

miRNA-Based Targeting for Improving Iron and Zinc Content in Wheat

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OPEN ACCESS

Keywords

Biofortification, Wheat, microRNA, Iron and Zinc, ZIP

How to cite this article:

Priyanka. 2026. miRNA-Based Targeting for Improving Iron and Zinc Content in Wheat. *Vigyan Varta* 7 (10): 103-106.

ABSTRACT

Iron (Fe) and zinc (Zn) deficiencies are major forms of hidden hunger, especially in populations that depend heavily on cereal-based diets. Wheat is therefore a strategic crop for biofortification, but grain Fe and Zn concentration is controlled by complex networks of uptake, chelation, transport, remobilization and storage. MicroRNAs (miRNAs) provide a precise post-transcriptional regulatory layer because they repress specific messenger RNAs by cleavage or translational inhibition. Recent wheat studies have identified Fe- and Zn-responsive miRNAs, including conserved families such as miR397, miR398 and miR408 and several wheat-specific novel miRNAs. These small RNAs are linked with multicopper oxidases, zinc-ion transport, transcription factors, E3 ubiquitin ligases, antioxidant defense and cellular detoxification. This review summarizes how miRNA-based targeting could complement conventional breeding, genomic selection and genome editing to increase Fe and Zn loading in wheat grain while maintaining yield and stress tolerance.

INTRODUCTION:

Wheat (*Triticum aestivum* L.) is one of the world's most important staple cereals and contributes a large share of daily calories in South Asia and

many other regions. However, polished or refined wheat-based diets often provide insufficient bioavailable Fe and Zn. Low Fe intake contributes to anemia and reduced work

capacity, whereas Zn deficiency affects immunity, growth and reproductive health. Genetic biofortification aims to increase the concentration and bioavailability of these micronutrients in the edible grain, making nutrition improvement part of the seed itself rather than a post-harvest addition.

Fe and Zn accumulation in wheat grain begins in the rhizosphere. Roots mobilize Fe through Strategy II phytosiderophore release and absorb Zn and Fe using transporter families such as ZIP/IRT-like proteins. Once inside the plant, metals are chelated by nicotianamine and related molecules, loaded into xylem and phloem, remobilized during grain filling, and finally stored in grain tissues. Candidate gene families repeatedly associated with this process include NAS, YSL, ZIP, VIT/VTL, NRAMP, MTP and ferritin-related pathways (Wani, 2022). Yet direct overexpression of transport genes can create trade-offs such as metal toxicity, poor grain filling or changes in other nutrients.

miRNAs offer a fine-tuning mechanism rather than a simple on/off switch. Because each miRNA may regulate several related targets and because miRNA expression is often tissue and stress specific, they can be used to redirect mineral flow only in desired organs or developmental windows. For wheat, the major opportunity is to identify miRNA target modules that enhance root acquisition, flag-leaf remobilization and grain loading without reducing yield. miRNA based biofortification is therefore best viewed as an additional layer integrated with QTL mapping, marker-assisted selection, genomic prediction and precise genome editing.

1. miRNA Regulation of Fe and Zn Homeostasis

Plant miRNAs are usually 20–24 nucleotide regulatory RNAs generated from MIR gene transcripts. After processing, mature miRNAs

guide Argonaute-containing complexes to complementary target transcripts. In nutrient stress, this system rapidly adjusts expression of transporters, redox enzymes, transcription factors and signaling proteins. For biofortification, the key question is not only whether a miRNA responds to Fe or Zn, but also whether manipulating the miRNA improves net grain mineral concentration and bioavailability.

Fe deficiency in wheat has been demonstrated to change the expression of a wide range of miRNAs in the roots and shoots. 105 differentially expressed miRNAs under Fe shortage were found in a small-RNA and transcriptome analysis, including nine novel miRNAs and expected targets such as multicopper oxidases, GRAS and WRKY transcription factors and E3 ubiquitin ligases (Sharma, 2024). This is significant because coordinated control of redox state, chelator synthesis, transporter expression and stress signaling is necessary for Fe homeostasis. While shoot-specific miRNAs may impact remobilization toward growing grains, root-specific miRNAs may impact uptake and rhizosphere response.

Wheat miRNA networks are also altered by zinc availability. 798 miRNAs under various Zn supplies, including 70 recognized and 728 unique candidates, were found in a recent bread-wheat study. Important miRNAs like miR397-5p and miR398 as well as new loci like 4D_25791 and 5A_27668, shown different reactions to excess, high and low zinc. Under low zinc their anticipated targets were enriched for zinc ion transmembrane transporter activity; under high or excess zinc, they were enriched for glutathione metabolism and ABC transporter pathways (Sun, 2026). These findings imply that the same miRNA family may be involved in detoxifying when there is an excess of zinc and Zn absorption when there is a deficit.

Table 1. Candidate miRNA modules for Fe/Zn biofortification in wheat

miRNA/module	Likely target process	Biofortification value	Caution
miR397 / miR408	Multicopper oxidases, laccase-like/redox targets	May adjust Fe-deficiency response and metal remobilization	Validate grain-stage effects
miR398	Cu/Zn-superoxide dismutase and oxidative-stress balance	Helps maintain ROS control during Fe/Zn stress	Avoid weakening stress tolerance
Fe-responsive wheat miRNAs	GRAS, WRKY, E3 ligases, MCOs	Markers or targets for Fe uptake and signaling	Strong tissue specificity
Zn-responsive novel miRNAs	Zn transport, detoxification and ABC-related pathways	Improve Zn-use efficiency and tolerance	Avoid Zn toxicity or Cd co-transport
Target-site edited transporter genes	ZIP, YSL, NAS/VIT regulatory regions	Release useful transporter expression from repression	Requires target validation

The table emphasizes candidate modules rather than ready-to-release traits. For each entry, the useful allele or edited variant must be tested across wheat backgrounds, soil types and grain-development stages.

2. miRNA-Based Targeting Strategies

The first strategy is expression-guided selection. Natural variation in MIR genes, promoter regions or target sites can be screened in diverse wheat germplasm. Lines showing favorable miRNA expression under low Fe or Zn, together with high grain mineral concentration, may be used in marker-assisted breeding. This approach is practical because it avoids transgenic deployment and can be combined with existing high Zn and high Fe QTL pipelines.

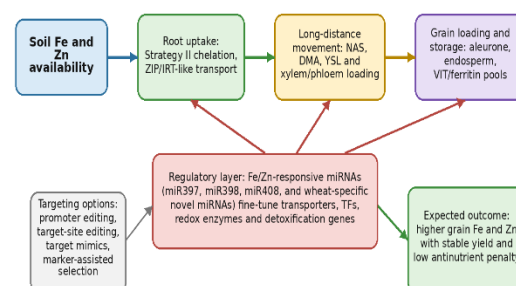
The second strategy is genome editing. CRISPR/Cas systems can be used to edit MIR promoters, mature miRNA sequences or miRNA-binding sites in target genes. For example, weakening a miRNA-binding site in a grain-loading transporter could increase expression during grain filling, while promoter

editing could restrict a miRNA response to roots or flag leaves. Such precision is important in hexaploid wheat, where homeologous genes on A, B and D genomes may have partially redundant or tissue-specific functions.

The third strategy is target mimicry or short tandem target mimic technology. Target mimics act as molecular sponges that reduce the activity of selected miRNAs. If a miRNA suppresses a transporter or chelator required for Fe/Zn movement to grain, a tissue-specific target mimic could release that target only in the organ of interest. Conversely, overexpression of beneficial miRNAs could suppress negative regulators of metal uptake or detoxification. In both cases, constitutive expression should be avoided unless strong evidence shows no yield or toxicity penalty.

A successful pipeline would begin with small-RNA sequencing under contrasting Fe and Zn supplies, followed by degradome sequencing or 5' RACE to confirm cleavage targets. Candidate miRNA-mRNA pairs should then be tested in roots, flag leaves, rachis and developing grains. Mineral concentration must be measured together with phytic acid, protein, yield, thousand-kernel weight and possible co-accumulation of undesirable metals such as cadmium. Only validated modules should move into breeding or editing programs.

miRNA-based targeting to improve Fe and Zn in wheat grain



Conceptual schematic prepared for this review; gene targets require genotype- and tissue-specific validation.

Figure 1. Conceptual route by which miRNA targeting can fine-tune Fe/Zn uptake, transport and grain loading in wheat.

3. Opportunities, Limitations and Future Scope

The strongest opportunity of miRNA-based targeting is regulatory precision. Wheat grain mineral content is influenced by environment, genotype and developmental stage; therefore, interventions that respond to nutrient status are preferable to permanent high expression of a single transporter. Fe and Zn also interact with nitrogen, phosphorus, sulfur and copper metabolism. miRNAs such as miR397, miR398 and miR408 sit near these cross-talk nodes, which makes them attractive but also risky targets. Their manipulation can improve mineral movement only if antioxidant capacity, growth and grain quality remain stable (Jamla *et al.*, 2023).

Many predicted miRNA targets that require experimental validation come from sequence complementarity and enrichment analysis. In wheat, homeolog-specific targeting complicates interpretation even more. A miRNA may work differently in grains and roots, or it may suppress a transporters A-genome copy but not its B- or D-genome copy. Multi-location studies are also necessary since Fe/Zn availability is influenced by soil pH, moisture, organic matter and fertilizer practices.

Future research should link miRNA profiles to transcriptomics, proteomics, grain ionome data and metabolite measurements like phytate and nicotianamine. The most beneficial products will probably combine altered miRNA regulatory regions that enhance endosperm loading and remobilization with traditional high Fe and Zn alleles. Wheat varieties that support nutrition security while maintaining farmer-preferred yield, disease resistance and processing quality can be produced using this integrated strategy.

CONCLUSION

miRNA-based targeting is a promising next-generation tool for Fe and Zn biofortification in wheat. Current evidence shows that Fe and Zn availability changes wheat miRNA expression and that these miRNAs regulate genes involved in transport, redox balance, signaling and detoxification. However, the approach should be used carefully: candidate miRNAs must be functionally validated, edited in a tissue-specific manner when possible and assessed for yield, bioavailability and metal safety. When combined with genomic breeding, miRNA regulation can help turn mineral homeostasis knowledge into nutritionally improved wheat cultivars.

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