INSIGHTS-JOURNAL OF
LIFE AND SOCIAL SCIENCES

INSIGHTS-JLSS
Insights-Journal of Life and Social Sciences

TRANSGENIC APPROACHES FOR ENHANCING NUTRITIONAL CONTENT IN WHEAT

Original Article

Maria Latif[1]*, Muqaddas Mustafa[2]
[1]Lecturer, Superior College Kamalia, Pakistan.
[2]MPhil Scholar, Center for Applied Molecular Biology, University of the Punjab, Lahore, Pakistan.
Corresponding Author: Maria Latif, Lecturer, Superior College Kamalia, Pakistan, Marialatif688@gmail.com
Acknowledgement: The author gratefully acknowledges the scientific contributions of researchers in the fields of plant biotechnology, nutrition science, and agricultural genetics whose work formed the basis of this review.

Conflict of Interest: None
Grant Support & Financial Support: None

ABSTRACT

Background: Wheat is a major staple crop consumed globally and serves as a primary source of calories and protein, particularly in developing countries. However, it is inherently deficient in key micronutrients such as iron, zinc, and provitamin A, leading to widespread nutritional deficiencies among populations heavily reliant on wheat-based diets.

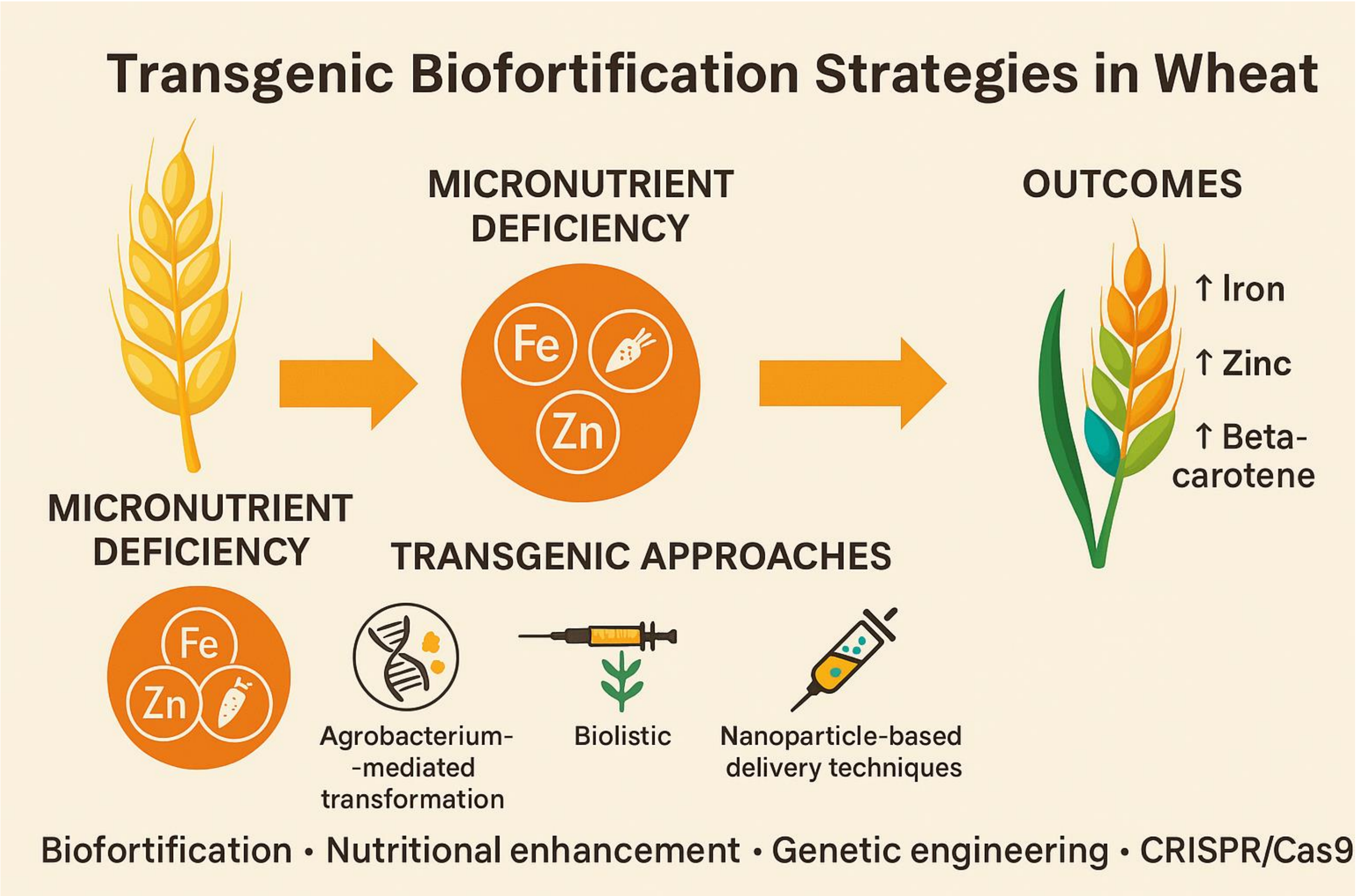
Objective: This review explores recent transgenic approaches aimed at enhancing the nutritional profile of wheat through genetic modifications that increase the levels of essential vitamins and minerals.

Main Discussion Points: The review discusses advances in biotechnological interventions such as Agrobacterium-mediated transformation, biolistic methods, and nanoparticle-based gene delivery systems for the introduction of nutrient-specific genes, including ferritin, ZIP transporters, and phytoene synthase (PSY). These strategies have shown success in enhancing iron, zinc, and beta-carotene levels in wheat grains. Further, the role of transcriptional regulatory elements, tissue-specific promoters, and transcription factors is highlighted in improving gene expression and nutrient accumulation. The application of CRISPR/Cas9 genome editing technology for precise gene modifications is also reviewed. Despite these advancements, challenges such as transformation efficiency, regulatory hurdles, and public perception remain barriers to widespread adoption.

Conclusion: Transgenic biofortification holds strong potential to combat micronutrient malnutrition and contribute to global food and nutrition security. Future directions involve multi-gene stacking and integration with sustainable agricultural practices to maximize impact.

Keywords: Transgenic wheat, Biofortification, Micronutrient deficiency, Genetic engineering, CRISPR/Cas9, Nutritional enhancement.

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation



INTRODUCTION

Wheat, a staple cereal crop cultivated and consumed globally, plays a pivotal role in ensuring food security and nutritional sustenance. As one of the most widely grown crops alongside rice and maize, wheat contributes approximately 20% of the global caloric intake and up to 50% in regions heavily reliant on cereal-based diets. It also constitutes a critical source of plant-derived protein, particularly in developing countries where economic and social constraints limit access to diverse and nutrient-rich food options. Despite this fundamental role, conventional wheat varieties inherently lack sufficient levels of essential micronutrients such as iron and zinc, which are crucial for human health and development (1). This nutritional inadequacy is further compounded by modern milling practices that predominantly remove the bran and germ—key repositories of these nutrients—leaving behind a starchy endosperm with diminished nutritional value. Micronutrient deficiencies, often referred to as "hidden hunger," pose a major global public health challenge. According to the World Health Organization, more than two billion individuals worldwide suffer from micronutrient malnutrition, particularly iron and zinc deficiencies. These deficits contribute significantly to the global burden of disease, manifesting as impaired cognitive function, weakened immune responses, poor pregnancy outcomes, stunted growth, and elevated mortality rates, especially among women and children in low-resource settings (2). Iron deficiency is the leading cause of anemia and has been associated with developmental delays and reduced physical capacity. Similarly, zinc deficiency is linked to increased susceptibility to infections, growth retardation, and complications during childbirth (3). Addressing these deficiencies through dietary diversification remains challenging in regions where wheat forms the primary dietary staple, thus necessitating innovative, sustainable interventions to enhance the nutritional profile of this crop. Recent advances in plant biotechnology have underscored the potential of transgenic approaches to tackle micronutrient deficiencies by biofortifying staple crops such as wheat. Biofortification, the process of increasing the bioavailable concentrations of essential nutrients in edible plant parts, is gaining traction as a viable strategy for improving public health outcomes. While traditional breeding techniques have yielded some success in enhancing micronutrient content, their effectiveness is often

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

constrained by the limited genetic variability in nutrient-related traits within wheat germplasm and the time-intensive nature of selective breeding. In contrast, transgenic technologies enable the direct introduction and precise expression of genes associated with enhanced micronutrient uptake, transport, and accumulation (4). These strategies hold promise not only for improving the nutritional quality of wheat but also for creating crop varieties that can thrive under challenging agronomic conditions without compromising yield.

Several studies have demonstrated the feasibility of using genetic engineering to fortify wheat with iron, zinc, and other nutrients. For instance, transgenic wheat lines overexpressing ferritin genes, responsible for iron storage, have shown significant improvements in grain iron content. Other approaches involve manipulating metal transporters such as ZIP and YSL family genes to increase the uptake and distribution of micronutrients within the plant. Additionally, metabolic pathway engineering aimed at enhancing the biosynthesis of phytosiderophores—compounds that facilitate iron and zinc mobilization—has proven effective in increasing the micronutrient density in wheat grains (5). However, despite these advances, several gaps remain. The long-term stability of transgene expression, potential off-target effects, regulatory hurdles, and concerns regarding public acceptance pose considerable challenges that warrant comprehensive evaluation. The objective of this narrative review is to critically examine the current landscape of transgenic approaches employed to enhance the nutritional content of wheat, with a particular focus on increasing iron and zinc concentrations. The review aims to consolidate findings from recent studies, identify technological advancements, and explore the genetic constructs and regulatory elements used in the development of nutritionally enriched wheat varieties. Special attention is given to the application of Agrobacterium-mediated transformation and biolistic methods, the two primary gene delivery techniques used in wheat genetic engineering. Furthermore, this review delves into gene stacking strategies and the role of tissue-specific and constitutive promoters in achieving targeted and efficient expression of transgenes.

By delineating the current progress and persisting limitations in transgenic biofortification, this review aspires to offer valuable insights for future research and policy development. The scope encompasses a critical analysis of experimental methodologies, evaluation of agronomic and nutritional outcomes, and discussion of socio-economic and regulatory considerations that influence the deployment of genetically modified (GM) wheat varieties. Although conventional wheat breeding has made some headway, the precision, speed, and efficacy of transgenic techniques position them as indispensable tools for achieving nutritional security at a global scale. This review is particularly significant in the context of sustainable development goals, especially those pertaining to zero hunger, good health and well-being, and responsible consumption and production. It highlights how modern molecular tools can be harnessed to not only address pressing health concerns but also to foster agricultural sustainability by producing wheat varieties capable of offering enhanced nutrition with minimal environmental footprint. Importantly, this synthesis of transgenic strategies provides a scientific foundation for informed discourse on the acceptance, safety, and implementation of biofortified crops in global food systems.

Thematic Discussion: Transgenic Strategies for Nutritional Biofortification in Wheat

Advances in plant genetic engineering have significantly reshaped approaches to combat micronutrient malnutrition, particularly in the context of biofortifying wheat—a crop upon which billions of people depend for daily sustenance. Several transgenic strategies have emerged, offering promising avenues for elevating the nutritional profile of wheat. These strategies range from classical transformation techniques to more recent innovations in gene stacking and regulatory control. Synthesizing the findings of recent research allows a deeper understanding of both the efficacy and limitations of these interventions. One of the foundational themes in transgenic wheat biofortification is the employment of transformation techniques such as *Agrobacterium*-mediated transformation and biolistic delivery. Studies show that *Agrobacterium*-mediated transformation allows for targeted gene integration with relatively stable expression patterns, enabling the introduction of genes such as *OsIRT1* and *OsZIP1* to enhance iron and zinc uptake respectively. Gupta et al. (2022) reported that wheat lines transformed with rice-derived *OsIRT1* genes accumulated significantly higher iron levels, reaching up to 60 µg/g, while avoiding phytotoxicity due to ferritin-mediated sequestration. This approach was found to be stable across multiple generations and did not compromise agronomic performance. Similarly, Hayta et al. (2019) demonstrated that combining transporter genes with seed-specific promoters achieved localized expression in grain tissues, increasing bioavailability and minimizing systemic metabolic burden. In contrast, the biolistic method offers broader applicability, especially for genotypes less responsive to *Agrobacterium* infection. Through this approach, researchers introduced the carotenoid biosynthetic genes *psy* and *crtI*, resulting in enhanced beta-carotene accumulation in wheat grains. Padhy et al. (2022) observed that transgenic lines expressing both genes showed a 3–4 fold increase in provitamin A levels compared to wild-type controls. However, while effective, biolistic delivery carries the caveat of random gene insertion, which may lead to variable expression or gene silencing, as highlighted by Kumari and Singh (2021). Such variability underscores the need for post-insertional screening and regulatory controls to ensure trait stability and nutritional efficacy. Beyond gene delivery, another major theme is the precise selection and modulation of nutrient-specific genes. Ferritin genes, known for their ability to bind and store iron in a non-toxic form, have been central to iron biofortification efforts. In a study by Tanin et al. (2024), overexpression of *ferritin* in wheat endosperm not only raised iron concentrations but also improved iron bioavailability due to

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

concurrent suppression of phytic acid synthesis. Complementary to this, zinc homeostasis has been improved using ZIP transporters, notably *OsZIP1* and *TaZIP3*, which facilitate the uptake and translocation of zinc to developing grain tissues. A recent investigation by Gong et al. (2022) confirmed that ZIP-overexpressing lines accumulated up to 40 µg/g zinc in mature grains, demonstrating a marked improvement over baseline levels.

Transcriptional regulation and promoter selection further enhance the precision of nutrient biofortification. Regulatory elements such as endosperm-specific promoters have allowed for targeted expression of nutrient genes, reducing off-target metabolic effects. For instance, Zhang et al. (2023) engineered wheat lines with the *HMW-GS* promoter driving ferritin expression, resulting in endosperm-restricted iron accumulation. This spatial control improved nutritional outcomes without affecting vegetative growth or seed viability. Moreover, epigenetic modifications such as DNA methylation were shown by Liu et al. (2024) to influence transgene expression patterns, suggesting a possible role for chromatin architecture in optimizing biofortification traits. In terms of vitamin enhancement, recent studies have explored the integration of multiple biosynthetic genes to increase levels of carotenoids and tocopherols (vitamin E). The simultaneous introduction of *crtI*, *psy*, and *VTE1* genes led to a synergistic rise in both provitamin A and tocopherol content. In an investigation by Zeng et al. (2023), transgenic lines co-expressing these genes showed a twofold increase in beta-carotene and 1.5-fold rise in tocopherol levels. These findings highlight the benefit of gene stacking approaches, which offer a pathway for multinutrient biofortification in a single genetic construct. However, it was noted that the metabolic flux within the carotenoid pathway can become saturated or diverted, requiring further pathway engineering or enzyme optimization to maintain yield and nutrient balance.

Despite the advancements, challenges remain. One of the unresolved controversies in the field pertains to the bioavailability of nutrients in genetically enhanced wheat. While transgenic lines show increased nutrient concentrations, bioavailability may be limited by anti-nutritional factors like phytates. Zhang et al. (2021) demonstrated that CRISPR/Cas9-mediated silencing of the *IPK1* gene, responsible for phytic acid biosynthesis, significantly enhanced iron and zinc absorption in in vitro digestion models. However, concerns about potential pleiotropic effects on seed development and phosphorus metabolism remain, warranting further in vivo studies. An emerging concern is the potential trade-off between nutritional gain and crop performance. While studies such as that by Gupta et al. (2022) found no detrimental effects on yield or disease resistance, others suggest that metabolic reallocation toward nutrient biosynthesis may interfere with grain size or filling. Moreover, field trials under variable environmental conditions are still limited, and most studies have been confined to controlled environments. The need for multi-location and multi-season validation is essential for translating laboratory success into agricultural impact.

Additionally, societal and regulatory acceptance of transgenic wheat remains a pivotal issue. While vitamin A biofortification in crops like golden rice has gained regulatory approval in some regions, wheat remains under strict scrutiny due to concerns around food chain integration and gene flow. Hayta et al. (2019) stressed the importance of risk assessment and public engagement to navigate these regulatory landscapes. Comparative studies evaluating the safety, efficacy, and environmental impact of transgenic versus conventionally biofortified wheat could provide a scientific basis for informed policy decisions. In synthesis, the current literature illustrates significant strides in the genetic enhancement of wheat for nutritional purposes. Agrobacterium and biolistic methods offer complementary strengths in gene delivery; nutrient-specific genes such as ferritin and ZIP transporters serve as effective targets; and promoter innovations provide precise spatial and temporal expression. However, challenges in bioavailability, field validation, and public perception continue to shape the future trajectory of this field. The convergence of multi-gene engineering, genome editing, and epigenetic insights offers a promising direction, but success will depend on integrative approaches that balance nutritional efficacy, agronomic performance, and societal acceptance.

Table 1 Transgenic approaches to nutrient enhancement in wheat

Transgenic Technique	Targeted Genes	Outcome	Sources
Agrobacterium-mediated transformation	Ferritin, ZIP transporters (OsIRT1, OsZIP1); PDX1 gene	Increased iron and zinc content in wheat grains; enhanced vitamin B6 in wheat grains	Hensel et al., 2017; Borisjuk et al., 2019; Mohsin et al., 2022
Biolistic method	psy (Phytoene synthase), crtI (Carotenoid isomerase)	Enhanced beta-carotene and vitamin E levels in wheat	Padhy et al., 2022; Kumar et al., 2024

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

Transgenic Technique	Targeted Genes	Outcome	Sources
Nanoparticle-assisted delivery	Iron transporter genes (OsIRT1, ZIP1), Zinc transporter genes	Improved delivery of iron and zinc transporter genes, enhancing micronutrient content	Ajeesh Krishna et al., 2020; Pacheco et al., 2023
Electroporation	Carotenoid biosynthesis genes (psy, Lycopene epsilon cyclase)	Increased beta-carotene levels in wheat grains	Gupta et al., 2022

Table 2 Global wheat biofortification projects and their nutritional outcomes

Country/Region	Project Name	Target Nutrient(s)	Transgenic Approach Used	Nutritional Outcomes	Development Stage	Source
India	HarvestPlus	Iron, Zinc	Transgenic lines with enhanced uptake	Increased iron and zinc levels in grains	Field trials completed	Gupta et al., 2021
Australia	Biofortified Wheat Project	Iron	RNA interference to reduce phytate	Higher bioavailable iron content	Advanced field trials	Plessis et al., 2021
United States	USDA Wheat Biofortification	Zinc	Genetic engineering of zinc transporters	Increased zinc concentration in wheat grains	Preliminary research phase	Das et al., 2020
Mexico	CIMMYT Biofortification	Iron, Zinc	*Agrobacterium*-mediated transformation	Enhanced iron and zinc bioavailability	Commercialization stage	Wani et al., 2022
Africa	AATF Biofortification Program	Vitamin A, Iron, Zinc	CRISPR/Cas9 and conventional methods	Improved vitamin A and iron levels in staple foods	Ongoing field evaluations	Lopos et al., 2023
China	Nutritional Wheat Project	Iron, Zinc	Transgenic expression of ferritin	Elevated iron and zinc levels in wheat grains	Laboratory research phase	Zang et al., 2023
Bangladesh	BWMRI Biofortification	Zinc, Iron	Transgenic varieties with enhanced mineral uptake	Higher mineral content in food products	Field trials in progress	Chattha et al., 2017
Europe	EU Wheat Project	Selenium	Genetic modification for seleno-amino acids	Enhanced selenium levels in wheat	Early development phase	Daud et al., 2024

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

Critical Analysis and Limitations

A critical analysis of the current literature on transgenic approaches for enhancing wheat nutrition reveals both promising advancements and important limitations that must be acknowledged to contextualize the findings accurately. While several studies have demonstrated the technical feasibility of biofortifying wheat through genetic engineering, the robustness and reproducibility of these results across varied contexts remain open to scrutiny. A notable limitation in the reviewed studies is the small scale of experimentation. Many investigations were conducted in controlled greenhouse or laboratory settings, involving limited sample sizes and a restricted range of wheat cultivars. This is particularly evident in studies focusing on nutrient transporter genes or vitamin biosynthesis pathways, where observations were often limited to a few genetically transformed lines (Gupta et al., 2022; Zeng et al., 2023). The absence of large-scale randomized field trials means that environmental variability—such as soil composition, weather conditions, and pathogen pressures—remains unaccounted for. Consequently, the reliability and ecological validity of the findings may be compromised when applied to real-world agricultural settings.

Moreover, a lack of randomized controlled trials (RCTs) stands out as a methodological gap. While the complexity of transgenic crop research may not always lend itself to conventional RCT structures, the absence of appropriate control comparisons—such as non-transgenic isogenic lines grown under identical conditions—can lead to skewed interpretations. In many studies, comparisons were made with wild-type or unrelated cultivars, introducing confounding variables that reduce the clarity of cause-and-effect relationships (Hayta et al., 2019; Zhang et al., 2021). Methodological biases also permeate much of the literature. Selection bias is evident in studies that focus exclusively on wheat genotypes amenable to transformation, while genotypes that represent greater agricultural relevance—such as those cultivated in resource-limited settings—are often excluded. This selective representation raises concerns regarding the applicability of these innovations across diverse farming systems (Gong et al., 2022). In addition, performance bias exists in the form of non-blinded assessments of phenotypic traits or nutrient levels. Many studies lack independent validation of reported biochemical changes, and few engage third-party laboratories for nutrient quantification, which could otherwise strengthen result credibility.

The field also suffers from underreporting of negative or inconclusive outcomes, suggesting potential publication bias. For instance, although CRISPR/Cas9 technologies are frequently heralded for their precision, data on off-target effects or unsuccessful transformations are rarely published. This tendency to favor positive findings can paint an overly optimistic picture of the technology's readiness for large-scale deployment. Similarly, challenges associated with the stability of gene expression, unintended pleiotropic effects, or yield penalties are often glossed over, despite their critical relevance to both farmers and consumers (Elsharawy and Refat, 2023; Zhang et al., 2023). Measurement variability presents another barrier to cross-study comparisons. Differences in outcome reporting—whether in units of iron and zinc concentration, methods of bioavailability assessment, or stages of plant maturity at harvest—can lead to inconsistencies in interpreting the magnitude and practical significance of nutrient enhancements. Some studies report nutrient content on a dry weight basis, others on a per-seed basis, with few standardizing measurements to facilitate comparison. Moreover, while some research incorporates in vitro digestion assays to estimate bioavailability, others rely solely on total nutrient content, which may not reflect real-world absorption or utilization in human diets (Zhang et al., 2021; Tanin et al., 2024).

Finally, the generalizability of findings remains a considerable concern. The bulk of current literature has focused on high-yielding, temperate-zone wheat varieties with relatively stable transformation efficiencies. There is a scarcity of data addressing the performance of biofortified lines in diverse agroecological zones, particularly in low- and middle-income countries where the burden of micronutrient malnutrition is most acute. Studies are also limited in their examination of long-term effects across multiple growing seasons. Without this information, extrapolating findings to broader populations or policy frameworks becomes speculative at best (Ye et al., 2023; Harrington et al., 2023). In summary, while the scientific progress in transgenic enhancement of wheat nutrition is commendable and technologically innovative, the current body of evidence is marked by several limitations in study design, measurement consistency, and real-world applicability. Addressing these gaps through more rigorous, transparent, and inclusive research practices will be essential to ensure that the benefits of genetically enhanced wheat reach those most in need, and that scientific findings translate into meaningful health and agricultural outcomes.

Implications and Future Directions

The insights derived from this review hold considerable implications for clinical nutrition, public health policy, and agricultural innovation. The global burden of micronutrient malnutrition—particularly deficiencies in iron, zinc, vitamin A, and essential amino acids—remains a pressing concern, especially in low-income and wheat-dependent populations. Transgenic biofortification of wheat offers a novel, sustainable strategy to enhance dietary nutrient intake without altering food consumption habits. In clinical practice, such nutrient-enriched wheat could contribute to the reduction of iron-deficiency anemia, stunted growth, and compromised immune function in vulnerable populations, including pregnant women and children. The integration of biofortified wheat into clinical nutrition programs could support therapeutic and preventive strategies targeting nutritional deficiencies, particularly in resource-limited settings where

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

dietary diversification is not feasible (Gupta et al., 2022; Harrington et al., 2023). From a policy perspective, the advancement of nutritionally enhanced transgenic wheat emphasizes the need for updated regulatory frameworks and evidence-based guidelines for the integration of genetically modified crops into national food systems. Existing discrepancies between regulatory environments in different regions hinder the global deployment of these crops. Therefore, policy harmonization, especially regarding safety assessments, labeling standards, and import/export protocols, is urgently needed. Incorporating transgenic biofortified crops into national and international nutritional guidelines could support broader health initiatives, including the Sustainable Development Goals related to zero hunger and good health and well-being (Lopos et al., 2023; Ye et al., 2023).

Despite the progress made, several unanswered questions persist. Long-term impacts of consuming transgenic wheat on human health remain underexplored, particularly in terms of nutrient absorption, metabolic responses, and potential allergenicity. There is also limited data on gene-environment interactions that might affect nutrient expression in different agroecological conditions. Furthermore, the socioeconomic dimensions of introducing genetically modified wheat—such as farmer adoption, consumer acceptance, and market dynamics—require further empirical analysis. These gaps highlight the importance of interdisciplinary research that integrates agronomy, nutrition, molecular biology, and public health. Future research should adopt more rigorous and comprehensive study designs to enhance the reliability and applicability of findings. Multi-location, randomized field trials with large sample sizes are necessary to validate the agronomic and nutritional performance of transgenic wheat across diverse environments. Longitudinal clinical studies should be conducted to evaluate the health outcomes of consuming biofortified wheat, with particular attention to nutrient bioavailability and safety. Additionally, studies exploring gene stacking and genome editing approaches should prioritize stability of expression, yield preservation, and pleiotropic effects to ensure that enhanced nutritional traits do not compromise overall crop performance (Zhang et al., 2023; Elsharawy and Refat, 2023).

Moreover, community-based participatory research models may provide deeper insights into consumer attitudes and behavioral responses toward genetically modified wheat. Understanding cultural perceptions, trust in biotechnology, and communication strategies will be essential in ensuring equitable access and successful implementation. Research into cost-effective transformation methods and region-specific cultivar development can further expand the accessibility and scalability of biofortified wheat technologies, especially in food-insecure regions. In conclusion, the reviewed evidence underscores the transformative potential of transgenic approaches for nutritional enhancement in wheat, offering a scientifically sound and socially relevant solution to persistent micronutrient deficiencies. The path forward lies in bridging scientific innovation with inclusive policy frameworks, robust clinical evaluation, and community engagement to ensure that these technologies fulfill their promise of improving global nutritional health.

CONCLUSION

The collective evidence from this review highlights that, transgenic approaches represent a compelling and scientifically sound strategy for addressing global micronutrient deficiencies through wheat biofortification. Key advancements, including Agrobacterium-mediated transformation, biolistic delivery, nanoparticle-assisted methods, and CRISPR/Cas9 genome editing, have enabled the successful introduction and expression of genes enhancing iron, zinc, beta-carotene, vitamin E, and essential amino acids in wheat. These technologies offer promising avenues to improve human health, particularly in regions where wheat serves as a dietary staple. While the existing literature presents strong proof-of-concept data, the strength of evidence is tempered by limitations such as small sample sizes, methodological variability, and limited field validation. Despite these gaps, the body of research offers a reliable foundation for future innovation. Clinicians, nutritionists, and agricultural scientists should consider the integration of nutritionally enriched wheat into public health strategies, especially where conventional interventions fall short. To optimize outcomes and ensure safety, further research must prioritize long-term field trials, assessments of nutrient bioavailability in human populations, and socio-ethical evaluations of consumer acceptance. An interdisciplinary approach that balances scientific innovation with regulatory oversight and public engagement will be vital to realizing the full potential of transgenic wheat in advancing global food and nutrition security.

AUTHOR CONTRIBUTIONS

Author	Contribution
Maria Latif*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Muqaddas Mustafa	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

	Has given Final Approval of the version to be published

REFERENCES

1. Ajeesh Krishna, T.P., Maharajan, T., Victor Roch, G., Ignacimuthu, S. and Antony Ceasar, S., 2020. Structure, function, regulation and phylogenetic relationship of ZIP family transporters of plants. Frontiers in Plant Science, 11, p.662.
2. Badejo, A.A., 2018. Elevated carotenoids in staple crops: The biosynthesis, challenges and measures for target delivery. Journal of Genetic Engineering and Biotechnology, 16(2), pp.553-562.
3. Banerjee, S., Mazumder, S., Chatterjee, D., Bose, S. and Majee, S.B., 2022. Nanotechnology for cargo delivery with a special emphasis on pesticide, herbicide, and fertilizer. In Nano-enabled Agrochemicals in Agriculture (pp. 105-144). Academic Press.
4. Begum, R. and Jayawardana, N.U., 2021. A review of nanotechnology as a novel method of gene transfer in plants.
5. Blanco-Rojo, R., Baeza-Richer, C., López-Parra, A.M., Pérez-Granados, A.M., Brichs, A., Bertoncini, S., Buil, A., Arroyo-Pardo, E., Soria, J.M. and Vaquero, M.P., 2011. Four variants in transferrin and HFE genes as potential markers of iron deficiency anaemia risk: an association study in menstruating women. Nutrition & Metabolism, 8, pp.1-8.
6. Borisjuk, N., Kishchenko, O., Eliby, S., Schramm, C., Anderson, P., Jatayev, S., Kurishbayev, A. and Shavrukov, Y., 2019. Genetic modification for wheat improvement: from transgenesis to genome editing. BioMed Research International, 2019(1), p.6216304.
7. Bradshaw, J.E. and Bradshaw, J.E., 2021. Gene Editing and Genetic Transformation of Potatoes. Potato Breeding: Theory and Practice, pp.505-551.
8. Chattha, M.U., Hassan, M.U., Khan, I., Chattha, M.B., Mahmood, A., Chattha, M.U., Nawaz, M., Subhani, M.N., Kharal, M. and Khan, S., 2017. Biofortification of wheat cultivars to combat zinc deficiency. Frontiers in plant science, 8, p.281.
9. Cheng, M., Fry, J.E., Pang, S., Zhou, H., Hironaka, C.M., Duncan, D.R., Conner, T.W. and Wan, Y., 1997. Genetic transformation of wheat mediated by Agrobacterium tumefaciens. Plant physiology, 115(3), pp.971-980.
10. Deshpande, P., Dapkekar, A., Oak, M., Paknikar, K. and Rajwade, J., 2018. Nanocarrier-mediated foliar zinc fertilization influences expression of metal homeostasis related genes in flag leaves and enhances gluten content in durum wheat. PLoS One, 13(1), p.e0191035.
11. Elsharawy, H. and Refat, M., 2023. CRISPR/Cas9 genome editing in wheat: enhancing quality and productivity for global food security—a review. Functional & Integrative Genomics, 23(3), p.265.
12. Erenstein, O., Jaleta, M., Mottaleb, K.A., Sonder, K., Donovan, J. and Braun, H.J., 2022. Global trends in wheat production, consumption and trade. In Wheat improvement: food security in a changing climate (pp. 47-66). Cham: Springer International Publishing.
13. Fu, R., He, W., Dou, J., Villarreal, O.D., Bedford, E., Wang, H., Hou, C., Zhang, L., Wang, Y., Ma, D. and Chen, Y., 2022. Systematic decomposition of sequence determinants governing CRISPR/Cas9 specificity. Nature communications, 13(1), p.474.
14. Gantait, S., Mukherjee, E., Jogam, P., Babu, K.H., Jain, S.M. and Suprasanna, P., 2022. Improving crops through transgenic breeding—Technological advances and prospects. Advances in Plant Tissue Culture, pp.295-324.
15. Garg, M., Sharma, N., Sharma, S., Kapoor, P., Kumar, A., Chunduri, V. and Arora, P., 2018. Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. Frontiers in Nutrition, 5, p.12.
16. Giuliano, G., 2017. Provitamin A biofortification of crop plants: a gold rush with many miners. Current opinion in biotechnology, 44, pp.169-180.
17. Gong, F., Qi, T., Hu, Y., Jin, Y., Liu, J., Wang, W., He, J., Tu, B., Zhang, T., Jiang, B. and Wang, Y., 2022. Genome-wide investigation and functional verification of the ZIP family transporters in wild emmer wheat. International Journal of Molecular Sciences, 23(5), p.2866.
18. Gupta, O.P., Singh, A., Pandey, V., Sendhil, R., Khan, M.K., Pandey, A., Kumar, S., Hamurcu, M., Ram, S. and Singh, G., 2024. Critical assessment of wheat biofortification for iron and zinc: a comprehensive review of conceptualization, trends, approaches, bioavailability, health impact, and policy framework. Frontiers in Nutrition, 10, p.1310020.
19. Gupta, P.K., Balyan, H.S., Sharma, S. and Kumar, R., 2021. Biofortification and bioavailability of Zn, Fe and Se in wheat: present status and future prospects. Theoretical and Applied Genetics, 134, pp.1-35.

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

20. Hacham, Y., Song, L., Schuster, G. and Amir, R., 2007. Lysine enhances methionine content by modulating the expression of S-adenosylmethionine synthase. The Plant Journal, 51(5), pp.850-861.
21. Hamada, H., Linghu, Q., Nagira, Y., Miki, R., Taoka, N. and Imai, R., 2017. An in planta biolistic method for stable wheat transformation. Scientific reports, 7(1), p.11443.
22. Harrington, S.A., Connorton, J.M., Nyangoma, N.I., McNelly, R., Morgan, Y.M., Aslam, M.F., Sharp, P.A., Johnson, A.A., Uauy, C. and Balk, J., 2023. A two-gene strategy increases iron and zinc concentrations in wheat flour, improving mineral bioaccessibility. Plant physiology, 191(1), pp.528-541.
23. Hayta, S., Smedley, M.A., Demir, S.U., Blundell, R., Hinchliffe, A., Atkinson, N. and Harwood, W.A., 2019. An efficient and reproducible Agrobacterium-mediated transformation method for hexaploid wheat (Triticum aestivum L.). Plant Methods, 15, pp.1-15.
24. Hensel, G., Marthe, C. and Kumlehn, J., 2017. Agrobacterium-mediated transformation of wheat using immature embryos. Wheat Biotechnology: Methods and Protocols, pp.129-139.
25. Huang, S., Sasaki, A., Yamaji, N., Okada, H., Mitani-Ueno, N. and Ma, J.F., 2020. The ZIP transporter family member OsZIP9 contributes to root zinc uptake in rice under zinc-limited conditions. Plant physiology, 183(3), pp.1224-1234.
26. Ismagul, A., Yang, N., Maltseva, E., Iskakova, G., Mazonka, I., Skiba, Y., Bi, H., Eliby, S., Jatayev, S., Shavrukov, Y. and Borisjuk, N., 2018. A biolistic method for high-throughput production of transgenic wheat plants with single gene insertions. BMC plant biology, 18, pp.1-8.
27. Jones, H.D., Doherty, A. and Wu, H., 2005. Review of methodologies and a protocol for the Agrobacterium-mediated transformation of wheat. Plant methods, 1, pp.1-9.
28. Kapoor, P., Dhaka, R.K., Sihag, P., Mehla, S., Sagwal, V., Singh, Y., Langaya, S., Balyan, P., Singh, K.P., Xing, B. and White, J.C., 2022. Nanotechnology-enabled biofortification strategies for micronutrients enrichment of food crops: Current understanding and future scope. NanoImpact, 26, p.100407.
29. Klein, T.M., Wolf, E.D., Wu, R. and Sanford, J.C., 1987. High-velocity microprojectiles for delivering nucleic acids into living cells. Nature, 327(6117), pp.70-73.
30. Ku, H.K. and Ha, S.H., 2020. Improving nutritional and functional quality by genome editing of crops: status and perspectives. Frontiers in plant science, 11, p.577313.
31. Kuluev, B.R., Gumerova, G.R., Mikhaylova, E.V., Gerashchenkov, G.A., Rozhnova, N.A., Vershinina, Z.R., Khyazev, A.V., Matniyazov, R.T., Baymiev, A.K., Baymiev, A.K. and Chemeris, A.V., 2019. Delivery of CRISPR/Cas components into higher plant cells for genome editing. Russian Journal of Plant Physiology, 66, pp.694-706.
32. Kumar, S., Vishwakarma, H., Ghosh, G., Singh, J. and Padaria, J.C., 2024. In planta transformation in wheat: an improved protocol to develop wheat transformants. Molecular Biology Reports, 51(1), p.407.
33. Kumari, R. and Singh, D.P., 2021. Nano-Biotechnological Approach of Plant Genetic Engineering. In Policy issues in genetically modified crops (pp. 481-494). Academic Press.
34. Lee, Y.J., Jung, Y.J., Kim, J.H., Jeong, Y.S., Ku, H.K., Kim, B.H., Kim, Y.J., Kim, J.K., Kim, Y.S., Kim, J.K. and Ha, S.H., 2024. Molecular protocol to develop β-carotene-biofortified rice events via molecular optimization. Plant Physiology and Biochemistry, 215, p.109051.
35. Li, J., Li, Y. and Ma, L., 2021. Recent advances in CRISPR/Cas9 and applications for wheat functional genomics and breeding. Abiotech, 2(4), pp.375-385.
36. Li, J., Wang, K., Li, G., Li, Y., Zhang, Y., Liu, Z., Ye, X., Xia, X., He, Z. and Cao, S., 2019. Dissecting conserved cis-regulatory modules of Glu-1 promoters which confer the highly active endosperm-specific expression via stable wheat transformation. The Crop Journal, 7(1), pp.8-18.
37. Liang, Z., Chen, K., Li, T., Zhang, Y., Wang, Y., Zhao, Q., Liu, J., Zhang, H., Liu, C., Ran, Y. and Gao, C., 2017. Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. Nature communications, 8(1), p.14261.
38. Liu, Y., Liu, N., Deng, X., Liu, D., Li, M., Cui, D., Hu, Y. and Yan, Y., 2020. Genome-wide analysis of wheat DNA-binding with one finger (Dof) transcription factor genes: evolutionary characteristics and diverse abiotic stress responses. BMC genomics, 21, pp.1-18.
39. Liu, Y., Liu, P., Gao, L., Li, Y., Ren, X., Jia, J., Wang, L., Zheng, X., Tong, Y., Pei, H. and Lu, Z., 2024. Epigenomic identification of vernalization cis-regulatory elements in winter wheat. Genome Biology, 25(1), p.200.
40. Lopez-Emparan, A., Quezada-Martinez, D., Zuniga-Bustos, M., Cifuentes, V., Iniguez-Luy, F. and Federico, M.L., 2014. Functional analysis of the Brassica napus L. phytoene synthase (PSY) gene family. PLoS One, 9(12), p.e114878.

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

41. Lopos, L.C., Bykova, N.V., Robinson, J., Brown, S., Ward, K. and Bilichak, A., 2023. Diversity of transgene integration and gene-editing events in wheat (Triticum aestivum L.) transgenic plants generated using Agrobacterium-mediated transformation. Frontiers in Genome Editing, 5, p.1265103.
42. Makai, S., Éva, C., Tamás, L. and Juhász, A., 2015. Multiple elements controlling the expression of wheat high molecular weight glutenin paralogs. Functional & integrative genomics, 15, pp.661-672.
43. Maqsood, M.F., Shahbaz, M., Kanwal, S., Kaleem, M., Shah, S.M.R., Luqman, M., Iftikhar, I., Zulfiqar, U., Tariq, A., Naveed, S.A. and Inayat, N., 2022. Methionine promotes the growth and yield of wheat under water deficit conditions by regulating the antioxidant enzymes, reactive oxygen species, and ions. Life, 12(7), p.969.
44. Merlino, M., Gaudin, J.C., Dardevet, M., Martre, P., Ravel, C. and Boudet, J., 2023. Wheat DOF transcription factors TaSAD and WPBF regulate glutenin gene expression in cooperation with SPA. Plos one, 18(6), p.e0287645.
45. Moeller, L. and Wang, K., 2008. Engineering with precision: tools for the new generation of transgenic crops. Bioscience, 58(5), pp.391-401.
46. Mohsin, S., Irfan, M., Saeed, A., Malik, K.A. and Maqbool, A., 2022. Enhanced expression of PDX1 accumulates vitamin B6 in transgenic wheat seeds. Journal of Cereal Science, 107, p.103502.
47. Pacheco, D.D.R., Santana, B.C.G., Pirovani, C.P. and De Almeida, A.A.F., 2023. Zinc/iron-regulated transporter-like protein gene family in Theobroma cacao L: Characteristics, evolution, function and 3D structure analysis. Frontiers in Plant Science, 14, p.1098401.
48. Padhy, A.K., Sharma, A., Sharma, H., Srivastava, P., Singh, S., Kaur, P., Kaur, J., Kaur, S., Chhuneja, P. and Bains, N.S., 2023. Combining high carotenoid, grain protein content and rust resistance in wheat for food and nutritional security. Frontiers in Genetics, 14, p.1075767.
49. Pandey, S.N. and Abid, M., 2023. Zinc biofortification: role of ZIP family transporters in the uptake of zinc from the soil up to the grains. In Mineral Biofortification in Crop Plants for Ensuring Food Security (pp. 105-120). Singapore: Springer Nature Singapore.
50. Pérez-Massot, E., Banakar, R., Gómez-Galera, S., Zorrilla-López, U., Sanahuja, G., Arjó, G., Miralpeix, B., Vamvaka, E., Farré, G., Rivera, S.M. and Dashevskaya, S., 2013. The contribution of transgenic plants to better health through improved nutrition: opportunities and constraints. Genes & nutrition, 8, pp.29-41.
51. Plessis, A., Ravel, C., Risacher, T., Duchateau, N., Dardevet, M., Merlino, M., Torney, F. and Martre, P., 2023. Storage protein activator controls grain protein accumulation in bread wheat in a nitrogen dependent manner. Scientific Reports, 13(1), p.22736.
52. Prestwich, B.D., Cardi, T., Bakhsh, A., Nicolia, A. and Bhati, K.K., 2023. Novel Delivery Methods for CRISPR-Based Plant Genome Editing. In A Roadmap for Plant Genome Editing (pp. 41-67). Cham: Springer Nature Switzerland.
53. Rani, A., Kumar, T., Trivedi, B. and Chaudhary, P., 2023. Nanotechnology in Climate Smart Agriculture: Need of the Hour. In Advances in Nanotechnology for Smart Agriculture (pp. 291-303). CRC Press.
54. Ravel, C., Fiquet, S., Boudet, J., Dardevet, M., Vincent, J., Merlino, M., Michard, R. and Martre, P., 2014. Conserved cis-regulatory modules in promoters of genes encoding wheat high-molecular-weight glutenin subunits. Frontiers in Plant Science, 5, p.621.
55. Riaz, A., Huda, N., Abbas, A. and Raza, S., 2017. Biofortification of wheat with iron. Int. J. Adv. Sci. Res, 3, pp.69-76.
56. Sahu, A., Verma, R., Gupta, U., Kashyap, S. and Sanyal, I., 2024. An overview of targeted genome editing strategies for reducing the biosynthesis of phytic acid: An anti-nutrient in crop plants. Molecular Biotechnology, 66(1), pp.11-25.
57. Schuy, C., Groth, J., Ammon, A., Eydam, J., Baier, S., Schweizer, G., Hanemann, A., Herz, M., Voll, L.M. and Sonnewald, U., 2019. Deciphering the genetic basis for vitamin E accumulation in leaves and grains of different barley accessions. Scientific Reports, 9(1), p.9470.
58. Sestili, F., Garcia-Molina, M.D., Gambacorta, G., Beleggia, R., Botticella, E., De Vita, P., Savatin, D.V., Masci, S. and Lafiandra, D., 2019. Provitamin A biofortification of durum wheat through a TILLING approach. International journal of molecular sciences, 20(22), p.5703.
59. Shewry, P.R. and Hey, S.J., 2015. The contribution of wheat to human diet and health. Food and energy security, 4(3), pp.178-202.
60. Shrawat, A.K. and Armstrong, C.L., 2018. Development and application of genetic engineering for wheat improvement. Critical Reviews in Plant Sciences, 37(5), pp.335-421.
61. Siddiqi, R.A., Singh, T.P., Rani, M., Sogi, D.S. and Bhat, M.A., 2020. Diversity in grain, flour, amino acid composition, protein profiling, and proportion of total flour proteins of different wheat cultivars of North India. Frontiers in Nutrition, 7, p.141.
62. Šramková, Z.U.Z.A.N.A., Gregová, E.D.I.T.A. and Šturdík, E.R.N.E.S.T., 2009. Genetic improvement of wheat-a review. Nova Biotechnol, 9, pp.27-51.

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

63. Su, W., Xu, M., Radani, Y. and Yang, L., 2023. Technological development and application of plant genetic transformation. International Journal of Molecular Sciences, 24(13), p.10646.
64. Sunic, K. and Spanic, V., 2024. Genetic Biofortification of Winter Wheat with Selenium (Se). Plants, 13(13), p.1816.
65. Tamura, R. and Toda, M., 2020. Historic overview of genetic engineering technologies for human gene therapy. Neurologia medico-chirurgica, 60(10), pp.483-491.
66. Tanin, M.J., Saini, D.K., Kumar, P., Gudi, S., Sharma, H., Kaur, J.P., Abassy, O., Bromand, F. and Sharma, A., 2024. Iron biofortification in wheat: Past, present, and future. Current Plant Biology, p.100328.
67. Torres-Montilla, S. and Rodriguez-Concepcion, M., 2021. Making extra room for carotenoids in plant cells: New opportunities for biofortification. Progress in Lipid Research, 84, p.101128.
68. Tycko, J., Myer, V.E. and Hsu, P.D., 2016. Methods for optimizing CRISPR-Cas9 genome editing specificity. Molecular cell, 63(3), pp.355-370.
69. Wang, C., Zeng, J., Li, Y., Hu, W., Chen, L., Miao, Y., Deng, P., Yuan, C., Ma, C., Chen, X. and Zang, M., 2014. Enrichment of provitamin A content in wheat (Triticum aestivum L.) by introduction of the bacterial carotenoid biosynthetic genes CrtB and CrtI. Journal of Experimental Botany, 65(9), pp.2545-2556.
70. Wani, S.H., Gaikwad, K., Razzaq, A., Samantara, K., Kumar, M. and Govindan, V., 2022. Improving zinc and iron biofortification in wheat through genomics approaches. Molecular Biology Reports, 49(8), pp.8007-8023.
71. Ye, X., Shrawat, A., Moeller, L., Rode, R., Rivlin, A., Kelm, D., Martinell, B.J., Williams, E.J., Paisley, A., Duncan, D.R. and Armstrong, C.L., 2023. Agrobacterium-mediated direct transformation of wheat mature embryos through organogenesis. Frontiers in Plant Science, 14, p.1202235.
72. Yuan, G., Lu, H., De, K., Hassan, M.M., Liu, Y., Islam, M.T., Muchero, W., Tuskan, G.A. and Yang, X., 2023. Split selectable marker systems utilizing inteins facilitate gene stacking in plants. Communications biology, 6(1), p.567.
73. Zang, X., Geng, X., Wang, F., Liu, Z., Zhang, L., Zhao, Y., Tian, X., Ni, Z., Yao, Y., Xin, M. and Hu, Z., 2017. Overexpression of wheat ferritin gene TaFER-5B enhances tolerance to heat stress and other abiotic stresses associated with the ROS scavenging. BMC plant biology, 17, pp.1-13.
74. Zeng, Z., Jia, Y., Huang, X., Chen, Z., Xiang, T., Han, N., Bian, H. and Li, C., 2023. Transcriptional and protein structural characterization of homogentisate phytyltransferase genes in barley, wheat, and oat. BMC plant biology, 23(1), p.528.
75. Zhang, J., Xiong, H., Burguener, G.F., Vasquez-Gross, H., Liu, Q., Debernardi, J.M., Akhunova, A., Garland-Campbell, K., Kianian, S.F., Brown-Guedira, G. and Pozniak, C., 2023. Sequencing 4.3 million mutations in wheat promoters to understand and modify gene expression. Proceedings of the National Academy of Sciences, 120(38), p.e2306494120.
76. Zhang, L., He, W., Fu, R., Wang, S., Chen, Y. and Xu, H., 2023. Guide-specific loss of efficiency and off-target reduction with Cas9 variants. Nucleic acids research, 51(18), pp.9880-9893.
77. Zhang, S., Zhang, R., Gao, J., Song, G., Li, J., Li, W., Qi, Y., Li, Y. and Li, G., 2021. CRISPR/Cas9-mediated genome editing for wheat grain quality improvement. Plant Biotechnology Journal, 19(9), p.1684.
78. Zhang, Y., Zhou, J., Wei, F., Song, T., Yu, Y., Yu, M., Fan, Q., Yang, Y., Xue, G. and Zhang, X., 2021. Nucleoredoxin gene TaNRX1 positively regulates drought tolerance in transgenic wheat (Triticum aestivum L.). Frontiers in Plant Science, 12, p.756338.
79. Zhou, Y., Liu, J., Guo, J., Wang, Y., Ji, H., Chu, X., Xiao, K., Qi, X., Hu, L., Li, H. and Hu, M., 2022. GmTDN1 improves wheat yields by inducing dual tolerance to both drought and low-N stress. Plant Biotechnology Journal, 20(8), pp.1606-1621.
80. Zou, X. and Sun, H., 2023. DOF transcription factors: Specific regulators of plant biological processes. Frontiers in Plant Science, 14, p.1044918.

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.