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## Haplotype diversity of microsatellite markers linked to QTLs controlling zinc content in rice grains

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### Abstract

Zinc deficiency is one of the serious problems of malnutrition in developing countries. Identifying genotypes with high levels of grain zinc in rice genetic resources and understanding the genetic basis of its accumulation is a prerequisite in genetically breeding programs of zinc. In this study, the haplotype diversity of three loci controlling zinc content were evaluated using 50 genotypes and 14 associated microsatellite markers. The grain zinc content was measured using atomic absorption with two replications. The Neemat and Dadras cultivars with the amount of 10.94 and 36.92 mg.Kg<sup>-1</sup> had the lowest and highest grain zinc content, respectively. According to the analysis of molecular, RM6925 was showed highest polymorphism information content (0.79). Haplotype of Mehr cultivar with allele combinations of 166-184-327-266 bp on chromosome 8 had the most similarity with haplotype of reference cultivar Dadras. This allele combination can be regarded as the effective combination of alleles for marker-assisted selection in the breeding programs of this microelement. Furthermore, the presence of allele combinations different from reference haplotype in cultivars with high zinc concentrations indicated the presence of new QTLs controlling grain zinc content in these rice cultivars.

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## Introduction

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 (Graham *et al.*, 1999; Chandel *et al.*, 2011). However, rice is a poor source of essential minerals such as zinc and iron (Bouis and Welch, 2010).

Zinc deficiency is serious nutritional problem for humans and is particularly prevalent among children and pregnant women, especially in developing countries (World Health Organization 2002, <http://www.who.int/whr/2002/>). Therefore, increasing the zinc content and bioavailability of cereals such as rice grains is an important target for breeders and offers the potential benefits to a large proportion of human population (Zhu *et al.*, 2007; Cakmak, 2008; Palmgren *et al.*, 2008). Traditional breeding, marker-assisted breeding, and plant transformation techniques and a combination of these techniques can be further exploited to mitigate the zinc deficiency in rice and humans. Many researchers have already evaluated genetic diversity for zinc content in cereal grains such as rice (Gregorio *et al.*, 2000; Zhang *et al.*, 2004), wheat (Cakmak *et al.*, 2000; Ortiz-Monasterio and Graham, 2000; Balint *et al.*, 2001) and reported the narrow genetic base for zinc in cultivated rice (Gregorio *et al.*, 2000; Ortiz-Monasterio and Graham, 2000; Stangoulis *et al.*, 2007).

Zinc content is quantitative trait which is affected by the environment (Gregorio *et al.*, 2000; Virk *et al.*, 2006, 2007). Quantitative trait locus (QTL) mapping is a powerful tool for understanding the genetic controls underlying complex traits (Yano and Sasaki, 1997; Yamamoto *et al.*, 2009). QTL mapping has been used extensively to identify genetic loci determining mineral content in rice (Ishikawa *et al.*, 2005; Lu *et al.*, 2008). Stangoulis *et al.* (2007) identified some QTLs for Fe, Zn, Mn and P in rice grain using a doubled-haploid population. Zinc content had two QTLs on chromosomes one and 12, explaining 15%

and 13% of the total phenotypic variation, respectively. The zinc QTL on chromosome 12 was co-located with the iron QTL. Garcia-Oliveira *et al.* (2009) reported three QTLs controlling zinc content in rice were mapped on chromosomes 5, 8, and 12. The QTL near marker RM152 on chromosome 8 accounted for 19% of phenotypic variation and the QTL that was located on chromosome 12 accounted for 9% of phenotypic variation. Susanto (2008) mapped two QTLs associated with zinc content in polished rice grain were detected on chromosomes 6 and 12.

In recent times there have been advances in development and mapping of QTLs controlling zinc content in rice. However, there are no reports on evaluation of haplotype variation of zinc QTLs in Iranian rice cultivars and other genotypes by reported linked molecular markers.

In this study, it has been evaluated genomic regions encompassing three known QTLs controlling zinc content that located on chromosome 6, 8 and 12 in rice by microsatellite or Simple Sequence Repeat (SSR) markers (Stangoulis *et al.*, 2007; Susanto, 2008; Garcia-Oliveira *et al.*, 2009; Ishikawa *et al.*, 2010).

The objectives of this research were to: (1) identify genotypes with high zinc content from a rice germplasm to exploit in breeding programs to enhance levels of zinc content, (2) investigate genetic relationships of rice genotypes with same zinc content, (3) analyze haplotype diversity of markers linked to three previously identified QTLs controlling zinc in rice grains with the aim of identifying and classifying allelic variation at these loci. The haplotype data presented here will provide the basis from which breeders may accurately identify, utilize and trace allelic variation at these loci in their breeding material (4) identify and utilize of novel alleles or allele combinations not previously deployed.

**Materials and methods**

*Plant materials*

Plant materials of this study were 50 different rice genotypes, including 26 Iranian landrace cultivars and 15 Iranian improved genotypes, 5 rice genotypes with different origin and 4 IRRI rice lines, which were

received from Rice Research Institute of Iran (IRRI) (Table 1). All genotypes were grown in the same soil and season. The grain zinc content of all the genotypes was measured with two replications using dry ash method and atomic absorption spectrophotometry (Munson *et al.*, 1990).

**Table 1.** The names of evaluated genotypes in this research and zinc averages (mg.kg<sup>-1</sup>).

Genotype	Zinc average	Genotype	Zinc average
Iranian landrace genotypes		Iranian improved genotypes	
Dadras	36.92	Bojar	34.30
Abjibuji	36.69	Mehr	33.19
Hassan molaeei	28.38	Haraz	22.17
Deilamani	26.81	111	19.85
Shahak	25.83	Gil1	18.85
Sangejo	25.57	Line 29 [from Mohammadi×(Amol3×Tarom)]	17.82
Aghaeii seiah	24.67	Gil3	16.37
Hassani	23.59	Line 27 [from Asgari× Ch21]	15.63
Champa budar	21.78	Dorfak	15.53
Ghashange	21.32	Khazar	15.04
Cheli	21.06	Sepidrud	13.18
Gharib	20.42	Fajr	13.13
Ghanbarak	20.29	Amol3	12.87
Hasani fumani	19.85	Dasht	11.46
Mosa tarom	19.36	Neemat	10.94
Rashti sard	19.08	IRRI lines	
Ramezanali tarom	18.85	Line 18 [from IRRI]	21.58
Ali kazemi	18.85	Line 13 [from IRRI]	17.74
Domsorkh	18.13	IR30	17.35
Shahpasand	17.33	IR24	13.85
Salari	16.71	Genotypes with different origin	
Farideh	16.12	Fuji minori	15.40
Sadri	16.07	Onda	20.01
Anbarbu	15.76	Zinet	14.75
Domsiah	15.68	Norin22	18.98
Hashemi	11.25	Century patna	17.48

*DNA extraction, SSR genotyping*

Template DNA for the polymerase chain reactions (PCR) was extracted from leaf tissue using the modified CTAB method (Saghai-Marooif *et al.*, 1984). SSR markers were chosen on the basis of their proximity to QTL regions associated with zinc content and genome specificity. The SSRs were both public and proprietary databases of SSRs from Gramene (<http://www.gramene.org>) and Rice Togo Browser (<http://agri-trait.dna.affrc.go.jp>). A set of 14 SSR assays was applied to a subset of 50 rice genotypes (Table 2). The PCR reaction was performed in a 15 µL volume using the Applied Biosystems Veriti thermocycler. Each 15 µL reaction contained 1.5 µL of 10 × PCR buffer, 0.5 µL of 50 mM MgCl<sub>2</sub>, 0.18 µL of 10 mM dNTP, 0.4 µL of each SSR primer (5 µM), 0.15

µL of 5U/ µL *Taq* DNA polymerase and 20 ng of genomic DNA. The amplification program consisted of an initial denaturation of 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 54-58 °C for 1 min, 72 °C for 1 min, and ending with a 72 °C for 7 min final extension. Amplicons were electrophoresed through a 3% MetaPhor agarose gel, and stained with ethidium bromide.

*Data analysis*

Analysis of variance for zinc content data were performed using SPSS program. The size of amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size markers, 100 bp DNA ladder using Alpha-Ease FC 5.0 software (Alpha

Innotech, USA). PowerMarker version 3.25 software was used to calculate the average number of alleles, major allele frequency, gene diversity, and polymorphism information content (PIC) values (Liu

and Muse, 2005). Haplotype diversity was analyzed according to McCartney *et al.* (2004) and Mohammadi-Nejad *et al.* (2010).

**Table 2.** List of SSR markers used, chromosome location, number of amplified alleles, polymorphic information content (PIC) and gene diversity.

Marker	chr	Major Allele Frequency	Allele NO	Gene diversity	PIC	Amplicon size range (bp)
RM8226	6	0.26	6	0.80	0.78	205-250
RM7193	6	0.5	8	0.69	0.65	122-186
RM152	8	0.38	5	0.71	0.66	132-159
RM337	8	0.46	5	0.69	0.64	154-457
RM6925	8	0.32	10	0.81	0.79	155-287
RM407	8	0.44	4	0.69	0.64	162-176
RM22253	8	0.42	3	0.64	0.56	306-327
RM22254	8	0.58	4	0.57	0.5	252-274
RM17	12	0.72	5	0.45	0.42	154-184
RM235	12	0.26	6	0.80	0.78	102-137
RM270	12	0.5	3	0.62	0.54	104-111
RM3331	12	0.31	5	0.78	0.74	128-144
RM1999	12	0.34	5	0.77	0.73	170-227
RM28722	12	0.78	2	0.34	0.28	183-200
Mean		0.45	5.1	0.67	0.62	

### Results

The grain zinc content of cultivars varied from 10.94 (Neemat cultivar) to 36.92 (Dadras cultivar) mg.kg<sup>-1</sup> (Table 1), and the average of grain zinc content was estimated 19.68 mg.kg<sup>-1</sup>. The analysis of variance results showed highly significant difference among the genotypes for grain zinc content and an average of grain zinc content in landraces (21.40 mg.kg<sup>-1</sup>) were significantly higher than improved cultivars (17.81 mg.kg<sup>-1</sup>).

The fourteen SSR markers revealed 71 alleles among the 50 rice genotypes. The number of microsatellite alleles varied from 2 to 10, which RM6925 produced the highest number of alleles and RM28722 produced the lowest (Table 2). PIC value ranged from 0.28 to 0.79, the highest value belonged to RM6925, while RM28722 showed the lowest PIC value. Therefore, the SSR marker RM6925 was found to be suitable for analysis of genetic diversity among the markers in this research.

In evaluation of haplotype diversity, Dadras cultivar with the highest grain zinc content used as reference

genotype. Haplotypes were sorted for each QTL by the size of their fragments. Allele combinations for each QTL were compared to the haplotype of reference genotype and similar allele combinations were grouped together. Three QTL regions were analyzed for haplotype diversity.

#### The QTL region on chromosome 8

It has been employed six SSR markers to haplotyping this locus in our genotypes. Allelic pattern (190-166-184-159-327-266 bp) was observed in Dadras at six SSR loci: RM337, RM407, RM6925, RM152, RM22253 and RM22254, respectively. Based on allelic pattern of this region, genotypes arranged in various haplotype groups. Twenty-three haplotypes were identified among the 50 rice genotypes (Table 3). None of the 50 genotypes showed Dadras haplotype in this QTL but haplotypes No. 2, 3, 4 and 5 were similar to Dadras haplotype in most SSRs.

Comparison of these genotypes with Dadras pattern showed Mehr cultivar, with high level of grain zinc content, amplified the same SSR alleles (166-184-327-266 bp) for RM407, RM6925, RM22253 and



**Table 5.** Rice haplotypes produced by SSR markers located on QTL region associated with zinc content on chromosome 12 with reference to Dadras cultivar\*.

RM3331	■	□	■	□	□	□	□	□	□	□	■	□	■	■	■	□	□	□	□
RM270	□	□	□	■	□	■	□	□	□	□	■	■	■	□	□	□	□	■	□
RM235	□	□	□	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□
RM1999	□	□	□	□	■	□	□	□	■	■	□	□	□	□	□	□	□	□	□
RM28722	□	□	□	□	□	□	□	□	□	□	■	■	■	□	□	□	□	■	□
RM17	□	□	□	□	□	□	□	□	□	■	■	■	□	□	□	■	■	□	□
Haplotype No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19

1: Dadras\*, 2: Deilamani, Sangejo, Mosa tarom, Domsorkh, Salari, 3: Hashemi, 4: Aghaeii seiah, 5: Hassan molaiei, Hassani, Ghanbarak, Rashti sard, Ramezanali tarom, Gil1, Farideh, 6: Fuji, 7: Abjibuji, 111, Line 27, Zinet, 8: Amol3, Dorfak, Bojar, sadri, 9: Haraz, Gil3, IR24, 10: Anbarbu, Hassani fumani, 11: Cheli, 12: Champa budar, 13: Line 18, Line 13, 14: Onda, 15: Ali Kazemi 16: Mehr, Ghashange, Gharib, Norin22, Cantry patna, Domseiah, Khazar, Dasht,..17: IR30, 18: Line 29, Shahpasand, Fajr, 19: Sepidrud, Neemat, Shahak.

*The QTL region on chromosome 6*

Two markers, RM8226 and RM7193, were used for haplotyping the QTL region on chromosome 6. Based on allelic pattern of this QTL, 50 genotypes arranged in four haplotype groups (Table 6). Aghaeii seiah cultivar with high grain zinc content showed Dadras haplotype in this QTL. Therefore, allele combinations 223-145 bp for markers RM8226 and RM7193, respectively, can be useful for grain zinc content breeding.

**Table 6.** Rice haplotypes produced by SSR markers located on QTL region associated with zinc content on chromosome 6 with reference to Dadras cultivar\*.

RM8226	■	■	□	□
RM7193	■	□	■	□
Haplotype No	1	2	3	4

1: Dadras\*, Aghaeii seiah, 2: Rashti sard, Domsorkh, 111, Sadri, Khazar, 3: Bojar, Mehr, Domseiah, Hassan molaiei, Hassani, Haraz, Cheli, Line 29, Gil3, Line 27, Dorfak, Sepidrud, Fajr, Amol3, Onda, Norin22, Line 18, Line 13, Century patna, IR24, IR30, 4: Other cultivars.

**Discussion**

Rice provides energy to almost half of the world’s population. Thus, increasing the zinc content of rice by traditional plant-breeding methods or molecular marker techniques has a great potential to mitigate wide spread zinc deficiency problem in humans (Ruel and Bouis, 1998; Bouis, 2000; Welch and Graham, 2004). Therefore, it is essential to understand genetic bases of accumulation of zinc. So far, several studies have reported the genetics of accumulation of zinc in the rice grains. Hanarida *et al.* (2002) evaluated 251 rice genotypes (local varieties, advanced lines, and improved varieties) and reported high variability for zinc (16.5-43 mg.Kg<sup>-1</sup>) content in rice grains. Similarly, Anandan *et al.* (2011) evaluated 202 rice genotypes and reported that zinc concentration ranged from 3.00 to 38.60 mg.kg<sup>-1</sup> with a mean value of 15.84 mg.kg<sup>-1</sup>. In present study, the average of zinc content (19.68 mg.kg<sup>-1</sup>) was higher than that was reported for 202 rice genotypes. The difference might be due to variation of genotypes and genotype × environment interaction. Similar to this results, Anandan *et al.* (2011) and Gregorio *et al.* (2000) indicated that most of the traditional varieties contained high zinc and iron in the grain, whereas the modern -released varieties produced lower

micronutrient content in the grain. These observations can be attributed to the fact that direct selection for high zinc content was not part of the previous rice breeding program and the variability in improved varieties for zinc content was only as a consequence of random drift or indirect selection effects.

In the current study, among the twenty-three haplotypes identified for the zinc QTL on chromosome 8, haplotype of Mehr cultivar (contained high zinc) showed same SSR alleles with Dadras haplotype. Based on the result it is concluded, RM407, RM6925, RM22253 and RM22254 markers by allelic pattern of 166-184-327-266 bp are helpful allele combinations for identifying and utilizing zinc sources in breeding programs. Further, some genotypes such as Abjibuji, Hassan molaeii, Deilamani, Sangejo, had distinct haplotype patterns in this QTL, well-known zinc sources, suggesting that they could carry new zinc QTLs. Therefore, these cultivars could potentially be exploited to identify new QTLs of grain zinc in Iranian rice.

So far, many studies assessed haplotype variation of genomic regions controlling numerous traits in cereals, but were not found any researches for haplotype diversity of micronutrients QTLs. Mohammadi-Nejad *et al.* (2010) studied haplotype diversity for saltol QTL controlling salinity tolerance in rice, they found total of 16 haplotype groups using 8 microsatellite markers and 30 genotypes. Also McCartney *et al.* (2004) identified 76 haplotypes among the 79 wheat lines for fusarium head blight resistance QTLs.

It is concluded that the genotypes belong to different haplotypes, such as Dadras and Neemat cultivars, can be used for hybridization to generate helpful recombinants in the segregating generations, the genetics and breeding programs for improvement of zinc content in rice grains.

## References

- Anandan A, Rajiv G, Eswaran R, Prakash M.** 2011. Genotypic variation and relationships between quality traits and trace elements in traditional and improved rice (*Oryza sativa* L.) genotypes. *Journal of food science* **76**, 122-130.
- Balint AF, Kovacs G, Erdei L, Sutka J.** 2001. Comparison of the Cu, Zn, Fe, Ca and Mg contents of the grains of wild, ancient and cultivated wheat species. *Cereal Research Communications* **29**, 375-382.
- Bouis HE, Welch RM.** 2010. Biofortification-a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Science* **50**, 20-32.
- Bouis HE.** 2000. Enrichment of food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. *Nutrition* **16**, 701-705.
- Cakmak I, Ozkan H, Braun HJ, Welch RM, Romheld V.** 2000. Zinc and iron concentrations in seeds of wild, primitive, and modern wheat. *Food and Nutrition Bulletin* **21**, 401-403.
- Cakmak I.** 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification. *Plant and Soil* **302**, 1-17.
- Chandel G, Samuel P, Dubey M, Meena R.** 2011. In silico expression analysis of QTL specific candidate genes for grain micronutrient (Fe/Zn) content using ESTs and MPSS signature analysis in rice (*Oryza sativa* L.). *Journal of Plant Genetics and Transgenics* **2**, 11-22.
- Garcia-Oliveira AL, Tan L, Fu Y, Sun C.** 2009. Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. *Journal of Integrative Plant Biology* **51**, 84-92.

- Graham RD, Senadhira D, Beebe S, Iglesias C, Monasterio I.** 1999. Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. *Field Crops Research* **60**, 57-80.
- Gregorio GB, Senadhira D, Htut T, Graham RD.** 2000. Breeding for trace mineral density in rice. *Food and Nutrition Bulletin* **21**, 382-386.
- Hanarida I, Indrasari SD, Daradjat A.** 2002. Indonesian final report: breeding for iron dense rice: a low cost, sustainable approach to reduce anemia in Asia. Indonesia: IFPRI (International Food Policy Research Institute), CRIFC (Central Research Institute for Food Crops), RIFCB (Research Institute for Food Crops Biotechnology), RIR (Research Institute for Rice).
- Ishikawa S, Abe T, Kuramata M, Yamaguchi M, Ando T, Yamamoto T.** 2009. A major quantitative trait locus for increasing cadmium-specific concentration in rice grain is located on the short arm of chromosome 7. *Journal of experimental botany* **61**, 923-934.
- Ishikawa S, Ae N, Yano M.** 2005. Chromosomal regions with quantitative trait loci controlling cadmium concentration in brown rice (*Oryza sativa* L.). *New Phytologist* **168**, 345-350.
- Liu K, Muse SV.** 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* **21**, 2128-2129.
- Lu K, Li L, Zheng X, Zhang Z, Mou T, Hu Z.** 2008. Quantitative trait loci controlling Cu, Ca, Zn, Mn, and Fe content in rice grains. *Journal of genetics* **87**, 305-310.
- McCartney CA, Sommers DJ, Fedak G, Cao W.** 2004. Haplotype diversity at Fusarium head blight resistance QTLs in wheat. *Theoretical and Applied Genetics* **109**, 261-271.
- Mohammadi-Nejad G, Singhb RK, Arzanic A, Rezaiee AM, Sabouri H, Gregoriob GB.** 2010. Evaluation of salinity tolerance in rice genotypes. *International Journal of Plant Production* **4**, 199-208.
- Munson RD, Nelson WL.** 1990. Principles and practices in plant analysis. In: Westerman RL, Ed. *Soil testing and plant analysis*, Soil Science Society of America 359-388.
- Ortiz-Monasterio JL, Graham RD.** 2000. Breeding for trace minerals in wheat. *Food & Nutrition Bulletin* **21**, 392-396.
- Palmgren MG, Clemens S, Williams LE, Krämer U, Borg S, Schjørring JK.** 2008. Zinc biofortification of cereals: problems and solutions. *Trends in plant science* **13**, 464-473.
- Ruel M, Bouis H.** 1998. Plant breeding: a long-term strategy for the control of zinc deficiency in vulnerable populations. *The American journal of clinical nutrition* **68**, 488-494.
- Saghai-Maroo MA, Soliman KM, Jorgensen RA, Allard RW.** 1984. Ribosomal DNA sepal-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences* **81**, 8014-8019.
- Stangoulis JC, Huynh BL, Welch RM, Choi EY, Graham RD.** 2007. Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. *Euphytica* **154**, 289-294.
- Susanto U.** 2008. Mapping of quantitative trait loci for high iron and zinc content in polished rice (*Oryza Sativa* L.) grain and some agronomic traits using simple sequence repeats markers, PhD thesis, Bogor agricultural University, Bogor.
- Virk PS, Barry G, Bouis H.** 2007. Genetic enhancement for the nutritional quality of rice. In:

Aggarwal PK, Ladha JK, Singh RK, Devakumar C, Hardy B, Ed. Proceedings of International Rice Research Conference (9-12 October 2006, New Delhi, India). Macmillan, India, 279-258.

**Virk PS, Barry G, Das A, Lee JH, Tan J.** 2006. Research status of micronutrient rice development in Asia, Proceedings of the International Symposium on Rice Biofortification Improving Human Health through Biofortified Rice (Suwon, Korea: 15 September 2006), Suwon, Korea, 123-148.

**Welch RM, Graham RD.** 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany* **55**, 353-364.

**Yamamoto T, Yonemaru J, Yano M.** 2009. Towards the understanding of complex traits in rice: Substantially or superficially? *DNA research* **16**, 141-154.

**Yano M, Sasaki T.** 1997. Genetic and molecular dissection of quantitative traits in rice. *Plant Molecular Biology* **35**, 145-153.

**Zhang MW, Guo BJ, Peng ZM.** 2004. Genetic effects on Fe, Zn, Mn and P content in indica black pericarp rice and their genetic correlations with grain characteristics. *Euphytica* **135**, 315-323.

**Zhu C, Naqvi S, Gomez-Galera S, Pelacho AM, Capell T, Christou P.** 2007. Transgenic strategies for the nutritional enhancement of plants. *Trends in plant science* **12**, 548-555.