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<u>Abstract</u>

Background: Understanding the relationships between nutrition, human health, and plant food source is among the highest priorities for public health. Therefore, enhancing the minerals content such as iron (Fe), zinc (Zn), and selenium (Se) in barley (*Hordeum vulgare* L.) grains is an urgent need to improve the nutritive value of barley grains in overcoming malnutrition and its potential consequencing. This study aimed to expedite biofortification of barley grains by elucidating the genetic basis of Zn, Fe, and Se accumulation in the grains, which will contribute to improve barley nutritional quality.

Results: A genome-wide association study (GWAS) was conducted to detect the genetic architecture for grain Zn, Fe, and Se accumulations in 216 spring barley accessions across two years. All the accessions were genotyped by single nucleotide polymorphisms (SNPs) molecular markers. Mineral heritability values ranging from moderate to high were revealed in both environments. Remarkably, there was a high natural phenotypic variation for all micronutrient accumulation in the used population. High-LD SNP markers (222 SNPs) were detected to be associated with all micronutrients in barley grains across the two environments plus BLUEs. Three genomic regions were detected based on LD, which was identified for the most effective markers that had associations with





more than one trait. The strongest SNP-trait associations were found to be physically located within genes that may be involved in grain Zn and Fe homeostasis. Two putative candidate genes were annotated as Basic helix loop helix (BHLH) family transcription factor and Squamosa promoter binding-like protein, respectively, and have been suggested as candidates for increased grain Zn, Fe, and Se accumulation.

Conclusions: These findings shed light on the genetic basis of Zn, Fe, and Se accumulation in barley grains and have the potential to assist plant breeders in selecting accessions with high micronutrient concentrations to enhance grain quality and, ultimately human health.

Keywords: Zinc, Iron, Selenium, Barley, Micronutrient, GWAS