JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

"Recent Advances In Biofortification Through Omics – A Mini Review."

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ABSTRACT

Insufficient intake of the essential micronutrients (vitamins and minerals) through diet is primarily responsible for causing micronutrient deficiency, also known as 'hidden hunger'. Hidden hunger manifests in its ugliest form in the developing countries, where the majority of the population is dependent on staple crops for their Recommended Dietary Allowance (RDA). To rectify the deficiency, Biofortification was introduced. It is the perfect amalgamation of breeding practices, hybridisation and biotechnology to enhance the overall bioavailability of the micronutrient(s). Although it produced results but it has several limitations; traditional crop breeding methods like hybridisation and pure-line selection have struggled to raise the levels of essential micronutrients. They focus only on improving nutritional content, ignoring other aspects such as metabolic pathways and nutrient accumulation. To overcome these challenges, a new wave of innovation—smart breeding for smart nutrition comes into play. It is the perfect amalgamation of cutting-edge multi-omics technologies encompassing genomics, transcriptomics, proteomics, and metabolomics. These technologies not just focus on improving nutrition by adding micronutrients in the crops and grains but often work on molecular levels by checking the faults at the genetic level, reprogramming metabolic pathways and identifying nutrient-related genes.

Key Words: Biofortification, Genomics, Hidden hunger, Micronutrients, Omics, Predictive breeding.

1. INTRODUCTION

Introduced globally in the early 2000s, 'Biofortification' is a technique through which the nutritional quality of food and staples can be enhanced using breeding, hybridisation and biotechnology. Its primary aim was to combat micronutrient deficiencies that lead to "malnutrition" [1].

Micronutrient deficiencies are also known as "hidden hunger". It remains a global health obstacle, with the most persistent deficiencies being those of Vitamin A, Zinc (Zn), and Iron (Fe) [2]. People in developing countries often bear the burden of these nutritional gaps because of primary reliance on staples for nutrition uptake, limited access to varied, nutrient-rich foods. Many struggle with stunted growth, weakened immune systems, and deprived overall health. The COVID-19 pandemic was instrumental in highlighting the severe impact of micronutrient deficiencies as it shifted the focus to nutritional supplementation for enhancing immune defence and physiological resilience [3].

Biofortification has emerged as a pioneering invention to tackle hidden hunger by improving the nutritional quality of staple crops. Traditional agronomic methods—such as applying nutrient-enriched fertilizers do offer a quick fix by enhancing micronutrient uptake, but their effects are often transient. Whereas, genetic approaches such as selective breeding and transgenic modification aim to develop inherently nutrient-dense crop varieties, providing a more sustainable and long-term solution. Despite their potential, these methods face limitations related to development timelines, scalability across diverse farming systems, and sensitivity to environmental conditions [4].

In our review, we have highlighted the role of multi-omics technologies—(meta)genomics, transcriptomics, proteomics, and metabolomics and their role in nurturing biofortification. These cutting-edge approaches are reforming biofortification through molecular precision and predictive breeding. These integrative tools enable high-throughput analysis across species and environments, accelerating the development of nutrient-rich crops [5]. Synthetic biology further enhances this framework by applying engineering principles through "*design-build-test-learn*" cycles to reprogram plant traits.

2. THE SUCCESS OF BIOFORTIFICATION:

(1) The Success of Conventional Breeding Practices- one of the most pervasive micronutrient deficiencies is of Iron (Fe) affecting about 69.1% of toddlers and 33% non-pregnant women. To combat this, millets were biofortified which led to the invention of **Iron Pearl Millets (IPM)**. A report published by ***Harvest Plus*** suggests that IPM increases the iron content by at least 30%. Pearl millet, commonly known as *bajra* in Hindi, is one of the staples in India [6]. Breeding efforts in pearl millet aim to boost iron and zinc levels to combat micronutrient deficiencies in populations dependent on this staple. In combination with genetic improvement, agronomic strategies such as soil management and micronutrient fertilization are being advanced to enhance both mineral content and bioavailability in grains, offering a sustainable solution to improve nutritional outcomes in susceptible communities [7].

(2)Zinc (Zn) deficiency in wheat used to be a major concern across many countries. The problem is intensified during post-harvest processing, where conventional milling removes the Zn-rich aleurone and bran layers, leading to a marked reduction in flour Zn content. As well, alterations in processing methods can cause further Zn fatalities, lowering the nutritional value of wheat-based diets for populations that rely heavily on them [8]. Conventional practices like soil application of zinc (Zn) fertilizer enhanced grain Zn concentration by approximately 29.1%, which remains below the biofortification target of $40\,\mathrm{mg\,kg^{-1}}$, necessary to meet human nutritional requirements [9]. In contrast, foliar Zn application and shared soil–foliar strategies result in significantly higher improvements of 55.2% and 62.3%, respectively, both exceeding the critical threshold of $40\,\mathrm{mg\,kg^{-1}}$. These findings emphasize the larger efficacy of foliar and integrated Zn fertilization approaches in achieving nutritionally relevant Zn enrichment in wheat grains [10].

3. THE CONTRIBUTION OF BIOFORTIFICATION IN SDG-2

In a study conducted by [11], it was stated that the United Nations' **Second Sustainable Development Goal (SDG 2)** objects to end hunger, achieve food security, improve nutritional content, and promote sustainable agriculture. A critical component of this goal is the elimination of *hidden hunger*. In response, many countries have executed a range of nutritional interventions. While dietary diversification and food supplementation remain important, biofortification has emerged as a particularly promising and sustainable long-term solution. Unlike conventional fortification, which adds nutrients during food processing, biofortification enhances the nutritional quality of crops during growth, ensuring nutrient-rich food reaches even remote populations where access to fortified products is limited.

Existing biofortification strategies include:

- **Agronomic biofortification**: Applying micronutrient-rich fertilizers to crops.
- **Conventional plant breeding**: Selecting and crossing varieties with naturally higher nutrient content.
- **Transgenic approaches**: Introducing genes that enhance nutrient biosynthesis or uptake.

These methods have been successfully applied to a wide range of staple crops, including cereals, legumes, oilseeds, vegetables, and fruits, demonstrating the adaptability and scalability of biofortification.

Among these, transgenic approaches stand out for their precision, speed, and potential to introduce novel traits beyond the limits of traditional breeding. To further enhance the effectiveness of transgenic biofortification, *multi-omics* technologies: genomics, transcriptomics, proteomics, and metabolomics are increasingly being integrated. These tools allow a deeper understanding of plant metabolic pathways and gene functions, mapping the location of desired genes, accelerating the development of nutrient-dense crop varieties personalized to specific nutritional needs and agroecological contexts.

4. THE INTEGRATION OF OMICS TECHNOLOGIES:

Genomics: QTL and GWAS are the principal genomics technologies applied in biofortification. QTL refers to *Quantitative Trait Loci*. A fundamental aim of QTL analysis is to govern the genetic make-up underlying phenotypic variation—specifically, whether apparent trait differences are driven by a small number of loci with significant effects or by several loci, each contributing only slightly. This distinction informs both the intricacy of trait inheritance and the strategies used for genetic improvement [12].

[13] in his research stated that the QTL analysis can help tell the chromosomal positions of target loci with no prior clues of the associated genes and can be used in breeding programs via MAS.

Furthermore, the patterns of linkage instability are influenced by Genome-wide association studies (GWAS) to discover genetic regions related to specific traits. By highlighting candidate genes' fundamental quantitative trait loci (QTLs), GWAS significantly speeds up gene cloning and trait enrichment efforts. Currently, this approach has become a widely implemented tool for dissecting complex traits in bread wheat [14].

(4.1) Revolutionary QTL studies from Indian field experiments:

A foundational study conducted in India by [15], employed a doubled haploid (DH) population (Berkut/Kirchauff), provided dynamic evidence for major-effect loci under real-world field conditions. Across two years and locations (2011–2013), this work identified three highly stable and major-effect QTLs.

Significantly, loci on chromosomes 1B and 2B established considerable influence on grain Zn concentration, accounting for up to 23.1% and 35.9% of the phenotypic variation (PVE), respectively. Significantly, the major Fe-regulating QTL co-localized with the 2B Zn QTL, explaining 22.2% PVE, suggesting a shared or closely linked genetic regulatory mechanism for both micronutrients. Further enriching the Indian genetic resource, using a recombinant inbred line (RIL) population (PBW 343/Kenya Swara), identified two novel and stable QTLs located on chromosomes 2Bc and 3AL, each contributing up to 15% PVE.

(4.2) Genome Wide Association Studies (GWAS):

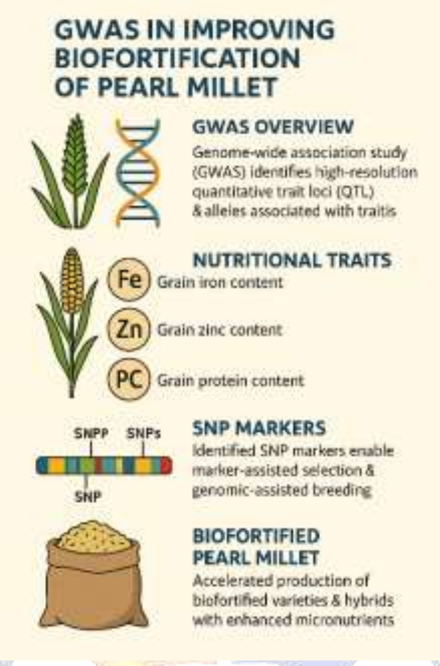
Genome-wide association studies (GWAS) have turned out to be a powerful tool for discovering the genetic makeup of complex traits. When it comes to wheat, researchers have long tried to locate the genes responsible for grain zinc (Zn) and iron (Fe) content using quantitative trait loci (QTL) mapping. While these traditional QTL studies have provided valuable insights, they come with limitations—mainly because they rely on bi-parental populations and offer only low-resolution mapping of genetic regions. GWAS, in contrast, offers a more polished and wide-ranging approach. It allows scientists to analyse genetic variations across a comprehensive and more diverse set of wheat lines, as well as landraces, elite cultivars, and trailblazing breeding materials. This means better persistence in identifying genetic markers, extensive allele coverage, and the ability to tap into natural genetic diversity [16].

GWAS has shown that this method offers high-resolution mapping of quantitative trait loci (QTL) and wide-ranging allele coverage. These strengths make GWAS an indispensable tool for enhancing iron (Fe) and zinc (Zn) concentrations in wheat, paving the way for more nutritious crop varieties [17].

(4.2.1) GWAS in Improving Biofortification of Pearl Millet: A Case Study

In reference to the study of [18], the current availability of the draft genome of pearl millet has paved the way for high-resolution genetic analysis, predominantly through the discovery of single-nucleotide polymorphisms (SNPs) and candidate genes. SNP markers are especially valuable in GWAS, genomic selection, and QTL mapping because of their richness and genome-wide availability.

GWAS have played a fundamental role in finding genomic regions associated with key nutritional traits in pearl millet, including grain iron (Fe), zinc (Zn), and protein content (PC). The identification and endorsement of single-nucleotide polymorphism (SNP) markers linked to these traits enable the expansion of robust diagnostic markers. These markers are highly valuable for marker-assisted selection (MAS) and genomic-assisted breeding approaches. By integrating these tools into breeding pipelines, researchers can accelerate the manufacture of biofortified pearl millet varieties and hybrids with enhanced micronutrient profiles. This advancement holds significant promise for enhancing nutritional security, particularly in regions where pearl millet serves as a dietary staple.



GWAS in improving biofortification of pearl millets, a study by [Pujar et al. 2020].

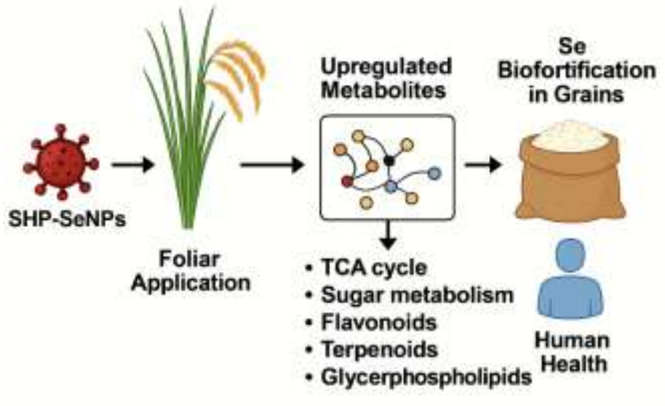
(4.3) Applying Metabolomics for Selenium biofortification:

Another important micronutrient is Selenium (Se). Although in developing countries, Se deficiency is rarely observed, in some parts of countries like Finland, New Zealand, Siberia, and China, the deficiency prevails. In China, about 72% of the soil is depleted in Se content, leading to **Keshan** and **Kashin-Beck** diseases.

To combat this, recent developments in nano-enabled biofortification highlight the role of **metabolomics** in explaining the metabolic pathways underlying nutrient assimilation and crop enhancement. [19] used *Phellinus igniarius* polysaccharide-stabilized selenium nanoparticles (SHP–SeNPs) for selenium enrichment in rice. Besides their physicochemical stability and superior bioavailability, SHP–SeNPs induced profound metabolic reprogramming in rice plants. In their study, foliar application of 45 mg/L SHP–SeNPs was done. This not only boosted traits such as chlorophyll content, soluble sugars, and protein accumulation, but also significantly increased grain yield and selenium deposition.

The workflow was simple; the central metabolic pathways, predominantly the TCA cycle, along with sugar metabolism, were activated, both of which are crucial for energy generation and carbon flux redistribution. Activation of these pathways enhances the plant's ability to efficiently generate ATP and utilize carbon skeletons, thereby supporting greater biosynthetic and growth demands during selenium uptake. Additionally, 16 core metabolites were upregulated, including flavonoids, terpenoids, and glycerophospholipids, which play essential roles in modulating plant growth, improving stress resilience, and facilitating nutrient transport, ultimately leading to improved selenium accumulation and overall plant health.

This study demonstrates how metabolomics can serve as a powerful analytic and predictive tool in biofortification research. By capturing dynamic changes in metabolite profiles, metabolomics enables the identification of biochemical markers and responsive pathways that can be targeted or enhanced through nano-formulation strategies. More broadly, integrating metabolomics into biofortification efforts may facilitate the development of crops with improved nutritional content and stress tolerance, offering a strategy to address micronutrient deficiencies and contribute to global food security [20].



Graphical representation of metabolomic study by Li. et al. 2025.

5. CONCLUSION

Biofortification has advanced into a multidisciplinary, evidence-driven approach that can sustainably decrease hidden hunger by making staple crops inherently more nutritious. Conventional breeding methods have shown success (e.g., iron-rich pearl millet, zinc wheat), but lack precision in understanding the molecular basis of nutrient accumulation.

Our review highlights how Omics have shifted the pace and accuracy of biofortification. QTL mapping identifies majoreffect loci in structured populations, and GWAS captures diverse alleles at high resolution and locates candidate genes for functional validation. Metabolomics is used to activate metabolic cycles crucial for nutrient assimilation and crop improvement. Omics-driven biofortification is an evolutionary approach in modern crop improvement, offering molecular-level precision and faster development of nutrient-rich cultivars.

We also emphasize the cooperative role of multiomics and synthetic biology. Omics-driven biofortification is a transformative approach in modern crop improvement, offering molecular-level precision and faster development of nutrient-rich cultivars. With coordinated science, policy, and delivery, biofortification can make a costeffective, durable contribution to SDG2, delivering nutrientdense staples to vulnerable populations and substantially reducing the global burden of micronutrient deficiency.

6. ACKNOWLEDGEMENT

The authors are highly indebted to President, and Department of Biotechnology, Isabella Thoburn College, Lucknow, India, for their great support and encouragement to complete this manuscript.

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