



ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2026): 4.82
 Indexing: Embase
 IJBS 2026; 8(10): 22-24
www.biologyjournal.net
 Received: 18-08-2026
 Accepted: 24-09-2026
 Published: 09-10-2026

Srujana Vemula

Ph.D, Professor Jayashankar
 Telangana Agricultural
 University (PJTU),
 Department of Genetics and
 Plant Breeding, Hyderabad,
 India

Dr. D Anil

Assistant Professor (PJTU),
 Department of Agronomy,
 Hyderabad, Telangana, India

Improvement of micronutrient use efficiency and nutritional quality in crops by genome editing approaches

Srujana Vemula¹, D Anil²

DOI: <https://www.doi.org/10.33545/26649926.2026.v8.i10a.1004>

ABSTRACT

Micronutrients are essential for plant growth, metabolism, productivity, and nutritional quality. However, their availability and utilization are influenced by soil properties and complex regulatory mechanisms. Conventional breeding and transgenic approaches have contributed to improving micronutrient acquisition and accumulation; however, genome-editing technologies, particularly CRISPR/Cas systems, provide precise tools for modifying genes involved in nutrient uptake, transport, signalling, homeostasis and storage. Recent studies have demonstrated the potential of genome editing to improve iron (Fe), zinc (Zn), and boron (B) utilization and to reduce the accumulation of toxic cadmium (Cd) in crops. Editing genes such as OsIRO3, OsVIT2, OsZIP9, BnaA9.WRKY47, and OsNramp5 has provided valuable insights into micronutrient regulation and crop improvement. Genome editing has also been used to modify phytic acid and starch composition, thereby improving nutritional quality. Overall, genome editing offers a promising approach for developing nutrient-efficient, nutritionally improved, and safer crops.

Keywords: Genome editing, CRISPR/Cas9, Micronutrient-use efficiency, Iron, Zinc, Boron, Cadmium, Biofortification

1. Introduction

Micronutrients are essential for normal plant growth and development even though they are required in relatively small amounts. Important micronutrients include iron (Fe), zinc (Zn), manganese (Mn), boron (B), copper (Cu), molybdenum (Mo), nickel (Ni), and chlorine (Cl). These elements participate in enzyme activity, photosynthesis, respiration, chlorophyll formation, cellular metabolism, and reproductive processes (White *et al.*, 2009) [1]. Deficiency of micronutrients can restrict crop growth and productivity and may also reduce the nutritional quality of harvested products. Therefore, improving micronutrient-use efficiency requires coordinated regulation of nutrient uptake, transport, utilization, storage, and redistribution. Plants maintain micronutrient homeostasis through transporters, transcription factors, signaling proteins, chelators, and storage mechanisms. For example, iron acquisition involves reduction and transport systems, whereas zinc uptake is controlled by several transporter families according to the zinc status of the plant. Boron uptake and distribution are regulated through boric-acid channels and borate transporters. Traditional breeding and transgenic approaches have been used to improve nutrient acquisition and nutritional quality. However, these approaches may be limited by complex genetic regulation and difficulties in modifying specific genes without affecting other traits. Genome-editing technologies have expanded the possibilities for targeted crop improvement by enabling specific mutations, deletions, substitutions, and regulatory changes at defined genomic loci. CRISPR/Cas9 has become one of the most widely used genome-editing systems because of its relative simplicity, efficiency, and ability to target multiple genes (Sathee *et al.*, 2022) [2].

2. Genome editing for micronutrient-use efficiency

Genome editing can modify genes encoding transporters, transcription factors, signaling proteins, and other regulators of micronutrient homeostasis. Such modifications can influence nutrient uptake, translocation, accumulation, and stress responses. In several cases, edited plants have shown improved nutritional traits without major yield penalties.

2.1 Iron use efficiency and biofortification

Iron is essential for photosynthesis, respiration, chlorophyll formation, electron transport, and several enzymatic reactions. Although soils may contain considerable amounts of iron, its availability is often low, particularly in alkaline and calcareous soils.

Corresponding Author:

Srujana Vemula

Ph.D, Professor
 Jayashankar Telangana
 Agricultural University
 (PJTU), Department of
 Genetics and Plant
 Breeding, Hyderabad, India

Genome editing has identified several important regulators of iron homeostasis. Editing of bHLH18, bHLH19, and bHLH20 in *Arabidopsis* improved responses to iron deficiency under certain conditions Cui *et al.* (2018) [3]. Similarly, disruption of OsIRO3 in rice altered iron-deficiency responses and iron-related gene expression (Wang *et al.*, 2020) [4].

The FEP1 gene is also involved in iron distribution. Genome-edited fep1 mutants showed impaired root-to-shoot Fe translocation, indicating that FEP1 contributes to systemic Fe homeostasis (Hirayama *et al.*, 2018) [5]. Similarly, the IRON MAN (IMA) gene family plays an important role in systemic regulation of iron uptake and transport (Grillet *et al.*, 2018) [6].

Genome editing also offers opportunities for iron biofortification. Che *et al.* (2021) [7] investigated the vacuolar iron transporter OsVIT2 in rice. Its targeted disruption increased iron accumulation in rice grains without reducing grain yield, demonstrating the potential of genome editing for iron biofortification. The IMA gene family also plays an important role in systemic regulation of iron uptake and transport.

4. Zinc use efficiency

Zinc is important for enzyme activity, protein synthesis, membrane stability, gene expression, and plant growth. Zinc deficiency can reduce crop productivity and nutritional quality.

The transcription factors bZIP19 and bZIP23 act as zinc sensors in *Arabidopsis* and regulate plant responses to Zn deficiency (Lilay *et al.*, 2021) [8]. These regulatory factors provide useful information for understanding how plants sense and respond to changes in Zn availability.

Huang *et al.* (2020) [9] used CRISPR/Cas9 to generate OsZIP9 knockout mutants in rice. Loss of OsZIP9 reduced Zn uptake under Zn-deficient conditions, confirming its role in root Zn acquisition. Interestingly, the mutants also showed changes in Fe accumulation, demonstrating the close relationship between Zn and Fe homeostasis. These findings indicate that improving zinc-use efficiency requires consideration of multiple transporters and regulatory pathways rather than a single gene.

5. Boron use efficiency

Boron is required for cell-wall formation, membrane integrity, reproductive development, and plant growth. Both boron deficiency and toxicity can negatively affect crops.

In *Brassica napus*, BnaA9.WRKY47 was identified as an important regulator of adaptation to low-boron conditions. CRISPR/Cas9 knockout increased sensitivity to boron deficiency, whereas increased expression improved boron accumulation. The transcription factor regulates BnaA3.NIP5;1, a boric-acid channel involved in boron uptake (Feng *et al.*, 2020) [10].

This study demonstrates how genome editing can identify transcriptional regulators of micronutrient uptake and provides a potential molecular approach for improving B-use efficiency in crops.

6. Reduction of cadmium accumulation

Cadmium is not an essential nutrient but is an important food-safety concern because it can accumulate in edible crop tissues. Genome editing can modify metal-transport pathways to reduce its accumulation.

Tang *et al.* (2017) [11] used CRISPR/Cas9 to knock out *OsNramp5*, a metal transporter involved in Cd uptake in rice. The edited plants showed substantially reduced Cd accumulation in roots, shoots, and grains while maintaining plant growth. Under Cd-contaminated conditions, the edited lines had considerably lower grain Cd concentrations than the wild type.

The *OsNramp5* study is an important example of using genome editing not only to improve essential micronutrient acquisition but also to reduce the accumulation of undesirable metals. Such approaches may be particularly useful for cultivation in areas affected by heavy-metal contamination.

7. Genome editing for phytic acid and nutritional quality

Genome editing can also improve nutritional quality by modifying compounds that influence mineral availability and grain composition. Phytic acid is the major storage form of phosphorus in cereal seeds. Although it serves an important physiological role in seeds, it can bind minerals and reduce their bioavailability in monogastric animals. Genome-editing technologies have therefore been investigated for reducing seed phytic acid.

Liang *et al.* (2014) [12] used TALEN and CRISPR/Cas9 systems to target phytic-acid biosynthesis genes including ZmMRP4, ZmIPK, and ZmIPK1A in maize. The results demonstrated that targeted genome modification can be used to alter phytic-acid metabolism and improve nutritional quality.

Similar approaches have been applied to wheat and rice. Genome editing of genes involved in phytic-acid biosynthesis can potentially improve the bioavailability of minerals such as Fe and Zn. CRISPR-based approaches for nutrient enrichment therefore provide opportunities to combine mineral biofortification with reduction of *anti-nutritional* compounds (Kumar *et al.*, 2022) [13].

8. Modification of amylose and amylopectin

Genome editing has also been used to modify starch composition in rice. The Wx/OsWaxy gene plays an important role in amylose synthesis. Editing of this gene can reduce amylose content and produce glutinous rice characteristics.

Yunyan *et al.* (2019) [14] used CRISPR/Cas9 to edit the Wx gene in rice varieties Suken 118 and Huaidao 5. The edited grains showed characteristics of glutinous rice and contained low amylose levels. Similarly, Ma *et al.* (2015) [15] used a CRISPR/Cas9 system to edit the OsWaxy gene and obtained mutants with substantially reduced amylose content.

Genome editing of starch-branching enzyme genes has also been used to alter resistant starch content. Sun *et al.* (2017) [16] reported that editing SBEIIb increased amylose and resistant starch levels. These findings demonstrate that genome editing can be used to develop crops with specific nutritional and industrial characteristics.

9. Advantages and future prospects

Genome editing offers several advantages for crop nutritional improvement. It allows specific genes to be modified without necessarily introducing foreign genes and can be used to investigate gene function and develop useful alleles. CRISPR/Cas technology can also be combined with promoter engineering, base editing, prime editing, CRISPR activation, and CRISPR interference to achieve more precise changes in gene expression (Sathee *et al.*, 2022) [2].

For micronutrient improvement, future strategies may focus on modifying regulatory genes rather than individual transporters alone. This may help coordinate nutrient uptake, transport, storage, and redistribution. Combining genome editing with conventional breeding, molecular breeding, and precision phenotyping can accelerate the development of nutrient-efficient varieties.

10. Challenges

Despite its potential, genome editing of micronutrient pathways has several challenges. Micronutrient metabolism is controlled by complex genetic networks, and modification of one gene can influence other nutrients. For example, the OsZIP9 study demonstrated interaction between Zn and Fe homeostasis. Similarly, modifications affecting Fe metabolism may influence Cu and other micronutrients.

Genotype × environment interactions are another important consideration. A gene modification that performs well under controlled conditions may show different effects under field conditions. Therefore, edited plants need to be evaluated for yield, nutrient concentration, stability, and quality under diverse environments.

Off-target effects, editing efficiency, regulatory requirements, consumer acceptance, and integration with breeding programs also need

consideration. Current genome-editing research is increasingly moving toward more precise technologies such as base and prime editing, which may allow specific nucleotide changes without creating conventional double-strand breaks.

11. Conclusion

Genome editing has emerged as an important molecular breeding tool for improving micronutrient-use efficiency and nutritional quality in crops. CRISPR/Cas9-based modification of genes such as bHLH18, bHLH19, bHLH20, OsIRO3, FEP1, OsVIT2, OsZIP9, BnaA9.WRKY47, and OsNramp5 has provided valuable information about nutrient uptake, transport, signaling, homeostasis, and toxic-metal accumulation.

Genome editing can contribute to Fe and Zn biofortification, improve B-use efficiency, and reduce Cd accumulation in edible tissues. It can also improve nutritional quality by modifying phytic acid and starch composition. However, nutrient interactions, genotype × environment effects, yield stability, and field performance must be carefully considered. Integration of genome editing with conventional breeding, molecular breeding, and precision phenotyping can facilitate the development of nutrient-efficient, nutritionally improved, and safer crop varieties.

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How to cite this article: Vemula S, Anil D. Improvement of micronutrient use efficiency and nutritional quality in crops by genome editing approaches. *International Journal of Biology Sciences*. 2026;8(10):22–24. doi: <https://www.doi.org/10.33545/26649926.2026.v8.i10a.1004>