	17.1 ± 1.8	13.2–21.9	10.5	0.60	0.29
	Mn	15.2 ± 0.0	24.3 ± 9.9	***	19.2 ± 2.2	13.8–25.3	11.5	−0.01	0.12
	Ca	165.0 ± 0.0	211.4 ± 14.6	***	155.9 ± 12.0	127.4–183.8	7.7	0.09	−0.05

[a] All values represent the mean ± standard deviation (SD) of three replicates (CNU) and two replicates (CNARES). Fe: iron; Zn: zinc; Mn: manganese; Ca: calcium; CV: coefficient of variation. [b] *** indicates a significant difference between Hwaseong and *O. rufipogon* at $p < 0.001$.

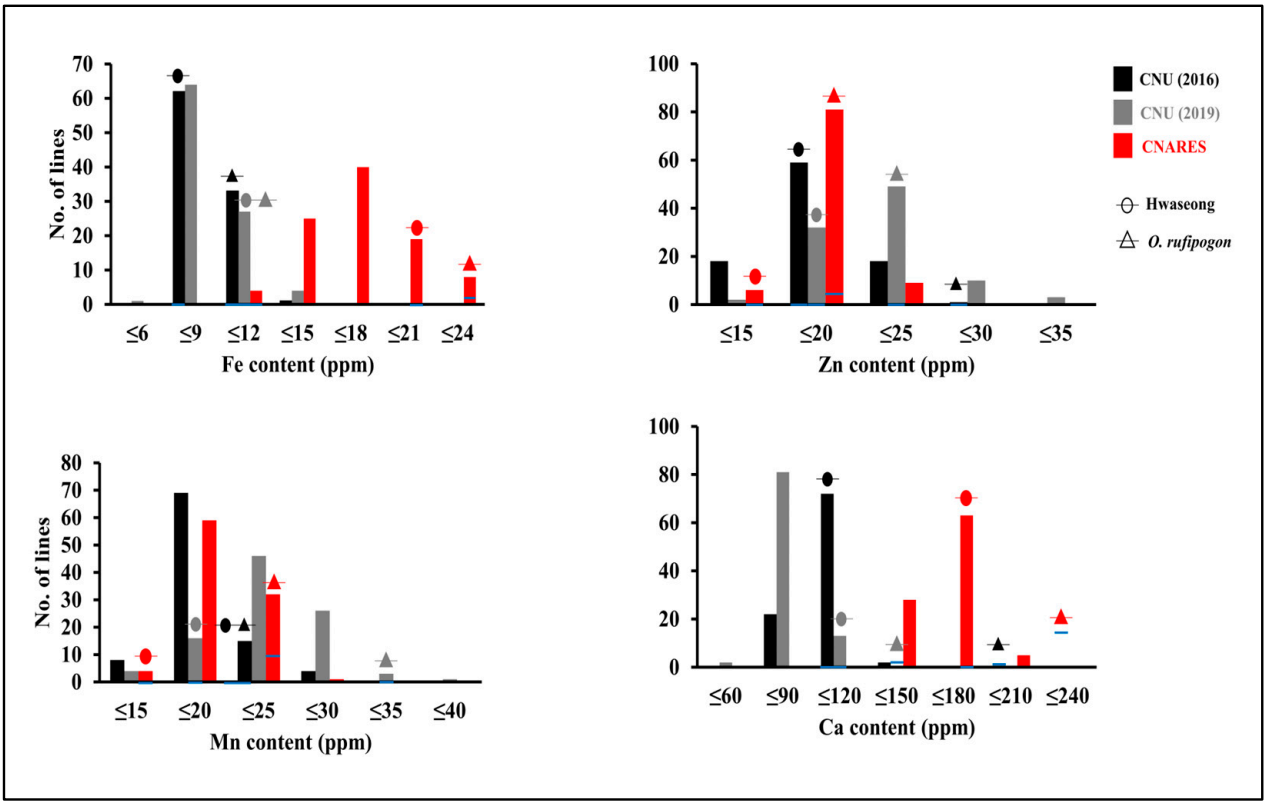


Figure 1. Frequency distribution of mineral element contents in brown rice in 96 ILs and parental lines. Values of Hwaseong and *O. rufipogon* represent mean ± standard deviation (horizontal bar line) of triplicate (CNU) and duplicate (CNARES) measurements.

3.2. Correlation among Four Mineral Elements

Correlation analysis of Fe, Zn, Mn, and Ca contents in the 96 ILs was performed (Table S5). Overall, a significant positive correlation was observed between Fe and Zn contents in ILs at both locations and years. A positive correlation between Fe and Zn contents has previously been reported, indicating that high Fe and Zn contents occur simultaneously in rice [34,35]. Across locations and years, a significant positive correlation was consistently detected between Mn and Ca contents. These results suggest that high Mn content might be accompanied by high Ca content in rice.

3.3. Mapping the QTLs for Mineral Element Contents

QTL analysis was performed to detect the genomic region controlling mineral element content in brown rice. QTL analysis using the genotype data from SSR and KASP markers and the mineral element content of each IL identified six QTLs on chromosomes 6, 8, and 10, consisting of one QTL for Fe, two QTLs for Zn, two QTLs for Mn, and one QTL for Ca (Table 2 and Figure 2). For Fe content, one QTL, *qFe10*, was found to explain 5.1% to 5.7% of the phenotypic variance. For Zn content, two QTLs, *qZn8* and *qZn10*, were found to explain 9.3% to 27.0% of the phenotypic variance. For Mn content, two QTLs, *qMn6* and *qMn10*, were found, explaining 4.8% to 39.2% of the phenotypic variance. For Ca content, one QTL, *qCa10*, was found, explaining 7.2% to 12.3% of the phenotypic variance. Neither *qZn8*, *qMn6*, *qMn10*, nor *qCa10* was detected in the ILs from CNU in 2019. The *qFe10* was not detected in ILs from CNARES. Comparative analysis of data across locations and years revealed that only *qZn10* was consistently detected, indicating that this stable QTL may have a potential value in rice breeding programs. All favorable QTL alleles were derived from *O. rufipogon*. In this study, two cases of QTL co-localization were identified on chromosome 10. The grain Fe and Zn showed the co-localized QTL *qFe10* and *qZn10*. The grain Mn and Ca showed the co-localized QTL *qMn10* and *qCa10* (Table 2).

Table 2. QTLs for grain mineral element contents detected in 96 ILs.

Trait [a]	QTL [b]	Chr	Marker Interval [c]	2016 CNU			2019 CNU			CNARES			Alelle Effect
				LOD	PVE (%)	Additive Effect	LOD	PVE (%)	Additive Effect	LOD	PVE (%)	Additive Effect	
Fe	*qFe10*	10	RM271-RM258	2.11	5.7	−2.7	4.94	5.1	−2.2	-	-	-	*O. rufipogon*
Zn	*qZn8*	8	KJ08_037-KJ08_043	2.04	9.5	−1.4	-	-	-	5.00	27.0	−1.4	*O. rufipogon*
	qZn10	10	RM271-RM258	5.54	21.0	−2.4	4.33	13.2	−5.2	2.65	9.3	−1.6	*O. rufipogon*
Mn	*qMn6*	6	RM276-RM539	11.38	39.2	−3.0	-	-	-	2.02	4.8	−0.8	*O. rufipogon*
	qMn10	10	RM147-KJ10_041	3.11	13.0	−1.7	-	-	-	2.49	7.7	−0.9	*O. rufipogon*
Ca	*qCa10*	10	KJ10_041-RM333	2.69	7.2	−4.7	-	-	-	2.75	12.3	−4.6	*O. rufipogon*

[a] Fe: Iron; Zn: Zinc; Mn: Manganese; Ca: Calcium. [b] *qFe*: QTL for iron; *qZn*: QTL for zinc; *qMn*: QTL for manganese; *qCa*: QTL for calcium. [c] Underlined marker represents the location of QTL detected by SMA. LOD, Logarithm of the odds. PVE, proportion of the phenotypic variance explained by the QTL. Additive effect is the additive effect of replacing a Hwaseong allele with an *O. rufipogon* allele.

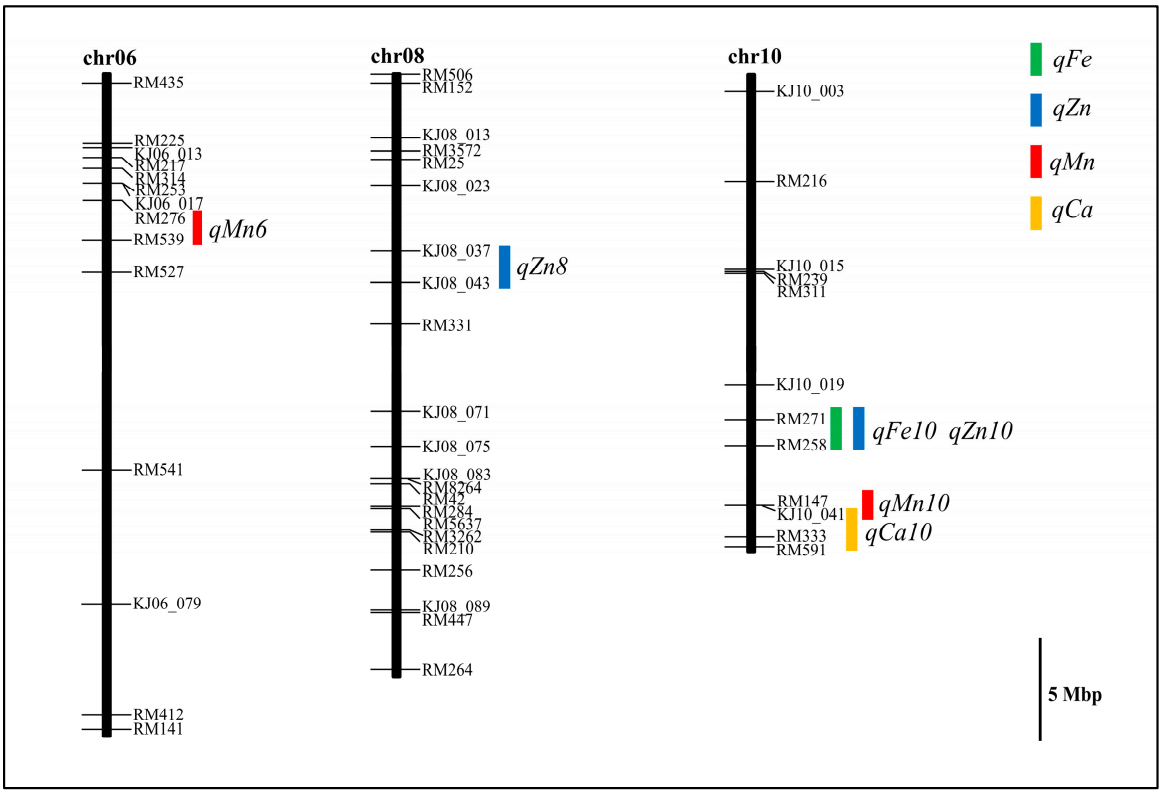
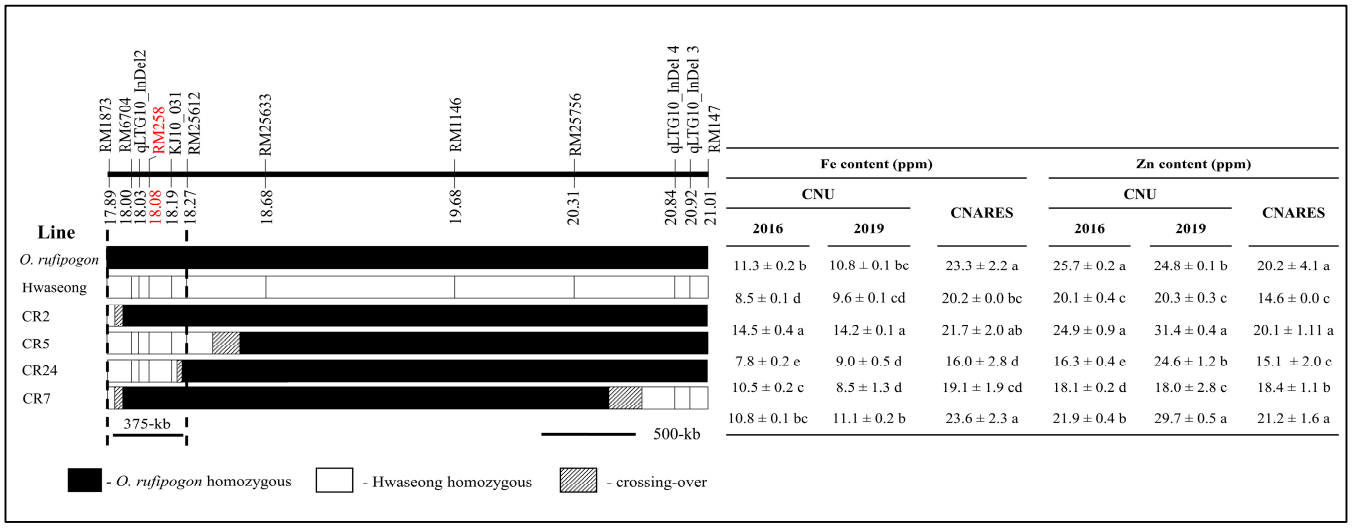


Figure 2. Map locations of the QTLs. QTLs are represented on the right side of the chromosome. *qFe*: QTL for iron; *qZn*: QTL for zinc; *qMn*: QTL for manganese; *qCa*: QTL for calcium.

3.4. Characterization and Validation of qFe10 and qZn10

We further defined the location of *qFe10* and *qZn10* using substitution mapping with additional markers. Among the 96 ILs, four (CR2, CR5, CR7, and CR24) were selected because they did not have the *O. rufipogon* segment on chromosome 8 linked to qZn8 of the Hwaseong background (Figure S2). The seed morphologies of the four selected ILs were phenotypically similar to that of Hwaseong (Figure S1). Two ILs (CR2 and CR7) carrying the *O. rufipogon* segment covering the region between the markers RM1873 and RM25612 showed higher Fe and Zn contents at both locations. CR24, which carries a shorter substituted segment of *O. rufipogon*, had lower Fe and Zn contents than CR2 and CR7. Significantly lower Fe and Zn concentrations were also observed in CR5, which carried

the Hwaseong segment encompassing the target region. These results further confirmed that the *O. rufipogon* segment between the markers RM1873 and RM25612 was associated with higher Fe and Zn levels (Figure 3). We also conducted hydroponic experiments to confirm the candidate region of *qFe10* and *qZn10* using two ILs (CR2 and CR5) and parental lines. Overall, irrespective of the genotype and Fe or Zn availability conditions, greater accumulation of Fe or Zn was observed in the roots than in shoots, indicating that the uptake of minerals involves the active transport of ions into root cells. Among the parental lines, *O. rufipogon* generally showed higher Fe and Zn contents in the root and shoot tissues than Hwaseong subjected to different treatments (-Fe, +Fe, -Zn, and +Zn) under greenhouse and growth chamber conditions (Tables 3 and 4). In addition, *O. rufipogon* had longer roots and shoots and higher root dry weight, culm dry weight, leaf dry weight, shoot dry weight, and root-to-shoot ratio than Hwaseong under all Fe and Zn treatments at greenhouse conditions. Although *O. rufipogon* had higher Fe and Zn contents in the root and shoot tissues than Hwaseong in the growth chamber, the differences in agro-morphological traits between *O. rufipogon* and Hwaseong were not significant, possibly because of the poor growth of *O. rufipogon* (Tables S6 and S7).



Line	Fe content (ppm)			Zn content (ppm)		
	CNU		CNARES	CNU		CNARES
	2016	2019		2016	2019	
O. rufipogon	11.3 ± 0.2 b	10.8 ± 0.1 bc	23.3 ± 2.2 a	25.7 ± 0.2 a	24.8 ± 0.1 b	20.2 ± 4.1 a
Hwaseong	8.5 ± 0.1 d	9.6 ± 0.1 cd	20.2 ± 0.0 bc	20.1 ± 0.4 c	20.3 ± 0.3 c	14.6 ± 0.0 c
CR2	14.5 ± 0.4 a	14.2 ± 0.1 a	21.7 ± 2.0 ab	24.9 ± 0.9 a	31.4 ± 0.4 a	20.1 ± 1.11 a
CR5	7.8 ± 0.2 e	9.0 ± 0.5 d	16.0 ± 2.8 d	16.3 ± 0.4 e	24.6 ± 1.2 b	15.1 ± 2.0 c
CR24	10.5 ± 0.2 c	8.5 ± 1.3 d	19.1 ± 1.9 cd	18.1 ± 0.2 d	18.0 ± 2.8 c	18.4 ± 1.1 b
CR7	10.8 ± 0.1 bc	11.1 ± 0.2 b	23.6 ± 2.3 a	21.9 ± 0.4 b	29.7 ± 0.5 a	21.2 ± 1.6 a

Figure 3. Graphical representation of four ILs and the map of the target region of *qFe10* and *qZn10*. Physical position (Mbp) is shown under the chromosome. The mean values in each column with different letters are significantly different from each other at $p = 0.05$ based on Tukey's test. Values represent mean ± standard deviation of triplicate (CNU) and duplicate (CNARES) measurements.

Table 3. Iron content in the root and shoot of rice genotypes grown under -Fe and +Fe treatments.

Line	Greenhouse				Growth Chamber			
	Root		Shoot		Root		Shoot	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
O. rufipogon	2445.6 ± 46.6 b	6776.5 ± 107.8 a	403.7 ± 3.71 b	554.2 ± 6.1 a	3130.1 ± 34.4 a	7388.9 ± 56.3 a	133.3 ± 0.1 a	345.9 ± 1.1 a
Hwaseong	2112.4 ± 12.7 c	5561.6 ± 38.0 c	236.5 ± 2.35 c	351.1 ± 8.5 d	2552.3 ± 17.1 d	5615.2 ± 109.2 d	113.7 ± 0.8 c	223.8 ± 1.9 c
CR2	3064.6 ± 41.2 a	6884.1 ± 149.8 a	420.0 ± 9.1 a	469.8 ± 3.2 b	2920.5 ± 47.1 b	6405.4 ± 45.3 b	115.9 ± 0.7 b	252.7 ± 0.6 b
CR5	1869.4 ± 19.4 d	6022.9 ± 90.9 b	226.0 ± 2.6 c	387.8 ± 3.4 c	2728.6 ± 46.1 c	6110.8 ± 54.6 c	112.3 ± 0.9 c	196.3 ± 1.1 d

Means in each column with different letters are significantly different from each other at $p = 0.05$ based on Tukey's test. Error bars represent the ± standard deviation of the mean. All values represent the mean of three replicates.

We also compared and evaluated the effects of *qFe10* and *qZn10* in Hwaseong and the two ILs (CR2 and CR5) on agro-morphological traits (Tables S6 and S7). Agro-morphological traits of CR2 and CR5 were similar to those of Hwaseong under different Fe and Zn treatments at both greenhouse and growth chamber conditions. However, the Fe and Zn contents in the root and shoot tissues of Hwaseong, CR2, and CR5 varied. CR2 had higher Fe and Zn contents in both the root and shoot tissues than Hwaseong and CR5 under all

Fe and Zn treatments at both greenhouse and growth chamber conditions. These results imply that CR2 has a Fe- and Zn-efficient genotype, emphasizing its Fe-responsive and Zn-responsive behavior (Tables 3 and 4). Typically, Fe- and Zn-efficient genotypes have better Fe and/or Zn uptake in the roots or effective use of Fe and/or Zn in the cells, as they could make Fe and/or Zn more readily available in the rhizosphere, making it easier to take up Fe and/or Zn from the soil. Thus, the hydroponic experiment findings strongly suggest that the 375-kb *O. rufipogon* segment between the markers RM1873 and RM25612 is the potential region responsible for enhancing Fe and Zn content in rice. *O. rufipogon qFe10* and *qZn10* could simultaneously increase the Fe and Zn contents in rice. Furthermore, we also found that the 375-kb *O. rufipogon* segment was not associated with deleterious traits such as fertility reduction [15].

Table 4. Zinc content in root and shoot of rice genotypes grown under -Zn and +Zn treatments.

Line	Greenhouse				Growth Chamber			
	Root		Shoot		Root		Shoot	
	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn
O. rufipogon	36.0 ± 0.3 a	31.8 ± 0.2 a	29.2 ± 0.2 a	42.7 ± 0.5 a	49.9 ± 0.1 b	86.7 ± 0.9 b	31.9 ± 0.5 a	60.2 ± 0.8 b
Hwaseong	25.6 ± 0.2 d	24.4 ± 0.2 c	13.4 ± 0.2 d	20.7 ± 0.1 c	43.7 ± 0.2 c	82.4 ± 0.0 c	24.5 ± 0.2 b	53.7 ± 0.4 c
CR2	33.8 ± 0.1 b	30.8 ± 0.1 b	18.3 ± 0.4 b	21.9 ± 0.2 b	50.9 ± 0.5 a	89.0 ± 0.7 a	32.1 ± 0.2 a	63.1 ± 0.6 a
CR5	30.2 ± 0.2 c	22.7 ± 0.2 d	16.5 ± 0.3 c	20.9 ± 0.0 c	34.4 ± 0.3 d	73.2 ± 0.3 d	23.9 ± 0.3 b	47.4 ± 0.4 d

Means in each column with different letters are significantly different from each other at $p = 0.05$ based on Tukey's test. Error bars represent the ± standard deviation of the mean. All values represent the mean of three replicates.

3.5. Candidate Gene Analysis Underlying qFe10 and qZn10

The 375-kb interval for *qFe10* and *qZn10* comprised 41 annotated genes based on the Rice Annotation Project Database, of which 15 genes were hypothetical protein and non-protein coding transcripts; thus, these genes were omitted prior to analysis (Table S8). Here, we also compared the function of the candidate genes in the current study to those from earlier studies on Fe and/or Zn uptake and transport. By whole genome sequencing, we identified the SNPs or InDels between Hwaseong and *O. rufipogon*. Out of 26, 17 genes had at least one nonsynonymous SNP or InDel in their DNA coding sequences (Table S9). Furthermore, we listed the spatiotemporal expression profile (Figure S3) and gene expression level (Figure S4) in different tissues in rice. Out of 17, four genes had no spatiotemporal expression profiles, namely LOC_Os10g34030 (*Os10g0481450*), LOC_Os10g34110 (*Os10g0482200*), LOC_Os10g34130 (*Os10g0482450*), and LOC_Os10g34150 (*Os10g0482700*) (Table S8). To consider these genes in the analysis, the expression level in different tissues in rice of the 17 putative candidate genes was collected from the Rice Expression Database. According to the spatiotemporal expression profile data, LOC_Os10g33960 displayed the highest expression in leaves (Figure S3g), while LOC_Os10g33920 displayed the highest expression in roots (Figure S3d). Both LOC_Os10g33940 (Figure S3f) and LOC_Os10g33960 (Figure S3g) were substantially expressed in the endosperm. Based on the results of gene expression, it revealed that LOC_Os10g33960, one of the 17 putative candidate genes, had the highest expression level in the seeds (Figure S4g), while LOC_Os10g33920 (Figure S4d) and LOC_Os10g33930 (Figure S4e) had high expression levels in the roots. Both LOC_Os10g33930 (Figure S4e) and LOC_Os10g33960 (Figure S4g) displayed high levels of expression in leaves.

By comparing the results of whole genome sequencing data, spatiotemporal expression profile, and gene expression level, four genes, namely LOC_Os10g33920 (protein of unknown function DUF250 domain-containing protein), LOC_Os10g33930 (protein of unknown function DUF1336 domain-containing protein), LOC_Os10g33940 (auxin response factor 22 [*OsARF22*]), and LOC_Os10g33960 (rice homeobox gene 9 [*OsHox9*]) were selected for qRT-PCR in both roots and shoots of *O. rufipogon*, Hwaseong, CR2, and CR5 under all Fe and Zn treatments at greenhouse conditions (Figures 4 and 5). Among the four selected genes, the transcription regulation gene LOC_Os10g33940, involved in hormone

stimulus-response, consistently displayed higher expression in *O. rufipogon* and CR2 than in Hwaseong and CR5 in both roots and shoots (Figures 4a–d and 5a–d). Additionally, *O. rufipogon* and CR2 consistently had higher expression of LOC_Os10g33960 in their shoots than Hwaseong and CR5, regardless of the Fe or Zn treatment (Figures 4a,c and 5a,c). The *O. rufipogon* and CR2 consistently had higher expression of LOC_Os10g33930 in their roots than Hwaseong and CR5, regardless of the Fe or Zn treatment (Figures 4b,d and 5b,d).

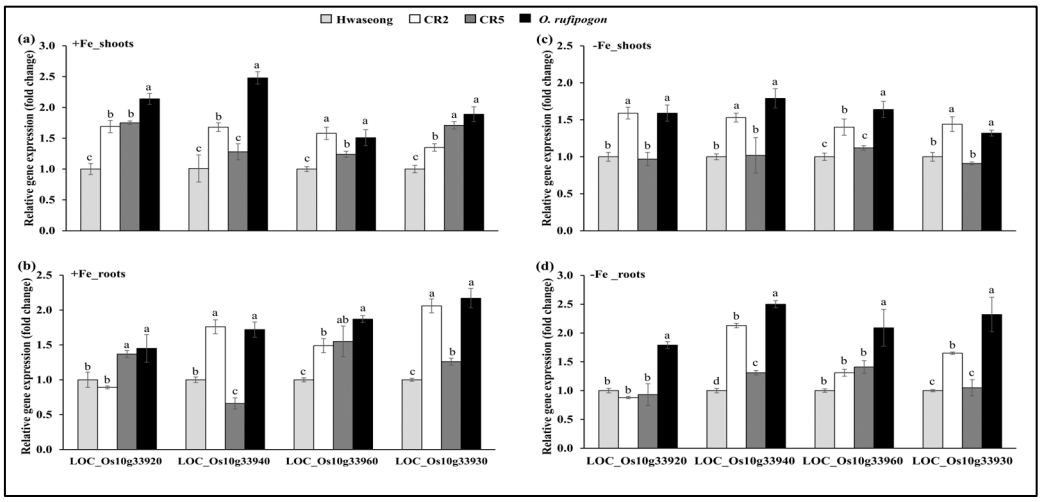


Figure 4. Quantitative real-time PCR analysis of the four candidate genes covering the *qFe10* region. Relative expression levels of the four candidate genes in shoot tissues under (**a**) +Fe and (**c**) -Fe treatment conditions. Relative expression levels of the four candidate genes in root tissues under (**b**) +Fe and (**d**) -Fe treatment conditions. The mean values with different letters are significantly different from each other at $p = 0.05$ based on Tukey's test. Error bars represent the ± standard deviation of the mean. All values represent the mean of three replicates.

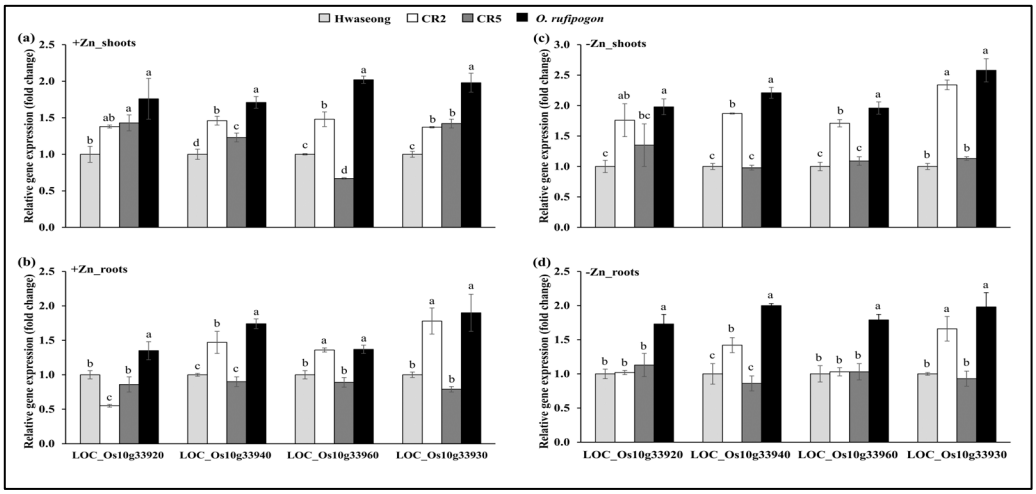


Figure 5. Quantitative real-time PCR analysis of the four candidate genes covering the *qZn10* region. Relative expression levels of the four candidate genes in shoot tissues under (**a**) +Zn and (**c**) -Zn treatment conditions. Relative expression levels of the four candidate genes in root tissues under (**b**) +Zn and (**d**) -Zn treatment conditions. The mean values with different letters are significantly different from each other at $p = 0.05$ based on Tukey's test. Error bars represent the ± standard deviation of the mean. All values represent the mean of three replicates.

4. Discussion

Malnutrition and chronic illnesses are widely considered global health challenges. Although rice is not the primary source of mineral elements, its nutritional value is still

important, as rice is the main food source in many developing countries. An essential strategy to address disease-related malnutrition is the selection of excellent genetic resources to develop rice varieties with improved mineral element content. This strategy has previously been applied for QTL mapping [2,17,18]. In this study, we focused on *O. rufipogon*, which is an important genetic resource for mineral elements. QTL analysis was performed using the 96 ILs evaluated at two locations.

We observed significant differences in mineral element content between the parental lines at the two locations (Table 1). *O. rufipogon* had consistently higher Fe, Zn, Mn, and Ca contents than Hwaseong across both locations and years. Previous studies have reported that wild rice species, such as *O. rufipogon*, *O. nivara*, *O. latifolia*, and *O. officinalis*, had mineral concentrations that were 4–5 times higher than those of cultivated rice [18,34,36,37]. Their poor yields are a significant drawback. Nevertheless, the results indicate that *O. rufipogon* is a potential genetic carrier for improving the nutritional quality of rice cultivars. The IL population in this study displayed a wide variation in all mineral element contents across locations, indicating the complexity and polygenic nature of the traits. The Fe and Ca contents observed in the ILs CNARES were higher than those from CNU. Differences in mineral levels may be attributed to tissue specificities, such as endosperm and embryo, grain position on the panicle, harvest time, soil properties, environment, and genotype × environment [34,38].

In plants, there are two types of interactions: (1) interactions between nutrients, where the amount of one nutrient influences how well the other nutrients are utilized and absorbed, and (2) interactions between ions, where the ions compete for absorption, adsorption, transport, and function in plant tissues [39]. In the present study, a significant correlation was observed between the macronutrient (Ca) and micronutrients (Fe, Zn, and Mn) (Table S5). Significant positive correlations were observed between Fe and Zn and between Mn and Ca in both locations and years, indicating that high Fe, Zn, Mn, and Ca contents occur concurrently in brown rice. Several studies have reported a significant positive correlation between Fe and Zn contents in brown rice [34,35,40] and genetic factors that increase Fe co-segregate with those that increase Zn in grains [1]. Zeng et al. (2005) [41] reported that Ca content is positively associated with Mn content in rice grains. A positive correlation has been observed between the uptake of Ca and Mn in P-deficient upland soils [42].

We identified six QTLs on chromosomes 6, 8, and 10, affecting Fe, Zn, Mn, and Ca contents in brown rice. The *O. rufipogon* alleles at all loci increased the Fe, Zn, Mn, and Ca contents (Table 2). Only *qZn10* was consistently detected in ILs across locations and years. Here, the number of QTLs may be underestimated because the ILs did not cover some of the *O. rufipogon* segments possibly associated with micronutrient contents. In addition, only major QTLs with significant phenotypic effects were detected in this study, as QTLs with minor effects may not meet the significance threshold of detection [43]. In a previous study, Buescher et al. (2010) [44] reported that alterations in the environment in which the plants were grown significantly influenced the correlation between mineral elements and the QTLs controlling mineral accumulation. Variations in the uptake of mineral elements in different environments have been reported in previous studies of *A. thaliana* [45] and maize [46]. QTLs detected across environments have been found to be stable and could play a key role in the accumulation of mineral elements [47].

Co-localization of QTLs controlling different traits is significant for the simultaneous improvement of the traits of interest, such as Fe and Zn contents, in rice breeding programs. The uptake, transport, and loading of different mineral elements may follow the same or similar pathways as those of the protein transporters. Therefore, they might share the same genomic regions, QTLs, and genes [48]. The occurrence of QTL co-localization has previously been reported in several studies [18,19,49,50]. In our study, co-localization of QTL for grain content was found for Fe and Zn and Mn and Ca. The co-localization of QTL may be due to the pleiotropic effect of a single gene, suggesting a common mechanism or pathway for their transport or tight linkage of multiple genes controlling different

traits [2,50]. In rice, citrate, 2′ deoxymugineic acid (DMA), and nicotianamine (NA) are the dominant Fe chelators that play significant roles in the long-distance transport of Fe in rice [51]. In graminaceous species, NA is the biosynthetic precursor of DMA, and both play a role in Zn uptake [52,53]. Because of their similar affinities to specific transporters and ligands that are responsible for their absorption and translocation, Fe and Zn homeostasis in plants may be closely connected [54–56]. He et al., (2021) [57] reported that although Mn and Ca have specific roles in plants, both have comparable ionic radii and binding coordination and share various transport mechanisms to cross organelle membranes. The co-localization of QTL will be useful for concurrently enhancing the mineral element content in rice.

The physical locations of the QTLs identified in the present study were compared with those of previously reported QTLs that affect rice mineral content (Table S10). Five out of six QTLs (*qFe10*, *qZn8*, *qZn10*, *qMn10*, and *qCa10*) in this study were in accordance with the chromosomal regions carrying the QTL reported from previous studies confirming the existence of these loci, while only one QTL (*qMn6*) may be novel. The *qFe10* identified in our study shared an overlapping region reported by Nawaz et al., (2015) [20]. For Zn, two QTLs overlapped with previously reported QTLs. The *qZn8* in this study shared the overlapping regions of the previously reported QTL between the markers RM152 and RM223 that was identified from BC_2F_3 mapping populations derived from the cross of *O. sativa* cv. Swarna, and *O. nivara* [18]. Another QTL for Zn was identified in the backcross inbred line population on chromosome 10 between the markers RM1125 and RM6704, which overlapped the *qZn10* identified in our present study [14]. Garcia et al., (2009) [2] identified QTLs for Mn and Ca in ILs, which were near the *qMn10* and *qCa10* identified in the present study. Notably, *qMn6* did not overlap with the reported QTLs, implying that this could be a potentially novel QTL for improving the nutritional quality of rice.

Plants have established several tolerance mechanisms to cope with various environments. In this study, a hydroponic experiment was conducted to validate the candidate region by assessing the response of *O. rufipogon*, Hwaseong, CR2, and CR5 to different Fe and Zn treatments, based on the assumption that genotypic differences were attributed to inherent differences in tolerance. A significant difference in response to different Fe and Zn treatments was observed between *O. rufipogon* and Hwaseong. Furthermore, *O. rufipogon* had higher Fe and Zn contents than Hwaseong under all Fe and Zn treatments (Tables 3 and 4). Between Hwaseong and the two ILs, CR2 containing *O. rufipogon qFe10* showed significantly higher responses than Hwaseong and CR5 despite receiving the same Fe treatments under greenhouse and growth chamber conditions (Table 3). These results could be attributed to the potential of different rice genotypes to upregulate Strategy I (reduction strategy), II (chelation strategy), or combinations of both strategies for Fe absorption and uptake, as well as differences in Fe-responsive mechanisms among rice genotypes [58]. Plants have developed different chelating and reducing mechanisms for Fe uptake to obtain Fe from the rhizosphere. Non-Poaceae family members follow Strategy I, which involves ferrous ions (Fe^{2+}), whereas Poaceae family members, such as rice and maize, follow Strategy II, which involves ferric ions (Fe^{3+}), and their root epidermis releases phytosiderophores, creating stable Fe(III) chelates in the rhizosphere [49,59]. Rice is often grown in anaerobic paddy fields, where abundant Fe^{2+} is easily accessible to plants because of its low redox potential. This could explain why rice plants have an Fe^{2+} uptake mechanism despite being graminaceous plants [60–62]. In addition, rice functions as an iron-regulated transporter 1 (IRT1) homolog that enables the direct uptake of Fe^{2+} from the rhizosphere, demonstrating distinct uptake strategies for Fe^{2+} and Fe^{3+} [49]. We also observed that CR2, harboring the *O. rufipogon qZn10* allele, showed a significantly higher response than Hwaseong and CR5, despite receiving the same Zn treatments under greenhouse and growth chamber conditions (Table 4). This could be associated with Zn efficiency among rice genotypes and could be attributed to varietal differences in Zn solubilization and the degree of phytosiderophore secretion released from the plant roots [63]. The uptake of Zn has been linked to various root-related processes, including the release of

low-molecular-weight organic acids, increased efflux and uptake of Zn ligands, and efflux of phytosiderophores to solubilize unavailable forms of Zn in soils [64–66]. Homeostasis, which controls Fe and Zn translocation, absorption, and transport within the plant system, is another factor contributing to the variation in Fe and Zn contents [1]. However, further research is required to elucidate the underlying mechanism and possible genes that cause CR2 to have elevated Fe and Zn levels. Overall, the findings confirmed that the 375-kb *O. rufipogon* segment between the markers RM1873 and RM25612 is the candidate region responsible for increasing Fe and Zn content in rice.

Identifying new genes associated with mineral element traits and their potential use in the future is crucial to increase the mineral element content in rice. To date, several genes responsible for increased Fe and/or Zn content in rice have been cloned, including *Oryza sativa* yellow-like stripe 2 (*OsYSL2*), *Oryza sativa nicotianamine synthase* (*OsNAS*), and *Oryza sativa* ferritin 2 (*Osfer2*). *OsYSL2* is a metal-nicotianamine transporter for rice that is controlled by Fe, is expressed in the phloem cells, and is necessary for Fe translocation to seeds, particularly in the endosperm [67,68]. Three *NAS* genes have been identified in rice: *OsNAS1*, *OsNAS2*, and *OsNAS3*. Johnson et al. (2011) [69] reported that the constitutive overexpression of the *OsNAS* gene family showed a single-gene strategy for successfully biofortifying rice endosperm with up to a four-fold increase in Fe concentration and a two-fold increase in Zn concentration. Paul et al. (2012) [70] reported that the rice ferritin gene *Osfer2* resulted in an accumulation of iron and zinc that was 2.09- and 1.37-fold, respectively. Map-based cloning has also been applied to rice to identify genes that have a substantial impact on Fe and/or Zn regulation, including nicotianamine aminotransferase (*NAAT1*) [71] and *Oryza sativa* short postembryonic root 1 (*OsSPR1*) [72].

This study investigated the Fe and/or Zn-related candidate genes using a combined analysis of earlier reports on Fe and/or Zn regulation, sequence variants in their promoter and DNA coding regions, tissue-specific expression profiles, and gene expression levels in different tissues in rice (Table S8). These factors are crucial for elucidating the dynamic nature of gene regulation that controls the expression of specific phenotypic traits and changes in the transcriptome profile of each organ or tissue during the entire growth process as a result of both intrinsic and extrinsic factors. In this study, we identified 17 candidate genes having at least one nonsynonymous SNP or InDel in their DNA coding sequences (Table S9). Among the candidate genes, twelve belong to a family or subgroup previously reported and associated with Fe and/or Zn regulation. It appears that most of the genes listed in Table S8 may have a function in rice Fe and/or Zn absorption and transport [73–83]. Of the 17 genes, LOC_Os10g33960 (*Rice Homeobox gene 9* (*OsHox9*)) was identified as the high potential candidate gene of *qFe10* and *qZn10* based on the spatiotemporal expression profile and gene expression as revealed by both the rice expression database and qRT-PCR analysis. Homeobox transcription factors are members of a broad gene family and are recognized to be essential for several stages of plant development. Members of the homeobox transcription factor family have been divided into 14 categories, including HD-ZIP and TALE super-classes. Different HD-ZIP super-class members control a number of plant developmental processes and abiotic stress responses [84–88]. In wheat, the homeobox-leucine zipper protein HOX4, which was annotated as TaHDZIP1 on chromosome 5A, was associated with grain Zn concentration [77]. Since we further discussed the potential candidate genes for *qFe10* and *qZn10*, it is important to elucidate how these candidate genes actually work to regulate the Fe and/or Zn content in rice. Gene cloning and overexpression will help extract more information about these candidate genes.

5. Conclusions

In conclusion, this study demonstrated that *O. rufipogon* could serve as a potential resource for identifying rare alleles to enhance mineral element content in rice. Substitution mapping and hydroponic experiments further confirmed the association of *qFe10* and *qZn10* in enhancing the mineral element content in rice. In addition, this study provided a repertoire of information on CR2. This isogenic line can be exploited in future rice breeding

programs as a possible genetic resource to concurrently increase the Fe and Zn contents in rice, particularly in countries that greatly depend on rice-based diets. Furthermore, this study demonstrated that the combined analysis of gene expression data, tissue-specific expression profiles, and whole genome sequencing data yielded useful information regarding potential candidate genes in the *qFe10* and *qZn10* regions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13010076/s1, Figure S1: Comparison of seed morphology of parental lines and four selected ILs used in substitution mapping. Scale bar: 10 mm; Figure S2: Graphical genotype of four ILs used in substitution mapping. White regions indicate the Hwaseong genetic background, and the black regions indicate *O. rufipogon* chromosomal segments; Figure S3: Expression patterns of the candidate genes covering the *qFe10* and *qZn10* regions on chromosome 10. Data were obtained from the Rice Expression Profile Database (RiceXPro) (https://ricexpro.dna.affrc.go.jp/ (accessed on 20 May 2022)); Figure S4: Expression levels of the candidate genes in different tissues in rice covering the *qFe10* and *qZn10* regions on chromosome 10. Data were obtained from the Rice Expression Database (http://expression.ic4r.org/index (accessed on 1 June 2022)); Table S1: List of SSR markers used in this study; Table S2: List of KASP markers used in this study and their chromosomal position; Table S3: List of InDel markers used in substitution mapping and their chromosomal position; Table S4: List of markers used in qRT-PCR; Table S5: Correlation analysis among grain mineral element contents in brown rice in 96 ILs; Table S6: Agro-morphological characterization of the parental lines and two introgression lines grown under -Fe and +Fe treatments; Table S7: Agro-morphological characterization of the parental lines and two introgression lines grown under -Zn and +Zn treatments; Table S8: List of genes and SNPs/InDels identified in the *qFe10* and *qZn10* region; Table S9: List of putative candidate genes covering the *qFe10* and *qZn10* region on chromosome 10 and their nucleotide sequence variation found in the DNA coding region between Hwaseong and *O. rufipogon*; Table S10: Comparison of the QTLs for mineral element contents in the present and previous studies.

Author Contributions: C.A. and S.-N.A. designed the experiments and wrote the manuscript. Y.-T.Y. performed mineral element evaluation at CNARES. H.-S.L., K.-C.S. and N.H.L. conducted the agronomic traits investigation and performed genotyping. J.-W.K., and H.-J.K. developed the introgression lines. All authors contributed to the article and approved the submitted version. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All data supporting the findings of this study are provided within the article and within its supplementary data.

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References

1. Welch, R.M.; Graham, R.D. Breeding for micronutrients in staple food crops from a human nutrition perspective. *J. Exp. Bot.* **2004**, *55*, 353–364. [CrossRef] [PubMed]
2. Garcia-Oliveira, A.L.; Tan, L.; Fu, Y.; Sun, C. Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. *J. Integr. Plant Biol.* **2009**, *51*, 84–92. [CrossRef] [PubMed]
3. Meng, F.; Wei, Y.; Yang, X. Iron content and bioavailability in rice. *J. Trace Elem. Med. Biol.* **2005**, *18*, 333–338. [CrossRef] [PubMed]
4. Adeyeye, E.I.; Arogundade, L.A.; Akintayo, E.T.; Aisida, O.A.; Alao, P.A. Calcium, zinc, and phytate interrelationship in some foods of major consumption in Nigeria. *Food. Chem.* **2000**, *71*, 435–441. [CrossRef]
5. Mahender, A.; Anandan, A.; Pradhan, S.K.; Pandit, E. Rice grain nutritional traits and their enhancement using relevant genes and QTLs through advanced approaches. *SpringerPlus* **2016**, *5*, 2086. [CrossRef] [PubMed]
6. Kennedy, G.; Nantel, G.; Shetty, P. The scourage of "hidden hunger": Global dimensions of micronutrient deficiencies. *Food Nutr. Agric.* **2003**, *32*, 8–16.
7. Huang, Y.; Sun, C.; Min, J.; Chen, Y.; Tong, C.; Bao, J. Association mapping of quantitative trait loci for mineral element contents in whole grain rice (*Oryza sativa* L.). *J. Agric. Food Chem.* **2015**, *63*, 10885–10892. [CrossRef]

8. Tan, Y.; Sun, L.; Song, Q.; Mao, D.; Zhou, J.; Jiang, Y.; Wang, J.; Fan, T.; Zhu, Q.; Huang, D.; et al. Genetic architecture of subspecies divergence in trace mineral accumulation and elemental correlations in the rice grain. *Theor. Appl. Genet.* **2020**, *133*, 529–545. [CrossRef]
9. Cheon, K.S.; Baek, J.H.; Cho, Y.I.; Jeong, Y.M.; Lee, Y.Y.; Oh, J.; Won, Y.J.; Kang, D.Y.; Oh, H.; Lim, S.; et al. Single Nucleotide Polymorphism (SNP) discovery and Kompetitive Allele-Specific PCR (KASP) marker development with Korean Japonica rice varieties. *Plant. Breed. Biotech.* **2018**, *6*, 391–403. [CrossRef]
10. Yang, G.; Chen, S.; Chen, L.; Sun, K.; Huang, C.; Zhou, D.; Huang, Y.; Wang, J.; Liu, Y.; Wang, H.; et al. Development of a core SNP arrays based on the KASP method for molecular breeding of rice. *Rice* **2019**, *12*, 21. [CrossRef]
11. Adeva, C.C.; Lee, H.S.; Kim, S.H.; Jeon, Y.A.; Shim, K.C.; Luong, N.H.; Kang, J.W.; Kim, C.S.; Cho, J.H.; Ahn, S.N. Two complementary genes, *SBE3* and *GBSS1* contribute to high amylose content in *japonica* cultivar Dodamssal. *Plant. Breed. Biotech.* **2020**, *8*, 354–367. [CrossRef]
12. Brar, D.S.; Khush, G. Alien introgression in rice. *Plant. Mol. Biol.* **1997**, *35*, 35–47. [CrossRef] [PubMed]
13. Xie, X.; Song, M.H.; Jin, F.; Ahn, S.N.; Suh, J.P.; Hwang, H.G.; Kim, Y.G.; McCouch, S.R. Fine mapping of a yield-enhancing QTL cluster associated with transgressive variation in an *Oryza sativa* x *O. rufipogon* cross. *Theor. Appl. Genet.* **2008**, *116*, 613–622. [CrossRef]
14. Hu, B.-L.; Huang, D.-R.; Xiao, Y.-Q.; Fan, Y.-Y.; Chen, D.-Z.; Zhuang, J.-Y. Mapping QTLs for mineral element contents in brown and milled rice using an *Oryza sativa* x *O. rufipogon* backcross inbred line population. *Cereal Res. Commun.* **2016**, *44*, 57–68. [CrossRef]
15. Yun, Y.T.; Chung, C.T.; Lee, Y.J.; Na, H.J.; Lee, J.C.; Lee, S.G.; Lee, K.W.; Yoon, Y.H.; Kang, J.W.; Lee, H.S.; et al. QTL mapping of grain quality traits using introgression lines carrying *Oryza rufipogon* chromosome segments in *japonica* rice. *Rice* **2016**, *9*, 62. [CrossRef]
16. Sweeney, M.; McCouch, S. The complex history of the domestication of rice. *Ann. Bot.* **2007**, *100*, 951–957. [CrossRef] [PubMed]
17. Ishikawa, R.; Iwata, M.; Taniko, K.; Monden, G.; Miyazaki, N.; Orn, C.; Tsujimura, Y.; Yoshida, S.; Ma, J.F.; Ishii, T. Detection of quantitative trait loci controlling grain zinc concentration using Australian wild rice, *Oryza meridionalis*, a potential genetic resource for biofortification of rice. *PLoS ONE* **2017**, *12*, e0187224. [CrossRef]
18. Swamy, B.P.M.; Kaladhar, K.; Anuradha, K.; Batchu, A.K.; Logvah, T.; Sarla, N. QTL analysis for grain iron and zinc concentrations in two *O. nivara* derived backcross populations. *Rice Sci.* **2018**, *25*, 197–207. [CrossRef]
19. Liu, X.; Fan, F.; Liu, M.; Long, W.; Yu, Y.; Yuan, H.; Pan, G.; Li, N.; Li, S.; Liu, J. Quantitative trait loci mapping of mineral element contents in brown rice using backcross inbred lines derived from *Oryza longistaminata*. *Front. Plant Sci.* **2020**, *11*, 1229. [CrossRef]
20. Nawaz, Z.; Kakar, K.U.; Li, X.B.; Li, S.L.; Zhang, B.; Shou, H.X.; Shu, Q.Y. Genome-wide association mapping of quantitative trait loci (QTLs) for contents of eight elements in brown rice (*Oryza sativa* L.). *J. Agric. Food Chem.* **2015**, *63*, 8008–8016. [CrossRef]
21. Cho, Y.C.; Suh, J.P.; Choi, I.S.; Hong, H.C.; Baek, M.K.; Kang, K.H.; Kim, Y.G.; Ahn, S.N.; Choi, H.C.; Hwang, H.G.; et al. QTLs analysis of yield and its related traits in wild rice relative *Oryza rufipogon*. *Treat. Crop Res.* **2003**, *4*, 19–29.
22. Causse, M.A.; Fulton, T.M.; Cho, Y.G.; Ahn, S.N.; Chunwonse, J.; Wu, K.; Xiao, J.; Yu, Z.; Ronald, P.C.; Harrington, S.E.; et al. Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* **1994**, *138*, 1251–1274. [CrossRef] [PubMed]
23. Panaud, O.; Chen, X.; McCouch, S.R. Development of microsatellite markers and characterization of simple sequence 399 length polymorphism (SSLP) in rice (*Oryza sativa* L.). *Mol. Gen. Genet.* **1996**, *252*, 597–607. [CrossRef]
24. McCouch, S.R.; Teytelman, L.; Xu, Y.; Lobos, K.B.; Clare, K.; Walton, M.; Fu, B.; Maghirang, R.; Li, Z.; Xing, Y.; et al. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Res.* **2002**, *9*, 199–207. [CrossRef] [PubMed]
25. Akhtamov, M. Quantitative Trait Loci Analysis for Germination and Coleoptile Length under Low-Temperature Condition Using Introgression Lines Derived from an Interspecific cross in Rice. Master's Thesis, Science in Agronomy, Master of Science-Chungnam National University, Daejeon, Republic of Korea, August 2020.
26. Akhtamov, M.; Adeva, C.; Shim, K.-C.; Lee, H.-S.; Kim, S.H.; Jeon, Y.-A.; Luong, N.H.; Kang, J.-W.; Lee, J.-Y.; Ahn, S.N. Characterization of quantitative trait loci for germination and coleoptile length under low-temperature condition using introgression lines derived from an interspecific cross in rice. *Genes* **2020**, *11*, 1200. [CrossRef]
27. Kwon, Y.-K.; Bong, Y.-S.; Lee, K.-S.; Hwang, G.-S. An integrated analysis for determining the geographical origin of medicinal herbs using ICP-AES/ICP-MS and ^{1}H NMR analysis. *Food Chem.* **2014**, *161*, 168–175. [CrossRef]
28. Meng, L.; Li, H.H.; Zhang, L.Y.; Wang, J.K. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *Crop J.* **2015**, *3*, 269–283. [CrossRef]
29. Yoshida, S.; Forno, D.A.; Cock, J.H.; Gomez, K.A. *Laboratory Manual for Physiological Studies of Rice*, 3rd ed.; The International Rice Research Institute: Los Baños, Laguna, Philippines, 1976; pp. 1–83.
30. Chen, L.; Wang, G.; Chen, P.; Zhu, H.; Wang, S.; Ding, Y. Shoot-root communication plays a key role in physiological alterations of rice (*Oryza sativa*) under iron deficiency. *Front. Plant Sci.* **2018**, *9*, 757. [CrossRef]
31. Moreno-Moyano, L.T.; Bonneau, J.P.; Sanchez-Palacios, J.T.; Tohme, J.; Johnson, A.A.T. Association of increased grain iron and zinc concentrations with agro-morphological traits of biofortified rice. *Front. Plant Sci.* **2016**, *7*, 1463. [CrossRef]
32. Jeon, Y.-A.; Lee, H.-S.; Kim, S.-H.; Shim, K.-C.; Kang, J.-W.; Kim, H.-J.; Tai, T.H.; Ahn, S.-N. Natural variation in rice ascorbate peroxidase gene APX9 is associated with a yield-enhancing QTL cluster. *J. Exp. Bot.* **2021**, *72*, 4254–4268. [CrossRef]

33. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **2001**, *25*, 402–408. [CrossRef]
34. Anuradha, K.; Agarwal, S.; Batchu, A.K.; Babu, A.P.; Swamy, B.P.M.; Longvah, T.; Sarla, N. Evaluating rice germplasm for iron and zinc concentration in brown rice and seed dimensions. *J. Phytol.* **2012**, *4*, 19–25.
35. Anuradha, K.; Agarwal, S.; Rao, Y.V.; Rao, K.V.; Viraktamath, B.C.; Sarla, N. Mapping QTLs and candidate genes for iron and zinc concentrations in unpolished rice of Madhukar × Swarna RILs. *Gene* **2012**, *508*, 233–240. [CrossRef] [PubMed]
36. Chandel, G.; Banerjee, S.; Verulkar, S.B. Expression profiling of metal homeostasis related candidate genes in rice (*Oryza* spp.) using semi quantitative RT-PCR analysis. *Rice Genet. Newsl.* **2010**, *25*, 44–47.
37. Neelamraju, S.; Swamy, B.P.M.; Kaladhar, K.; Anuradha, K.; Rao, V.Y.; Batchu, A.K.; Agarwal, S.; Babu, A.P.; Sudhakar, T.; Sreenu, K.; et al. Increasing iron and zinc in rice grains using deep water rices and wild species-identifying genomic segments and candidate genes. *Qual. Assur. Saf. Crops Foods* **2012**, *4*, 138. [CrossRef]
38. Lu, L.; Tian, S.; Liao, H.; Zhang, J.; Yang, X.; Labavitch, J.M.; Chen, W. Analysis of metal element distributions in rice (*Oryza sativa* L.) seeds and relocation during germination based on X-ray fluorescence imaging of Zn, Fe, K, Ca, and Mn. *PLoS ONE* **2013**, *8*, e57360. [CrossRef]
39. Fageria, V.D. Nutrient interactions in crop plants. *J. Plant Nutr.* **2001**, *24*, 1269–1290. [CrossRef]
40. Stangoulis, J.C.R.; Huynh, B.-L.; Welch, R.M.; Choi, E.-Y.; Graham, R.D. Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. *Euphytica* **2007**, *154*, 289–294. [CrossRef]
41. Zeng, Y.-W.; Shen, S.-Q.; Wang, L.-X.; Liu, J.-F.; Pu, X.-Y.; Du, J.; Qiu, M. Correlation of plant morphological and grain quality traits with mineral element contents in Yunnan rice. *Rice Sci.* **2005**, *12*, 101–106.
42. Majumder, N.D.; Rakshit, S.C.; Borthakur, D.N. Genetic effect on uptake of selected nutrients in some rice (*O. sativa* L.) varieties in phosphorus deficient soils. *Plant Soil* **1990**, *123*, 117–120. [CrossRef]
43. Collard, B.C.Y.; Jahufer, M.Z.Z.; Brouwer, J.B.; Pang, E.C.K. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica* **2005**, *142*, 169–196. [CrossRef]
44. Buescher, E.; Achberger, T.; Amusan, I.; Giannini, A.; Ochsenfeld, C.; Rus, A.; Lahner, B.; Hoekenga, O.; Yakubova, E.; Harper, J.F.; et al. Natural genetic variation in selected populations of *Arabidopsis thaliana* is associated with ionomic differences. *PLoS ONE* **2010**, *5*, e11081. [CrossRef]
45. Loudet, O.; Saliba-Colombani, V.; Camilleri, C.; Calenge, F.; Gaudon, V.; Koprivova, A.; North, K.A.; Kopriva, S.; Daniel-Vedele, F. Natural variation for sulfate content in *Arabidopsis thaliana* is highly controlled by APR2. *Nat. Genet.* **2007**, *39*, 896–900. [CrossRef] [PubMed]
46. Martinez-Trujillo, M.; Carreon-Abud, Y. Effect of mineral nutrients on the uptake of Cr(VI) b maize plants. *N Biotechnol.* **2015**, *32*, 396–402. [CrossRef] [PubMed]
47. Phuke, R.M.; Anuradha, K.; Radhika, K.; Jabeen, F.; Anuradha, G.; Ramesh, T.; Hariprasanna, K.; Mehtre, S.P.; Deshpande, S.P.; Anil, G.; et al. Genetic variability, genotype × environment Interaction, correlation, and GGE biplot analysis for grain iron and zinc concentration and other agronomic traits in RIL population of sorghum (*Sorghum bicolor* L. Moench). *Front. Plant Sci.* **2017**, *8*, 712. [CrossRef]
48. Dixit, S.; Singh, U.M.; Abbai, R.; Ram, T.; Singh, V.K.; Paul, A.; Virk, P.S.; Kumar, A. Identification of genomic region(s) responsible for high iron and zinc content in rice. *Sci. Rep.* **2019**, *9*, 8136. [CrossRef]
49. Garcia-Oliveira, A.L.; Chander, S.; Ortiz, R.; Menkir, A.; Gedil, M. Genetic basis and breeding perspectives of grain and zinc enrichment in cereals. *Front. Plant Sci.* **2018**, *9*, 937. [CrossRef]
50. Calayugan, M.I.C.; Formantes, A.K.; Amparado, A.; Descalsota-Empleo, G.I.; Nha, C.T.; Inabangan-Asilo, M.A.; Swe, Z.M.; Hernandez, J.E.; Borromeo, T.H.; Lalusin, A.G.; et al. Genetic analysis of agronomic traits and grain iron and zinc concentrations in a doubled haploid population of rice (*Oryza sativa* L.). *Sci. Rep.* **2020**, *10*, 2283. [CrossRef]
51. Kobayashi, T.; Itai, R.N.; Nishizawa, N.K. Iron deficiency responses in rice roots. *Rice* **2014**, *7*, 27. [CrossRef]
52. Suzuki, M.; Tsukamoto, T.; Inoue, H.; Watanabe, S.; Matsuhashi, S.; Takahashi, M.; Nakanishi, H.; Mori, S.; Nishizawa, N.K. Deoxymugineic acid increases Zn translocation in Zn-deficient rice plants. *Plant Mol. Biol.* **2008**, *66*, 609–617. [CrossRef]
53. Beasley, J.T.; Hart, J.J.; Tako, E.; Glahn, R.P.; Johnson, A.A.T. Investigation of nicotianamine and 2'deoxymugineic acid as enhancers of iron bioavailability in Caco-2 cells. *Nutrients* **2019**, *11*, 1502. [CrossRef]
54. Lee, S.; An, G. Over-expression of *OsIRT1* leads to increased iron and zinc accumulations in rice. *Plant Cell Environ.* **2009**, *32*, 408–416. [CrossRef]
55. Haydon, M.J.; Kawachi, M.; Wirtz, M.; Hillmer, S.; Hell, R.; Kramer, U. Vacuolar nicotianamine has critical and distinct roles under iron deficiency and for zinc sequestration in *Arabidopsis*. *Plant Cell* **2012**, *24*, 724–737. [CrossRef] [PubMed]
56. Wu, Q.; Liu, C.; Wang, Z.; Gao, T.; Liu, Y.; Xia, Y.; Yin, R.; Qi, M. Zinc regulation of iron uptake and translocation in rice (*Oryza sativa* L.): Implication from stable iron isotopes and transporter genes. *Environ. Pollut.* **2022**, *297*, 118818. [CrossRef] [PubMed]
57. He, J.; Rossner, N.; Hoang, M.T.T.; Alejandro, S.; Peiter, E. Transport, functions, and interaction of calcium and manganese in plant organellar compartments. *Plant Physiol.* **2021**, *187*, 1940–1972. [CrossRef] [PubMed]
58. Pereira, M.P.; Santos, C.; Gomes, A.; Vasconcelos, M.W. Cultivar variability of iron uptake mechanisms in rice (*Oryza sativa* L.). *Plant Physiol. Biochem.* **2014**, *85*, 21–30. [CrossRef] [PubMed]
59. Roberts, L.A.; Pierson, A.J.; Panaviene, Z.; Walker, E.L. Yellow stripe1. Expanded roles for the maize iron- phytosiderophore transporter. *Plant Physiol.* **2004**, *135*, 112–120. [CrossRef]

60. Bughio, N.; Yamaguchi, H.; Nishizawa, N.K.; Nakanishi, H.; Mori, S. Cloning an iron-regulated metal transporter from rice. *J. Exp. Bot.* **2002**, *53*, 1677–1682. [CrossRef]
61. Ishimaru, Y.; Suzuki, M.; Tsukamoto, T.; Suzuki, K.; Nakazono, M.; Kobayashi, T.; Wada, Y.; Watanabe, S.; Matsuhashi, S.; Takahashi, M.; et al. Rice plants take up iron as an Fe^{3+}-phytosiderophore and as Fe^{2+}. *Plant J.* **2006**, *45*, 335–346. [CrossRef]
62. Masuda, H.; Aung, M.S.; Kobayashi, T.; Hamada, T.; Nishizawa, N.K. Enhancement of iron acquisition in rice by the mugineic acid synthase gene with ferric iron reductase gene and OsIRO2 confers tolerance in submerged and nonsubmerged calcareous soils. *Front. Plant Sci.* **2019**, *10*, 1179. [CrossRef]
63. Arnold, T.; Kirk, G.J.D.; Wissuwa, M.; Frei, M.; Zhao, F.J.; Mason, T.F.D.; Weiss, D.J. Evidence for the mechanisms of zinc uptake by rice using isotope fractionation. *Plant Cell. Environ.* **2010**, *33*, 370–381. [CrossRef] [PubMed]
64. Widodo, B.; Broadley, M.R.; Rose, T.; Frei, M.; Pariasca-Tanaka, J.; Yoshihashi, T.; Thomson, M.; Hammond, J.P.; Aprile, A.; Close, T.J.; et al. Response to zinc deficiency of two rice lines with contrasting tolerance is determined by root growth maintenance and organic acid exudation rates, and not by zinc-transporter activity. *New Phytol.* **2010**, *186*, 400–414. [CrossRef] [PubMed]
65. Impa, S.M.; Johnson-Beebout, S.E. Mitigating zinc deficiency and achieving high grain Zn in rice through integration of soil chemistry and plant physiology research. *Plant Soil* **2012**, *361*, 3–41. [CrossRef]
66. Johnson-Beebout, S.; Goloran, J.B.; Rubianes, F.H.; Jacob, J.D.C.; Castillo, O.B. Zn uptake behavior of rice genotypes and its implication on grain Zn biofortification. *Sci. Rep.* **2016**, *6*, 38301. [CrossRef] [PubMed]
67. Koike, S.; Inoue, H.; Mizuno, D.; Takahashi, M.; Nakanishi, H.; Mori, S.; Nishizawa, N.K. OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. *Plant J.* **2004**, *39*, 415–424. [CrossRef]
68. Ishimaru, Y.; Masuda, H.; Bashir, K.; Inoue, H.; Tsukamoto, T.; Takahashi, M.; Nakanishi, H.; Aoki, N.; Hirose, T.; Ohsugi, R.; et al. Rice metal-nicotianamine transporter, OsYSL2, is required for the long-distance transport of iron and manganese. *Plant J.* **2010**, *62*, 379–390. [CrossRef]
69. Johnson, A.A.T.; Kyriacou, B.; Callahan, D.L.; Carruthers, L.; Stangoulis, J.; Lombi, E.; Tester, M. Constitutive overexpression of the OsNAS gene family reveals single-gene strategies for effective iron- and zinc-biofortification of rice endosperm. *PLoS ONE* **2011**, *6*, e24476. [CrossRef]
70. Paul, S.; Ali, N.; Gayen, D.; Datta, S.K.; Datta, K. Molecular breeding of Osfer2 gene to increase iron nutrition in rice grain. *GM Crops Food* **2012**, *3*, 310–316. [CrossRef]
71. Cheng, L.; Wang, F.; Shou, H.; Huang, F.; Zheng, L.; He, F.; Li, J.; Zhao, F.-J.; Ueno, D.; Ma, J.F.; et al. Mutation in nicotianamine aminotransferase stimulated the Fe (II) acquisition system and led to iron accumulation in rice. *Plant Physiol.* **2007**, *145*, 1647–1657. [CrossRef]
72. Jia, L.; Wu, Z.; Hao, X.; Carrie, C.; Zheng, L.; Whelan, J.; Wu, Y.; Wang, S.; Wu, P.; Mao, C. Identification of a novel mitochondrial protein, short postembryonic roots 1 (SPR1), involved in root development and iron homeostasis in *Oryza sativa*. *New Phytol.* **2011**, *189*, 843–855. [CrossRef]
73. Shen, J.; Xu, X.; Li, T.; Cao, D.; Han, Z. An MYB transcription factor from *Mallus xiaojinensis* has a potential role in iron nutrition. *J. Integr. Plant Biol.* **2008**, *50*, 1300–1306. [CrossRef] [PubMed]
74. Ying, S. Genome-wide identification and transcriptional analysis of Arabidopsis *DUF506* gene family. *Int. J. Mol. Sci.* **2021**, *22*, 11442. [CrossRef] [PubMed]
75. Qi, Y.; Wang, S.; Shen, C.; Zhang, S.; Chen, Y.; Xu, Y.; Liu, Y.; Wu, Y.; Jiang, D. OsARF12, a transcription activator on auxin response gene, regulates root elongation and affects iron accumulation in rice (*Oryza sativa*). *New Phytol.* **2011**, *193*, 109–120. [CrossRef] [PubMed]
76. Shen, C.; Yue, R.; Sun, T.; Zhang, L.; Yang, Y.; Wang, H. OsARF16, a transcription factor regulating auxin redistribution, is required for iron deficiency response in rice (*Oryza sativa* L.). *Plant Sci.* **2015**, *231*, 148–158. [CrossRef]
77. Alomari, D.Z.; Eggert, K.; von Wiren, N.; Alqudah, A.M.; Polley, A.; Plieske, J.; Ganal, M.W.; Pillen, K.; Roder, M.S. Identifying candidate genes for enhancing grain Zn concentration in wheat. *Front. Plant. Sci.* **2018**, *9*, 1313. [CrossRef]
78. Cu, S.T.; Guild, G.; Nicolson, A.; Velu, G.; Singh, R.; Stangoulis, J. Genetic dissection of zinc, iron, copper, manganese and phosphorus in wheat (*Triticum eastivum* L.) grain and rachis at two developmental stages. *Plant Sci.* **2020**, *291*, 110338. [CrossRef]
79. Pujar, M.; Gangaprasad, S.; Govindaraj, M.; Gangurde, S.S.; Kanatti, A.; Kudapa, H. Genome-wide association study uncovers genomic regions associated with grain iron, zinc and protein content in pearl millet. *Sci. Rep.* **2020**, *10*, 19473. [CrossRef]
80. Mohiuddin, S.J.; Haque, M.A.; Haque, M.M.; Islam, M.T.; Biswas, P.S. Genetic analysis reveals a major effect QTL associated with high grain zinc content in rice. *Plant. Breed. Biotech.* **2020**, *8*, 327–340. [CrossRef]
81. Gollhofer, J.; Schlawicke, C.; Jungnick, N.; Schmidt, W.; Buckhout, T.J. Members of a small family of nodulin-like genes are regulated under iron deficiency in roots of *Arabidopsis thaliana*. *Plant Physiol. Biochem.* **2011**, *49*, 557–564. [CrossRef]
82. Yu, H.; Yang, J.; Shi, Y.; Donelson, J.; Thompson, S.M.; Sprague, S.; Roshan, T.; Wang, D.-L.; Liu, J.; Park, S.; et al. Arabidopsis Glutaredoxin S17 contributes to vegetative growth, mineral accumulation, and redox balance during iron deficiency. *Front. Plant Sci.* **2017**, *8*, 1045. [CrossRef]
83. Ramireddy, E.; Galuszka, P.; Schmulling, T. Zn-fortified cereals in field-grown barley by enhanced root cytokinin breakdown. *Plant Signal Behav.* **2018**, *13*, 11. [CrossRef] [PubMed]
84. Gehring, W.J.; Affolter, M.; Burglin, T. Homeodomain proteins. *Annu. Rev. Biochem.* **1994**, *63*, 487–526. [CrossRef] [PubMed]
85. Nam, J.; Nei, M. Evolutionary change of the numbers of homeobox genes in bilateral animals. *Mol. Biol. Evol.* **2005**, *22*, 2386–2394. [CrossRef] [PubMed]

86. Jain, M.; Tyagi, A.K.; Khurana, J.P. Genome-wide identification, classification, evolutionary expansion and expression analyses of homeobox genes in rice. *FEBS J.* **2008**, *275*, 2845–2861. [CrossRef] [PubMed]
87. Mukherjee, K.; Brocchieri, L.; Burglin, T.R. A comprehensive classification and evolutionary analysis of plant homeobox genes. *Mol. Biol. Evol.* **2009**, *26*, 2775–2794. [CrossRef] [PubMed]
88. Bhattacharjee, A.; Khurana, J.P.; Jain, M. Characterization of rice homeobox genes, *OsHOX22* and *OsHOX24*, and over-expression of *OsHOX24* in transgenic Arabidopsis suggest their role in abiotic stress response. *Front. Plant. Sci.* **2016**, *7*, 627. [CrossRef]