

Genome-wide association of iron content in rice grains grown in Southern Brazil

Abstract – The objective of this work was to map the chromosomal regions responsible for iron accumulation in rice grains, in Southern Brazil. Eighty-one rice accessions were genotyped and phenotyped for Fe accumulation. Single nucleotide polymorphisms were mapped in the whole grain on chromosomes 1, 5, 6, and 10, from which 13 candidate genes were identified. Some of the genes, such as *Os10g0406800*, seem to have a relationship with Fe homeostasis, while others are related to other metabolic processes or have an unknown function.

Index terms: *Oryza sativa*, brown rice, candidate genes, genotyping, mapping.

Associação genômica ampla para teor de ferro em grãos de arroz na região Sul do Brasil

Resumo – O objetivo deste trabalho foi mapear regiões cromossômicas responsáveis pelo acúmulo de ferro em grãos de arroz, na região Sul do Brasil. Oitenta e um acessos de arroz foram genotipados e fenotipados quanto ao acúmulo de Fe. Polimorfismos de nucleotídeos únicos foram mapeados no grão integral, nos cromossomos 1, 5, 6 e 10, dos quais 13 genes candidatos foram identificados. Alguns dos genes, como o *Os10g0406800*, parecem ter relação com a homeostase do Fe, enquanto outros têm relação com outros processos metabólicos ou função desconhecida.

Termos para indexação: *Oryza sativa*, arroz integral, genes candidatos, genotipagem, mapeamento.

Rice (*Oryza sativa* L.) is considered a staple food that contributes up to 70% of daily calories to more than half of the world population. However, rice helps to solve the hunger problem, but not the “hidden hunger” that happens when a body is deprived of essential micronutrients as iron (Fe), which has serious consequences for the human health (Majumder et al., 2019).

Biofortification is the most effective and lasting approach for developing countries to alleviate the “hidden hunger”. This strategy increases the concentration and bioavailability of minerals such as Fe in parts of edible plants. Biofortification can be achieved through the agronomic practices or genetic improvement that allow of the development of new cultivars with greater mineral accumulation. The genetic biofortification with Fe is particularly important in staple food such as rice (Swamy et al., 2018).

The identification of new genes associated with Fe accumulation in rice grains can help breeders in the development of biofortified cultivars. The identification of chromosomal regions and, in some cases, the

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specific alleles associated with higher accumulation of Fe in different crops, has been made possible by using tools such as the genome-wide association study (GWAS) and quantitative trait loci (QTL) mapping (Connorton & Balk, 2019). Different studies using QTL mapping methods based on biparental populations and GWAS studies have identified QTLs that control Fe accumulation in rice, as reviewed by Talukdar et al. (2022). Up to this research, no GWAS studies on Brazilian rice cultivars, for Fe accumulation in grains, have been published.

The objective of this work was to map chromosomal regions responsible for the Fe accumulation in rice grains in southern Brazil.

A total of 81 accessions of *O. sativa indica* and *O. sativa japonica* subspecies, mainly *O. sativa indica*, were used in the present study. The rice accessions were cultivated in fields at the experimental station Terras Baixas of Embrapa Clima Temperado, located near the municipality of Pelotas, in the state of Rio Grande do Sul, Brazil, in the 2016/2017 and 2018/2019 harvest seasons, according to described by Oliveira et al. (2021). Sample and flour preparation from brown and polished rice grains were carried out at the Universidade Federal de Pelotas. The quantification of Fe in rice grains was performed in the Environmental BR laboratory, at Universidade Federal do ABC, in the state of São Paulo, Brazil, according to Oliveira et al. (2021).

Genotyping was performed at the Genotyping Services Laboratory of the International Rice Research Institute, Philippines, using 7098 single nucleotide polymorphisms (SNP) markers from 7K Infinium SNP genotyping platform (Illumina). Genotyping data were filtered using Tassel v.5.2.41 (Bradbury et al., 2007), by which accessions with call rates < 0.75, SNPs with missing data > 20%, and minor allele frequency ≤ 5% were removed, remaining 4093 SNP markers, as presented by Venske et al. (2019).

The population structure matrix was generated from the Bayesian clustering method using the fastStructure software (Raj et al., 2014). From k=1 to k=10, the model components used to explain the structure in data were selected with the command chooseK.py (Venske et al., 2019). Kinship matrix was obtained using the Tassel v.5.2.44 software (Bradbury et al., 2007). These two matrices are included in the association analysis to decrease false positives.

Associative mapping analyses were also performed in the Tassel v.5.2.44 software, using a linear mixed model. This model includes phenotyping data (Fe levels), genotyping data (SNPs), population structure and kinship matrices. The results are presented in Manhattan plot. The significance threshold was determined using the Bonferroni correction methods (BC), by the equation $p\text{-value BC} = 0.05/k$, where k is the total number of markers (Alqudah et al., 2020).

The Rice Annotation Project database (rap-db, 2023) was used for the dissection of chromosomal regions that showed SNPs associated with Fe. To identify candidate genes linked to the SNPs associated to Fe, the physical location of each SNP on every chromosome was verified. From this position, a region of 10 kb for each side was analyzed.

Population structure and linkage disequilibrium, as well as genetic diversity for Fe in grain for this panel were previously reported by Venske et al. (2019) and Oliveira et al. (2021). Based on the Bonferroni correction methods, a threshold of 5 was applied to the statistic $-\log_{10}(p\text{-value})$ for declaring significant QTLs. No significant SNPs associated with Fe were found for polished grains. Significant SNPs were detected for brown rice, using the threshold above described (Figure 1).

Significant SNPs associated with Fe in brown rice were mapped on chromosomes 1, 5, 6, and 10 (Figure 1 and Table 1). Bollinedi et al. (2020) detected one SNP associated with Fe on chromosome 6; however, it is located on the opposite arms of the chromosome. In another study, two SNPs associated with Fe were found on the short arm on chromosome 1 (Cu et al. 2021), while in the present study, the SNP is on the long arm.

The dissection of a 20 kb region from significant SNPs location on the chromosomes 1, 5, 6, and 10, allowed of the identification of 1, 2, 7, and 3 genes, respectively (Table 1). On chromosome 1, the gene *Os01g0653800* encodes WD40 repeat-like domain containing protein. In rice, the *OsWDL* gene, a WD40-repeat protein family member, is involved in GA biosynthesis and signaling, increasing the expression of the GA-induced genes – *OsEP3A* and $\alpha\text{-amylase}$ –, with a positive role in seedling development and grain size (He & Ho, 2018). By these results, the role of WD genes was shown in grains.

Next to SNP on chromosome 5, two genes with unknown function were identified. Among the

seven genes found close to SNP on chromosome 6, those encoding Granule-bound starch synthase I (*Os06g0133000*) and Phosphate-induced protein 1 conserved region family protein (*Os06g0133600*) stand out. Granule-bound starch synthase I (*Os06g0133000*) is responsible for the amylose synthesis and contributes to the amylopectin synthesis (Liu et al., 2013). A study by Quan et al. (2019) showed that most *Phosphate-induced 1 (PHI-1)* genes in rice are intensively expressed during the panicle development.

The *Armadillo-like helical domain containing (Os10g0406800)* is one of the genes located next to SNP on chromosome 10. Armadillo domains belong to a class of helix-forming proteins, which act in protein-protein interactions. These proteins play an important role for the development, cell division, cell death, and lateral root grow (Jia et al., 2011). A study conducted by Jia et al. (2011) reported that *Short postembryonic roots 1 (OsSPRI)*, an Armadillo-

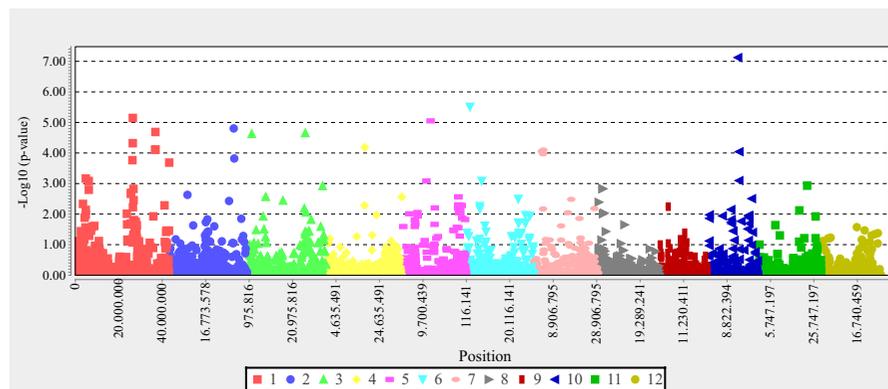


Figure 1. Manhattan plot of $-\text{Log}_{10}(\text{p-value})$ vs chromosomal position of SNP markers associated with Fe content in grains of brown rice (*Oryza sativa*), in a diverse panel grown in southern Brazil.

Table 1. Annotation of genes at RAP-DB (The Rice Annotation Project Database), mined within the QTL mapped for grain Fe content in brown rice (*Oryza sativa*), in a diverse panel grown in southern Brazil.

Chromosome	Gene ID	Transcript variants	Annotation	Gene Position
1	<i>Os01g0653800</i>	<i>Os01t0653800-01</i>	Similar to predicted protein; WD40 repeat-like	26,457,976~26,465,951
		<i>Os01t0653800-02</i>	domain containing protein.	26,453,784~26,465,951
		<i>Os01t0653800-03</i>	Similar to Peptidase S8 and S53, subtilisin, kexin.	26,454,207~26,459,369
5	<i>Os05g0296900</i>	<i>Os05t0296900-01</i>	Hypothetical conserved gene.	13,229,992~13,230,715
	<i>Os05g0297001</i>	<i>Os05t0297001-01</i>	Similar to predicted protein.	13,232,746~13,234,909
6	<i>Os06g0133000</i>	<i>Os06t0133000-01</i>	Granule-bound starch synthase 1, Synthesis of	1,765,622~1,770,574
		<i>Os06t0133000-02</i>	amylose in endosperm.	1,766,470~1,770,653
	<i>Os06g0133100</i>	<i>Os06t0133100-01</i>	Hypothetical protein.	1,769,541~1,770,459
	<i>Os06g0133200</i>	<i>Os06t0133200-01</i>	Conserved hypothetical protein.	1,776,726~1,777,351
	<i>Os06g0133300</i>	<i>Os06t0133300-00</i>	Hypothetical conserved gene.	1,780,570~1,780,854
	<i>Os06g0134400</i>	<i>Os06t0134400-01</i>	Protein of unknown function DUF3615 domain	1,784,324~1,784,813
			containing protein.	
	<i>Os06g0133500</i>	<i>Os06t0133500-00</i>	Conserved hypothetical protein.	1,785,455~1,785,802
<i>Os06g0133600</i>	<i>Os06t0133600-01</i>	Phosphate-induced protein 1 Conserved region	1,789,916~1,791,294	
		family protein.	1,789,946~1,791,291	
10	<i>Os10g0406800</i>	<i>Os10t0406800-01</i>	Armadillo-like helical domain containing protein.	13,919,961~13,925,636
	<i>Os10g0406701</i>	<i>Os10t0406701-01</i>	Conserved hypothetical protein.	13,908,015~13,912,718
	<i>Os10g0406600</i>	<i>Os10t0406600-01</i>	Complex 1 LYR protein family protein.	13,905,198~13,906,597

containing gene, encodes a mitochondrial protein, and it is involved in root development and Fe homeostasis in rice.

In the present work, 13 candidate genes for Fe accumulation in brown rice grains, located on chromosomes 1, 5, 6 and 10, are reported. None of these genes have been associated with Fe accumulation in grains in previous studies. Certain genes have unknown function, other are associated with grain size, development, and composition, and some other genes seem to show a relationship with Fe homeostasis (*Os10g0406800*). The exact role of these genes for Fe accumulation in rice grains should be elucidated in further studies.

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References

- ALQUDAH, A.M.; SALLAM, A.; BAENZIGER, P.S.; BÖRNER, A. GWAS: fast-forwarding gene identification and characterization in temperate cereals: lessons from barley: a review. **Journal of Advanced Research**, v.22, p.119-135, 2020. DOI: <https://doi.org/10.1016/j.jare.2019.10.013>.
- BOLLINEDI, H.; YADAV, A.K.; VINOD, K.K.; GOPALA KRISHNAN, S.; BHOWMICK, P.K.; NAGARAJAN, M.; NEERAJA, C.N.; ELLUR, R.K.; SINGH, A.K. Genome-wide association study reveals novel marker-trait associations (MTAs) governing the localization of Fe and Zn in the rice grain. **Frontiers in Genetics**, v.11, art.213, 2020. DOI: <https://doi.org/10.3389/fgene.2020.00213>.
- BRADBURY, P.J.; ZHANG, Z.; KROON, D.E.; CASSTEVENS, T.M.; RAMDOSS, Y.; BUCKLER, E.S. TASSEL: software for association mapping of complex traits in diverse samples. **Bioinformatics**, v.23, p.2633-2635, 2007. DOI: <https://doi.org/10.1093/bioinformatics/btm308>.
- CONNORTON, J.M.; BALK, J. Iron biofortification of staple crops: lessons and challenges in plant genetics. **Plant & Cell Physiology**, v.60, p.1447-1456, 2019. DOI: <https://doi.org/10.1093/pcp/pcz079>.
- CU, S.T.; WARNOCK, N.I.; PASUQUIN, J.; DINGKUHN, M.; STANGOULIS, J. A high-resolution genome-wide association study of the grain ionome and agronomic traits in rice *Oryza sativa* subsp. *indica*. **Scientific Reports**, v.11, art.19230, 2021. DOI: <https://doi.org/10.1038/s41598-021-98573-w>.
- HE, S.-L.; HO, S.-L. Functional characterization of a WD-repeat protein gene (*OsWD1*) in rice. **American Journal of Agriculture and Forestry**, v.6, p.18-27, 2018. DOI: <https://doi.org/10.11648/J.AJAF.20180602.11>.
- 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 Phytologist**, v.189, p.843-855, 2011. DOI: <https://doi.org/10.1111/j.1469-8137.2010.03513.x>.
- LIU, D.-R.; HUANG, W.-X.; CAI, X.-L. Oligomerization of rice granule-bound starch synthase 1 modulates its activity regulation. **Plant Science**, v.210, p.141-150, 2013. DOI: <https://doi.org/10.1016/j.plantsci.2013.05.019>.
- MAJUMDER, S.; DATTA, K.; DATTA, S.K. Rice biofortification: high iron, zinc, and vitamin-A to fight against “hidden hunger”. **Agronomy**, v.9, art.803, 2019. DOI: <https://doi.org/10.3390/agronomy9120803>.
- OLIVEIRA, V.F. de; BUSANELLO, C.; VIANA, V.E.; STAFEN, C.F.; PEDROLO, A.M.; PANIZ, F.P.; PEDRON, T.; PEREIRA, R.M.; ROSA, S.A.; MAGALHÃES JÚNIOR, A.M. de; OLIVEIRA, A.C. de; BATISTA, B.L.; PEGORARO, C. Assessing mineral and toxic elements content in rice grains grown in southern Brazil. **Journal of Food Composition and Analysis**, v.100, art.103914, 2021. DOI: <https://doi.org/10.1016/j.jfca.2021.103914>.
- QUAN, C.; BAI, Z.; ZHENG, S.; ZHOU, J.; YU, Q.; XU, Z.; GAO, X.; LI, L.; ZHU, J.; JIA, X.; CHEN, R. Genome-wide analysis and environmental response profiling of phosphate-induced-1 family genes in rice (*Oryza sativa*). **Biotechnology & Biotechnological Equipment**, v.33, p.627-638, 2019. DOI: <https://doi.org/10.1080/13102818.2019.1604157>.
- RAJ, A.; STEPHENS, M.; PRITCHARD, J.K. fastSTRUCTURE: variational inference of population structure in large SNP data sets. **Genetics**, v.197, p.573-589, 2014. DOI: <https://doi.org/10.1534/genetics.114.164350>.
- RAP-DB. **The Rice Annotation Project Database**. Available at: <http://rapdb.dna.affrc.go.jp/>. Accessed on: Aug. 25 2023.
- SWAMY, B.P.M.; KALADHAR, K.; ANURADHA, K.; BATCHU, A.K.; LONGVAH, T.; SARLA, N. QTL analysis for grain iron and zinc concentrations in two *O. nivara* derived backcross populations. **Rice Science**, v.25, p.197-207, 2018. DOI: <https://doi.org/10.1016/j.rsci.2018.06.003>.
- TALUKDAR, P.; TRAVIS, A.J.; HOSSAIN, M.; ISLAM, M.R.; NORTON, G.J.; PRICE, A.H. Identification of genomic loci regulating grain iron content in *aus* rice under two irrigation management systems. **Food and Energy Security**, v.11, e329, 2022. DOI: <https://doi.org/10.1002/fes3.329>.

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disequilibrium, and population structure in a panel of Brazilian rice accessions. **Journal of Applied Genetics**, v.60, p.27-31, 2019. DOI: <https://doi.org/10.1007/s13353-018-0475-0>.
