CHAPTER-3

Advances in Plant Nutrition and Metabolic Engineering: From Bio-fortification to Regulatory Innovations

Muhammad Tayyab Mateen[1*], Liaba Nasir[2], Waqas Mushtaq[3] Javaria Ashiq[4], Adeel Hafeez[5], Hassan Mumtaz[1] Muhammad Akmal Sajid[6] Muhammad Usman Amjad[6] Maham Shakeel[6] and Kashaf Ihsan[7]

[1] Department of Agronomy, College of Agriculture, University of Sargodha, Sargodha, 40100, **Pakistan**
[2] Nuclear Institute for Agriculture and Biology College, Pakistan Institute of Engineering and Applied Sciences (NIAB-C, PIEAS), Faisalabad, **Pakistan**
[3] Jiangxi Agricultural University, Key Laboratory of Crop Physiology, Ecology, and Genetic Breeding, Ministry of Education College of Agronomy, 1101 Fang Zhimin Avenue, Economic and technological Development Zone, Nanchang 330045, **China**
[4] Wheat Research Institute, Ayub Agricultural Research Institute, Jhang Road Faisalabad, **Pakistan**
[5] Department of Plant breeding and Genetics, College of Agriculture, University of Sargodha, Sargodha, 40100, **Pakistan**
[6] Department of Botany, Government College University Faisalabad (GCUF), Faisalabad, **Pakistan**
[7] Department of Bioinformatics and Biotechnology, Government College University Faisalabad (GCUF), Faisalabad, **Pakistan**

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Correspondence: hmateen005@gmail.com
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Summary

The recent advancements in plant nutrition sciences and metabolic engineering have provided novel platforms to improve the micronutrient profile, biochemical performance and metabolic stability of crops. Genetic, physiological and molecular advances in bio-fortification have made targeted nutrient enrichment of agronomic performance uncompromising. Meanwhile, metabolic pathway engineering, with the aid of genomics, transcriptomics, and highly accurate genome editing, has increased the ability to manipulate metabolic fluxes and optimize nutrient uptake in different environmental conditions. These developments have resulted a more mechanistic concept of metabolic regulation in crop systems, especially with regard to nutrient transporters, enzymatic bottlenecks and cross-talk in pathways. There has been a parallel development of regulatory frameworks in response to which nutrient-enhanced and metabolically engineered crops are being reassessed, approved and incorporated into agriculture. New models of scientific oversight are beginning to distinguish more and more between the traditional forms of transgenic constructs and specific genome edits, allowing a more liberal process of evaluation of low-risk, targeted amendments. These new regulations are also leading to the responsible use of engineered nutritional characteristics and a focus on transparency, environmental fitness, and traceability. Together, the developments in molecular design, optimization of nutrient use and progressive regulation are transforming the extent of plant nutritional enhancement and making metabolic engineering a core element in the future of agricultural innovation.

Keywords: Plant nutrition, metabolic engineering, biofortification, plant metabolism, functional genomics, metabolomics, nutrient use efficiency, synthetic biology, CRISPR, regulatory networks, sustainable agriculture, crop nutritional quality, precision nutrition, food security,

1 Introduction

1.1 Climate-Driven Challenges and the Persistence of Micronutrient Malnutrition

The world is increasingly facing pressure on global agriculture to provide high density food products in the face of the fast changing climate, soil erosion, and ever growing population. Some micronutrient deficiencies, specifically, iron, zinc, iodine, and vitamin A are still influential sources of hidden hunger, as they affect billions of people around the world and the health and productivity of the person (Welch and Graham, 2004; White and Broadley, 2009). High yield varieties have been a major priority in the practice of modern agriculture and this has led to dilution of vital micronutrients in staple crops, particularly with intensive production systems (Fan et al., 2008). At the same time, global food supply chains are becoming more and more destabilized by occurrences of extreme weather like heatwaves, irregular rainfall, flooding, and extended drought, which are threatening the nutritional quality of crops (Ray et al., 2015). Even climatic variability alone is projected to result in the loss of rice (1.6 million tons) and wheat (5 million tons) per year, which in turn leads to a substantial decline in global availability of calories and complicates the task of providing food to the project of 10 billion people by 2050 (Ray et al., 2015; Arora, 2019). Micronutrient malnutrition has been particularly acute in those areas that rely on cereal-based nutrition, and iron and zinc deficits are the only two factors that impact almost two billion individuals (Gregory et al., 2017). Though the traditional breeding strategies have been more concerned with increasing yields stability in the face of changing climatic conditions, important physiological functions related to grain production, including carbon assimilation and source-sink interactions and nutrient partitioning are very susceptible to environmental stress (Ullah and Chenu, 2019). Consequently, climate-related changes that take place in photosynthesis, transpiration, and metabolism may reduce nutrient levels in consumable plant tissues further (Alae-Carew et al., 2020).

1.2 Advancements in Metabolic Engineering for Nutritional Enhancement

Nonetheless metabolic engineering has been found to be among the most useful methods towards attaining such a dual objective through allowing the fine-tuning of biosynthetic pathways that produce vitamins, minerals, amino acids, antioxidants and other bioactive molecules. Metabolic engineering makes it easier to accumulate critical micronutrients and phytonutrients and enhance plant responses to environmental stresses through specific manipulation of important enzymes and control nodes (Ricroch et al., 2014; Liu et al., 2021). Deposition The following advanced tools have been developed: metabolic flux modeling, transgene stacking, and CRISPR/Cas-mediated gene editing can enable researchers to design complex pathways with high accuracy while being able to express valuable traits by the crop without disrupting primary metabolism. Such engineered advances are able to boost the defense systems of plants through optimizing the stress-response cascades, refining the antioxidative potential, and optimizing the metabolism of protective secondary metabolites (Bhat et al., 2022). Application of metabolic engineering and plant nutrition science also increases the possibilities of fighting malnutrition. Genome sequencing, transcriptomics, metabolomics, and systems biology insights have provided an opportunity to map the complex metabolic networks and to determine strategic points of

intervention that can be used to enhance nutritional outcome (Sweetlove et al., 2017). Advanced edit technologies and specifically CRISPR-based editing methods have hastened the creation of nutritionally enriched cultivars designed to have an increased level of micronutrients with a limited degree of genetic perturbation (Zhu et al., 2020). This is a vital ability because nowadays, food security can only be achieved by not only enhancing the production of calories but also enhancing the nutritional quality of the staple diets. Most commonly eaten cereals (rice, wheat, and maize) do not contain sufficient vitamins and minerals, which are not sufficient to address the nutritional requirements of the population (Muthayya et al., 2013; Poole et al., 2021). Plant metabolic engineering thus offers a strong platform towards increase of crops with greater quantities of nutrients indispensable in the environment and resiliency to environmental stressors. This integrated science is one of the significant improvements of sustainable food systems that can sustain the global food under the rapidly changing climatic conditions.

2 Fundamental Concepts of Plant Nutrition

A paradigm shift is currently being experienced in the concept of what plant nutrient is. Conventionally, the Arnon-Stout definition focused solely on the essential elements the ones strictly necessitated in the life cycle of a plant (Arnon and Stout, 1939). Recent investigations have contended, however, that such limited perspective does not take into consideration the extended functional roles of most minerals which, although not essential to survival, play important roles in improving agronomic performance. These encompass factors that enhance nutrient utilization efficiencies, high-stress tolerance, and quality of crops, hence connecting plant nutrition more intimately with the objectives of sustainable farming and the one nutrition concept that merges plant, animal, and human nutrition (Brown, Zhao and Dobermann, 2021). This redefinition indicates a progressive change in the theory of plant nutrition, starting with the classical experiments of de Saussure, Sprengel and Liebig, who provided the chemical basis of mineral nutrition, and later formalized with omission experiments in nutrient solutions of Arnon and Stout, who provided the classical requirements of essentiality. Although these criteria, which fail to complete the life cycle under the influence of the absence of a nutrient, their irreplaceability by other elements, and bio-chemical activity were valid at that time, current studies have found context-dependent nutrient functions that push these limits (van der Ploeg et al., 1999; Brown, Zhao and Dobermann, 2021).

2.1 Physiological and Rhizosphere-Mediated Mechanisms

Physiologically, there is much beyond mere acquisition and metabolism of plant nutrition. Complex root structures, minerals transportation proteins, chelators and vascularization networks coordinate uptake and assimilation of minerals. Nutrients can affect photosynthesis, respiration, signaling, and source-sink partitioning, and positive elements like silicon and sodium regulate the stress resilience through the effect on transporter expression, cell wall and antioxidant systems, especially when the environmental conditions are not favorable (Ma and Yamaji, 2008; Broadley et al., 2012). One of the main arenas through which these interactions are enhanced is the rhizosphere which is a highly dynamic zone around the roots of plants. Plant-soil interactions are concentrated in the rhizosphere which includes both beneficial, antagonistic, and neutral interactions and plays a major role in determining the fate, behavior, and uptake of

nutrients (Baetz, 2016; Kayler et al., 2017; Rugova et al., 2017). There are many biological and ecological changes in this zone as shown in figure 1. that control the activity of the microorganisms, the nutrient content, and the growth of the plants (Callesen et al., 2016; Huang et al., 2016). These mechanisms are significantly different to those occurring in bulk soil, which demonstrates the distinct microenvironment between root and soil (Ibekwe et al., 2017; Rugova et al., 2017). Rhizosphere is a dynamic interface between soil and plant roots, root systems with microorganisms and between soil and root-invertebrate (Bais et al., 2006; Hartmann et al., 2008; Singh et al., 2016; Rugova et al., 2017). Rhizosphere conditions significantly affect the fate and behavior of organic compounds that are emitted by roots and microbe (Cai et al., 2017). The zone contains abundant low-molecular-weight water-soluble compounds, such as secondary metabolites (flavonoids, glucosinolates and terpenoids), gaseous molecules (CO2, H2) and inorganic ions (HCO3-, OH-, H+), amino acids, organic acid anions and carbohydrates (Jha et al., 2015; Baetz, 2016; Haichar et al., 2014; Montiel-Rozas et al Substances of high molecular weight like mucilage, polysaccharides and proteins also enrich the rhizosphere, which helps in mobilizing nutrients and microbial symbioses (Jha et al., 2015).

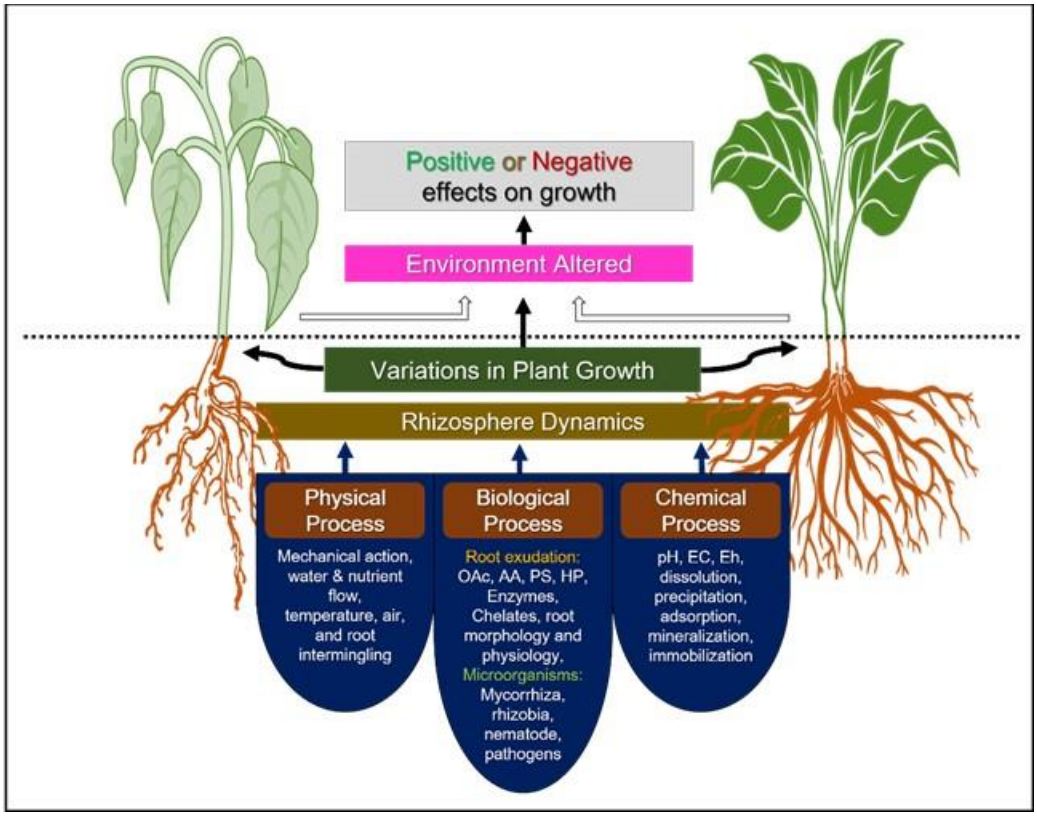

Figure 1. Rhizosphere Dynamics Governing Nutrient Uptake

Plants need proper balance of macro and micronutrients in order to grow, metabolize and be resilient. Although their roles vary in concentration and function within the body, macronutrients like nitrogen, phosphorus, potassium, calcium, magnesium and sulphur act as structural and metabolic structures whereas micronutrients like zinc, iron, copper, manganese, boron and molybdenum are catalytic and regulatory elements in enzymatic pathways (Marschner, 2012).

The deficiencies cause dysfunction in the body including chlorosis, poor growth, retarded photosynthesis, enzymatic inhibition, and decreased reproductive ability (Alloway, 2008). The initial stage of nutrient uptake is at the root-soil interface via special transporters and channels which is controlled by electrochemical gradients and subsequently in the xylem and phloem through translocation to different tissues where assimilation pathways such as reduction of nitrogen and biosynthesis of amino acids occur (Leustek et al., 2000). The efficiency of uptake is determined by the properties of the soil, genetics of the plant, root structure, and interaction of mycorrhizal fungi, and these symbioses are important in increasing the availability of phosphorus and micronutrients (Smith and Read, 2008). Nutrients are strongly linked to the primary metabolism, and they influence photosynthesis, carbon allocation, and sugar, amino acid, lipids and secondary metabolite biosynthesis all of which define crop productivity and nutritional quality (Hermans et al., 2006).

2.2 Climate-Modulated Nutrient Fluxes

Mineral nutrients also play the role of essential components of macromolecules, such as nucleic acids, phospholipids, individual amino acids, and co-enzymes, and they are important to cellular metabolism, chlorophyll formation, redox regulation, intactness of plasma membranes, and osmotic potential (Soares et al., 2019). The nutritional status of soils and their diversity are diverse across plant species and largely depend on climate, as shown in figure 2., which subsequently impacts the nutrient cycles and the productivity of plants (Dotaniya et al., 2016; Gong and Gao, 2019). Climatic change also brings new challenges by changing the supply and dynamics of major nutrients like carbon, nitrogen, and phosphorus, which are vital in the development of plants (Koller and Phoenix, 2017). The dynamics of soil nutrients are highly affected by temperature and the composition of nutrients.

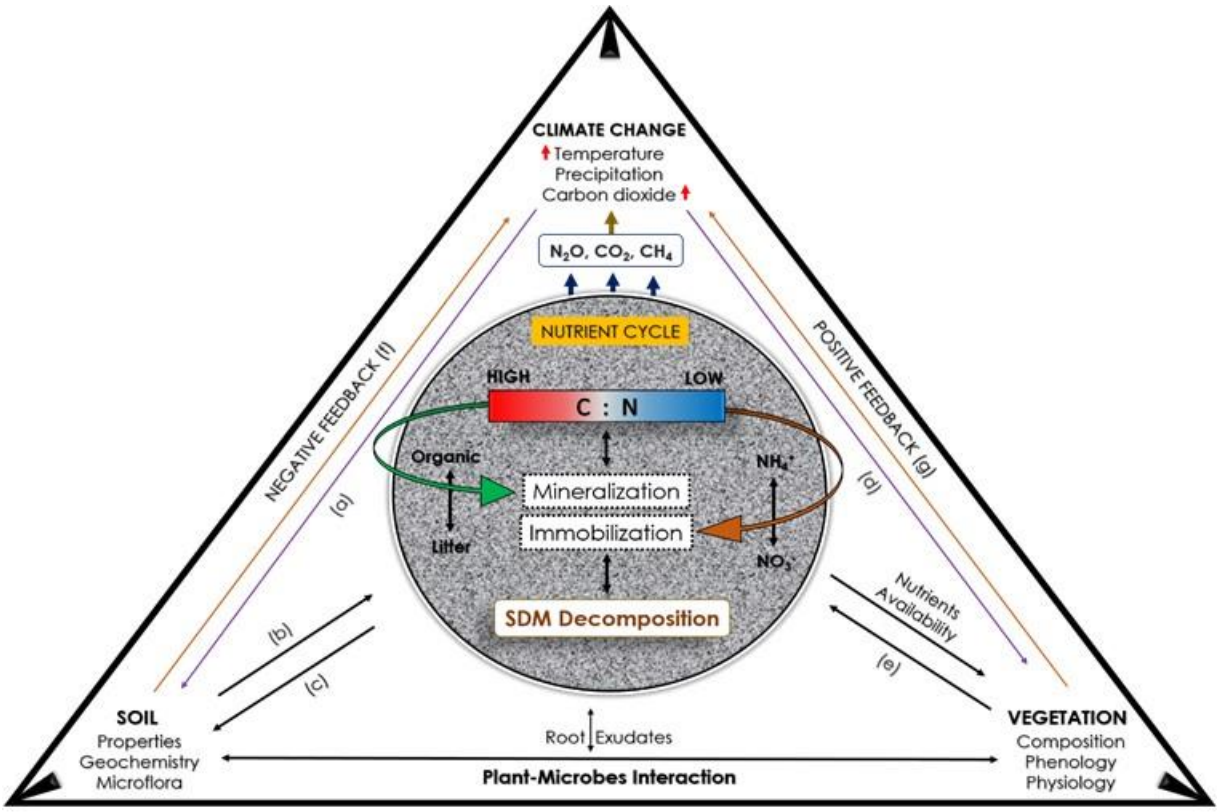

Figure 2. Dynamic root-microbiome response under climate dynamics

Increased temperatures will increase microbial activity which increases mineralization of organic matter, whereas high carbon levels, especially elevated C:N ratios favor microbial immobilization, temporarily decreasing nutrient availability. Conversely, adequate nitrogen will enhance mineralization, which will enhance the availability and the release of nutrients to be absorbed by plants. This combination of factors keeps the balance between the release and retention of the nutrients, which eventually determine the soil fertility and growth of plants. The presence of a functional balance between the shoot and root development has guaranteed the optimal nutrient acquisition and utilization since photosynthetic efficiency and leaf biomass are closely associated with the weight of the roots and the capacity to absorb nutrients (Pilbeam, 2014). Climatic factors consequently affect any plant growth, metabolism, physiology and yield, and thus the interdependence between nutrition and environmental factors (Alshaal et al., 2017).

3 Bio-fortification and Nutritional Enhancement Strategies

An increase in atmospheric CO_2 may increase root and shoot development which may improve nutrient uptake though this is dependent on adequate nitrogen presence. Such developments notwithstanding, there is a difficulty in converting scientific knowledge into farm production. A significant number of regulations governing the use of fertilizers are still based on old definitions of fertilizers that only include classical basic constituents and restrict the innovativeness of nutrient products and application plans that may enhance crop toughness, production, and quality (White and Broadley, 2009; Brown, Zhao and Dobermann, 2021).

Table 1. Advances in Bio-fortification and Nutrient Enhancement Strategies (2015–2025)

Strategy	Mechanism / Approach	Target nutrients / traits	Reference
Conventional breeding bio-fortification	MAS, QTL mapping, genomic selection to accumulate nutrient-dense alleles	Fe, Zn, pro-Vitamin A, quality protein	(Bouis & Saltzman, 2017; Birol et al., 2021; Dwivedi, 2023).
Transgenic metabolic engineering	Introducing or enhancing nutrient biosynthetic pathways via multigene constructs	Pro-Vitamin A, folate, iron chelators	(Paine et al., 2005; Storozhenko et al., 2007).
Genome editing (CRISPR/Cas)	Precise edits to endogenous nutrient-related genes; base/prime editing	Amino acids, Fe/Zn transport, carotenoids	(Kumar et al., 2022; Chen, 2024).
Agronomic bio-fortification	Micronutrient fertilizers, foliar sprays, seed priming	Zn, Se, I, occasionally Fe	(Mao, 2014; Ram, 2024).
Microbial / Rhizosphere interventions (PGPR, AMF, SynComs)	PGPR/AMF-mediated nutrient solubilization; SynCom assembly; metagenomic selection	N fixation (indirect), P, Fe, Zn	(Berendsen et al., 2012; Decleyre et al., 2015; Finkel et al., 2019).
Nano-enabled nutrient delivery	Controlled-release nanofertilizers and seed coatings	Zn, Fe; NUE improvements	(Rameshaiah et al., 2015).
Post-harvest nutrient retention strategies	Improved storage, processing, handling	Pro-Vitamin A, Fe, Zn	(Huey et al., 2023).
Integrated multi-pathway bio-fortification	Combined breeding, agronomy, microbiome, and value-chain interventions	Fe, Zn, pro-Vitamin A, Se, I	(Birol et al., 2021; Ram, 2024).

Even with development, nutritional enhancement methods have challenges of biosafety, regulatory and acceptance by the people. There are issues associated with the risk of gene flows, allergenicity, environmental effects, and stability of engineered products in the long run (Ricroch et al., 2014).

4 Metabolic Pathway Engineering in Plants

4.1 Overview of primary and secondary metabolic

Plant metabolism engineering is a complex concept that has a complex basis since the plant metabolism is a mass of many biochemical reactions that are present in plants to enable their growth, development, and adaptation to the environment (Xu, 2018). The major metabolic pathways including photosynthesis, respiration, and nitrogen assimilation are the primary energy and structural materials that are involved in the functioning of cells. Secondary metabolic pathways, in contrast, produce specific compounds, which form a basis of defense, signaling, and adaptation to stress (Sung et al., 2015).

4.2 Techniques for pathway modification

Recent innovations in metabolomics and flux analysis methods have provided an unprecedented amount of insights into the active regulation of these pathways, allowing the researchers to break down metabolic fluxes with more than ever before (Lee et al., 2003; Xu et al., 2021). In this

metabolic model, the possibility of re-directing fluxes by reengineering particular pathways can be used to achieve useful nutritional, biochemical or physiological ends. The main and auxiliary metabolic processes which affect amino acids, vitamins, antioxidants, pigments and other bioactive metabolites have been engineered to improve the nutritional value. The engineering of folate, vitamin E, carotenoids, flavonoids, and glucosinolates are not only effective interventions but serious enhancements to the nutritional and functional value of crops (Zhu et al., 2018). The methods of controlling these pathways are based on altering the expression of rate-limiting enzymes, biosynthetic regulators or pathway bottlenecks (K.-T. Fan et al., 2024). Gene overexpression is commonly applied to increase metabolic flow on a target product and boost the production of vitamins, antioxidants, and essential amino acids (K.-T. Fan et al., 2024). In tandem with this method, the silencing or knockout technology of RNA interference (RNAi) and CRISPR/Cas9 could help to shut down the competing pathways or remove the unwanted intermediates (Xu et al., 2022). Pathway precision can also be further promoted by the metabolic level of control in that engineering transcription factors, regulatory RNAs, and promoters refines the patterns of gene expression and pathway sensitivity (Mouchiroud et al., 2014). Other technologies like enzyme engineering and development of synthetic scaffolds enhance the use of metabolic channeling, which enhances the efficiency of multi-step reactions by physically co-locating enzymes (Mouchiroud et al., 2014). Synthetic biology and genome editing have solidified these methods, which are giving more and more advanced methods of customizing plant biosynthetic systems.

4.3 Systems biology and omics tools in metabolic reconstruction

The conception and advancement of the genetic transformation instruments have also played a significant role in facilitating metabolic engineering. Agrobacterium-mediated transformation is a popular method of integrating genes into stables, and the optimization of its efficiency and host range has been able to continue to expand the range of its application (Rahman et al., 2024). In species that do not respond to Agrobacterium, particle bombardment is an option, which has been improved of late by advancements in the sphere of particles design and delivery, and significantly enhanced success rates (Ozyigit & Yucebilgili Kurtoglu, 2020). The advent of the CRISPR/Cas9 system has become a significant boost to the field of plant biotechnology, providing the most precise and versatile gene editing experience (Perez Rojo et al., 2018). The development of new variations (base editing, prime editing, and others) further improve the precision with the ability to make single-nucleotide changes without causing a double-strand break (Anzalone et al., 2019). RNA interference, virus-induced gene silencing (VIGS), and newer optogenetic or chemogenetic tools provide temporal and spatial regulation of gene activity and pathway engineering is now more flexible and dynamic than ever (K. Fan, 2024). The systems of nanomaterials delivery also demonstrate the potential of enhancing the efficiency of the transformation, as well as increasing the ability to target tissues (Haider et al., 2025). Combining these tools of transformation with a high-throughput phenotyping and genome-wide association study increases the rate of gene-to-function discovery and enabled more specific reconstruction of metabolic pathways (Siddique et al., 2023).

4.4 Metabolic Engineering Challenges and Innovations

Engineering of metabolic pathways has provided valuable breakthroughs in the enhancement of

important nutritional characteristics. An example is the creation of amino acid biosynthetic pathways like the lysine and methionine biosynthetic pathways is done by modifying enzymes like dihydrodipicolinate synthase as well as relieving feedback inhibition (Ufaz and Galili, 2008). Enhancing vitamins- primarily the pro-Vitamin A and folate have been accomplished using multi-enzyme-transgenic methods that increase specific biosynthetic pathways (Storozhenko et al., 2007). Likewise, secondary metabolite biosyntheses such as flavonoids and anthocyanins, have been remodeled to yield crops with antioxidant functionality with added health value (Butelli et al., 2008). In addition to specific characteristics, the ability of systems biology and multi-omics methods is to reconstruct whole metabolic networks with the incorporation of transcriptomic, proteomic and metabolomic data. These instruments enlighten metabolic plasticity and pinpoint areas of leverage in order to come up with engineering that is more robust and stable (Weckwerth, 2011). Although impressive advances have been made, there are still problems regarding stable expression, reduction of unintended metabolic burdens and trade-offs that impact the growth or fitness of plants. With the further integration of more sophisticated genome editing, modeling, and synthetic biology technologies, metabolic engineering is expected to develop into more predictable and sustainable manipulation of biological pathways of plants biochemistry.

5 Harnessing Innovation in Plant Growth and Nutrient Signaling

5.1 Metabolism modulated by internal and external PGRs

To capitalize on how innovation in plant development and nutrient signaling can be harnessed, hormone biology needs to be combined with innovations in rhizosphere microbiome engineering since both systems are at the core of regulating nutrient uptake and stress response as well as plant performance. The auxins and cytokinins, gibberellins and abscisic acid are plant growth regulators (PGRs) that adjust important processes such as root system architecture, stomatal behavior and nutrient transporter activity, thus influencing nutrient acquisition and allocation (Taiz et al., 2015). These responses are further refined by extensive crosstalk of hormonal pathways and networks of nutrient signaling; auxin controls reactions to phosphate by remodeling root, cytokinins regulate nitrate uptake, assimilation and remobilization (Kiba et al., 2011). In addition to these regulatory mechanisms, strategic exploitation of the native microorganisms will improve nutrient dynamics, by selecting and bringing in useful microbial strains that inherently get attached to the plant species. These crop-specific microbial communities are found in maize, rice, and sugarcane and help in forming strong microbiomes in the tissues of plants and the rhizosphere (Elsayed et al., 2020; dos Santos et al., 2022; Medina and Kutuzova, 2022). The current approaches in microbial engineering make the active control over the rhizosphere environment more and more possible, as the key components of the core microbiome are identified, and the desirable consortia can be assembled that would be able to have stable interactions with the roots of plants. Strategies like plant growth with chosen bacteria and incorporation of microbial inoculants with current communities have been backed with the use of sophisticated tools as they demonstrate all the microbial diversity and capabilities. PhytoChip and GeoChip are functional gene microarrays that map the processes in the microbes and help to understand the role of particular taxa in ecosystem functioning (Decleyre et al., 2015; Meidute et al., 2018). In situ visualization methods based on fluorescence also enable real-time

dynamic monitoring of the colonization of roots by microbes, which will deepen the comprehension of the plant-microbe interaction in natural conditions (Hardoim et al., 2015b; Sayre et al., 2023; Haney, 2021b). These insights are supported by the molecular manipulation methods, including gene silencing, transgenic techniques, and CRISPR/Cas9 gene editing, to enable the targeted manipulation of plant characteristics, such as root exudate profiles, which affect the microbiome composition, nutrient intake, and resistance to stress (Lo et al., 2022; Chen et al., 2024).

5.2 Synthetic growth regulators enabled by biotechnology innovations

Synthetic biology also broadens the ability to improve nutrient signaling by allowing the creation of engineered signaling circuits, synthetic hormone receptors, and inducible promoters which offer very precise temporal regulation of the physiological responses (Park et al., 2017). Such advances are in addition to the microbial engineering to enhance the sustainability of agriculture. Genetic engineering and CRISPR-based genome editing can provide answers to the problems of pest resistance and nutrient inefficiency, as well as stresses caused by climate, and eventually result in increased yield and enhanced food security (Santin et al., 2016; Aplakidou et al., 2024). RNA interference (RNAi) also can be utilized to enhance the plant disease resistance by targeting transcriptional and post-transcriptional silencing of genes (Mason-Jones et al., 2022; Wang M. et al., 2023). The idea of engineering rhizosphere microbiomes further puts these methods into context by demonstrating how the focus on traditional soil management and strategies that involve the use of microbes have evolved into highly specialized plant breeding to favorable root exudates and microstructure-specific inoculants (Singer et al., 2016). Modern day engineering focuses on the use of functional profiling, metagenomics, biochemical assays, and high-resolution characterization as the tools to guide selection of microbial strains guided by genomes (Berendsen et al., 2012; Bulgarelli et al., 2013; Muller et al., 2016; Zhang et al., 2020; Solomon and Janda, 2024b). Genome sequencing detects plant growth-promoting and stress-reducing genes (Hacquard et al., 2015), whereas CRISPR-mediated trait optimization makes sure that the useful qualities are optimized on-field (Calvin and Bassham, 1962; Geldner, 2013; Carabotti et al., 2015; Berendsen et al., 2018; Cryan et al., 2019; Hulme et al., 2020). The functional expression of important genes and proteins is then confirmed through transcriptomic and proteomic profiling, which dictates the derivation of microbial strains that are active, have biocontrol activity, and are physiologically robust (Yuan et al., 2018). These technologies highlight the expanding opportunities of microbiome engineering combined with hormonal and metabolic cues to support sustainable agriculture and to satisfy the rising global need of resilient and resource-efficient agriculture (Bais et al., 2010; Hiruma et al., 2016; Bakker et al., 2018; Stringlis et al., 2018b; Finkel et al., 2019). Lastly, another important interface is in place between plant signaling pathways and engineered rhizosphere functions: so-called root exudate-mediated assembly of synthetic microbial communities (SynComs). Root exudates (a mix of various sugars, amino acids, organic acids and secondary metabolites) are recognized to selectively attract microbial companions and form SynCom structure (Marin et al., 2021; Hu et al., 2022). Temporal changes in exudates composition allow plants to enlist taxa that offer nutrient cycling, pathogen suppressing, and stress relieving qualities. Nevertheless, carbohydrates invite the microbes that live on sugar, whereas organic acids like malic and citric acid attract phosphate-solubilizing

bacteria (Yuan et al., 2021). Microbial competition and spatial organization in the rhizosphere are also regulated by exudates and contribute to cooperative metabolic networks as well as prevent pathogenic organisms (Wang and Kuzyakov, 2023; Wang and Kuzyakov, 2024b). Exudates maintain the activity of microbes by providing carbon, nitrogen, and energy resources once SynComs have been established (Cornforth and Foster, 2013), and triggering nitrogen fixation and phosphate mobilization genes (Heilbronner et al., 2021; Pantigoso et al., 2022a; Timofeeva et al., 2023). Combined, these innovations demonstrate the strong synergy of plant hormone signaling, designed microbial communities and contemporary genomic technology to provide a revolution in the sustainable management of nutrients and higher agricultural yield.

6 Advancements in Nutri-Metabolomics

6.1 Genome-Based Nutritional Engineering

The scope of a plant nutritional engineering has been extended recently through synthetic biology, genome-wide association studies, and metabolic network modeling. GWAS and QTL mapping have also shown the presence of major genetic loci that regulate the accumulation of micronutrients, which have opened the way to molecular breeding of crops with high nutrition (Garcia-Oliveira et al., 2009). The transcriptomics, metabolomics, and proteomics types of omics technologies enable a high-resolution monitoring of the nutrient-sensitive genes and pathways to enhance precision in trait improvement (Fernie and Stitt, 2012). Optimizing the use of fertilizer and increasing the bioavailability of nutrients are some of the uses of precision agriculture tools, such as remote sensing, spectral imaging, sensor-based nutrient monitoring systems, which complement metabolic engineering (Zhang and Kovacs, 2012). The combination of field level precision control and genetically modified nutrition characteristics will hold promise of more efficient and sustainable production systems at crop level.

6.2 Precision Agriculture and Omics Integration

Regulatory innovations in modern agricultural biotechnology are becoming more and more favourable to the precision-based approaches where the emphasis is put on process-based assessment frameworks being replaced by product-based assessment frameworks. Some nations such as Argentina, Brazil, the United States, and Japan are now concentrating on the end attributes of genome-edited products and not on the breeding method applied, especially in cases where no foreign DNA is implanted. This model was first crafted in Argentina, which has created a system of case-by-case determination to avoid subjecting a variety of CRISPR-edited crops to GMO regulations, leading to much shorter approval periods and faster introduction of products (Whelan and Lema, 2015). Science-based guidance followed by Japan, which excludes objects of CRISPR-editing plants, high-GABA tomatoes, of GMO regulation, creates the possibility of faster commercialization of nutritionally and functionally enhanced cultivars (Mizuno et al., 2020). These changes in policies have resulted in a favorable condition of innovations aimed at nutrient composition, resilience to stress and enhanced metabolic characteristics. Parallel regulatory innovations have been developed in microbial biotechnology especially in engineered microbial inoculants and synthetic microbial communities (SynComs).

6.3 Regulatory Innovations and Bio-fortification

The Regulation (EU 2019/1009) by the European Union developed a specific system to regulate the bio-stimulants, where they must be identified genomic, certifying quality, and documenting functional efficacy, which separates bio-stimulants and fertilizers/pesticides and promotes innovation (du Jardin, 2015). Likewise, the U.S Environmental Protection Agency has included genomics-based characterization and ecological-risk modeling into the process of microbial pesticides and engineered strains, thus allowing the incorporation of safer microorganisms to improve nutrient signaling, root development, and plant physiological stress (Gray and Limonard, 2019). Such regulation is a systematic route to the development of rhizosphere engineering, microbial consortium utilization and technology of root-microbe interaction as well as adaption of advanced strategies combined with modern genetics, as shown in Figure 3.

Important policy shifts have also been favorable to bio-fortification and nutritional enhancement and have placed agriculture in closer relation to the strategies of the public health. Some global programs like HarvestPlus have cooperated with regulatory agencies to come up with standards of verifying micronutrient enrichment like zinc, iron, and pro-Vitamin-A that permit such crops to be accessed by national procurement programs like school feeding and social safety-net programs (Bouis and Saltzman, 2017). The mandatory labeling and quality standards of biofortified crops and flour blends have been developed in India by the Food Safety and Standards Authority of India (FSSAI); thus enabling nutritionally enhanced products to enter mainstream markets and government supply chains (Govender et al., 2021). Such regulations enhance consumer confidence and allow the sale of nutritionally enriched cultivars at scales that allow the regulatory innovation to become one of the core areas of promoting food and nutritional security through biotechnology.

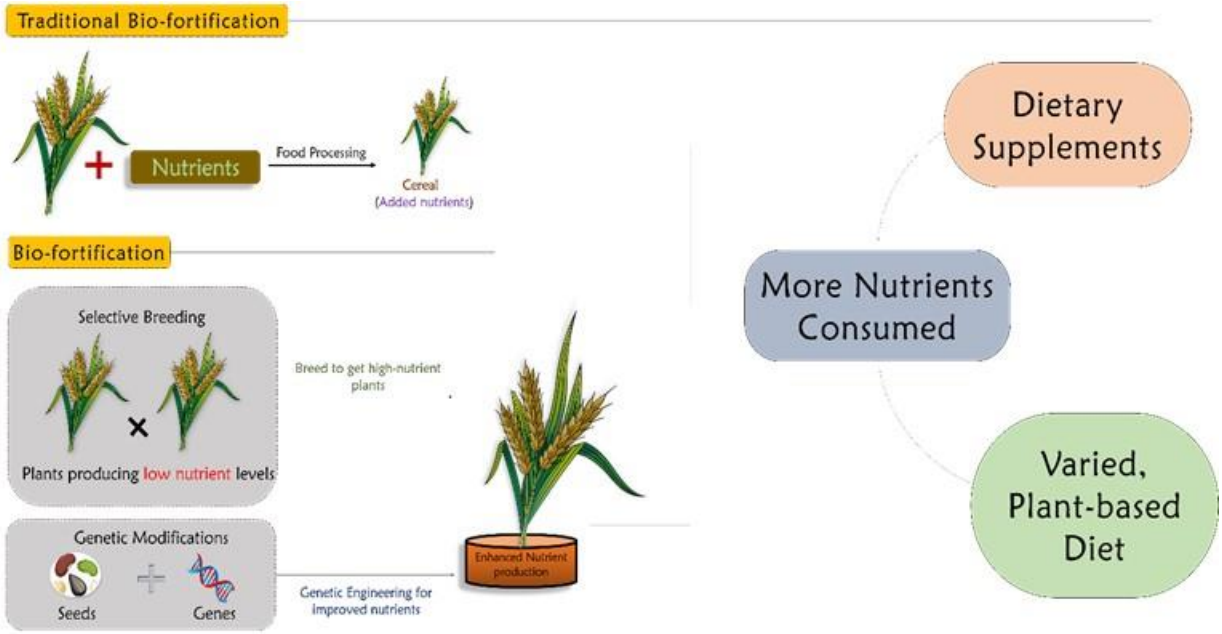

Figure 3. Advancements in Bio-fortification strategies for modern-day agriculture

6.4 Emerging Challenges, Ethical Dimensions, and Future Pathways

Important ethical, biosafety and regulatory issues are introduced with the introduction of nutritional and metabolic engineering in plants. The subject of genetically modified and bioengineered crops is being subject to questioning by the masses, and the fears are that there may be health hazards, gene transfer to wild stocks, ecosystem imbalances, and the intellectual property (IP) (Qaim, 2020). Open communication, democratic managerial practices and high levels of risk evaluation measures are needed to establish confidence of the society and to maintain safety. Green management of nutrition needs to incorporate environmental management. Crop engineering to increase the nutrient density of crops must not affect biodiversity, soil health, and ecosystem services. Climate-resistant, nutrition-enhanced crops necessitate the use of a combined approach to biotechnology, agroecology and the principles of the circular management of nutrients (Zhu et al., 2020; Ricroch et al., 2014). The future spatial of research is to have a combination of multi-omics analysis and prediction modeling to further speed up the discovery of the major regulatory nodes in nutrient and stress resilience pathways. Precision agriculture can be used with synthetic biology and CRISPR/Cas genome editing to adjust the nutrient bioavailability and metabolic flux to variable environmental conditions (Liu et al., 2021; Sweetlove et al., 2017). Global food and nutrition security will be a central objective of interdisciplinary collaboration in the fields of plant science, nutrition, biotechnology, and socio-economic.

7 Conclusion

Considerable advancements have been done in the last decade covering the science of nutrients, nutrition and metabolic engineering of plants, shifting the strategies of crop nutritional quality, resistance, and metabolic efficacy. Bio-fortification using traditional breeding methods or transgenic systems and genome editing have proved to be very effective in increasing the micronutrient level and efficiency in nutrient utilization in key staples. Those developments represent a more accurate biological picture of the nutrient uptake, transport and assimilation pathways, which makes it possible to manipulate major control mechanisms and biosynthetic cascades to produce improved nutritional characteristics without reducing agronomic traits. Simultaneously, the technology of metabolic engineering has become a strong platform, which can be used to reengineer intricate metabolic networks to maximize phytonutrient storage, stress-responsive metabolites, and metabolic homeostasis in plants. CRISPR/Cas systems, artificial promoters, and multi-gene pathway engineering have increased the ability to easily control flux using both primary and secondary metabolic pathways at a level never before achieved. This has enabled scientists to deal with the qualities like improved pro-vitamin A production, improved iron-zinc co-fortification and improved antioxidant metabolite, and these have led directly to nutritional security around the world and climate-adaptive crop production. Regulatory innovations have incorporated into the process of making sure that these technologies are deployed in a responsible manner. The current direction of metabolic engineering and bio-fortification studies is now determined by contemporary biosafety systems, regulation of individual traits, guidelines on genome-editing and harmonized international standards. The current regulatory changes especially those that are shifting towards product but not process based assessment are creating an ecosystem that promotes balanced scientific advancement and

safety, transparency and societal acceptance. With the global agriculture faced with issues like climate variability, micronutrient malnutrition, and rising food demand, convergence of nutritional genomics, metabolism engineering tool and future-thinking regulatory architecture, the research is likely to keep driving radical innovations in sustainable crop enhancement.

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