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Regional Training Course on Efficient Screening Methods for Improved Nutritional Quality in Mutant Populations

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Principles of Biofortification and Nutritional Quality-Improvement in Food Crops

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Nutritional quality classification

Macronutrients

Carbohydrates

Proteins

Fats

Micronutrient

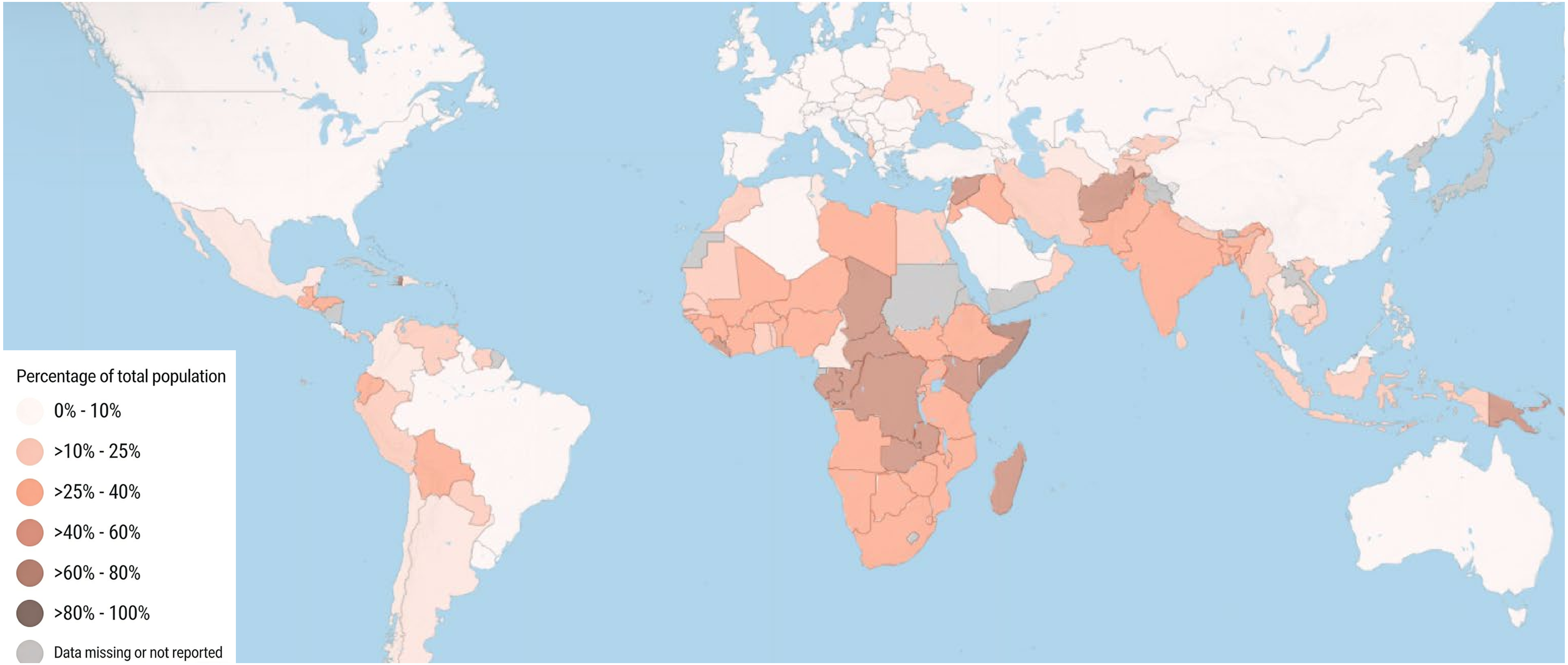
Vitamins

Minerals

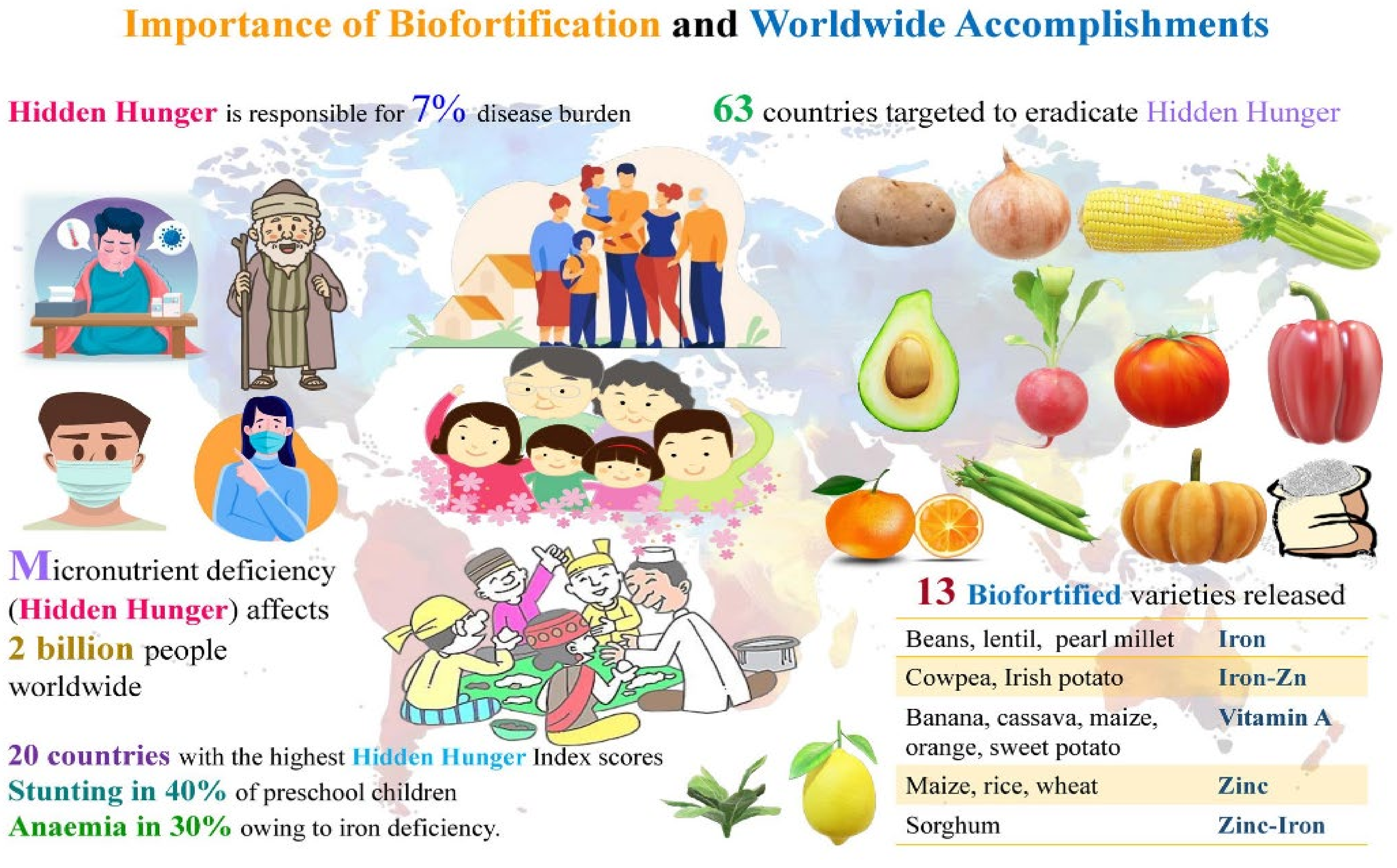
The Hidden Hunger Crisis

- **Hidden Hunger： refers to a form of malnutrition where individuals consume enough calories to meet their energy needs, but their diets lack essential vitamins and minerals such as iron, zinc, iodine, and vitamin A.**
- **Hidden Hunger is "invisible" — its effects are chronic, subtle, and often go unnoticed until they manifest as developmental delays, cognitive impairment,weakened immunity, or increased disease risk.**
- **According to the FAO (2025) flagship report, more than 2 billion people worldwide suffer from micronutrient malnutrition (hidden hunger) despite adequate calorie intake.**

Prevalence of undernourishment (percent) (3-year average) 2022-2024



source:FAO



（source： Asad Azeem et al., 2025; DOI: 10.1007/s44372-025-00276-

Biofortification

Definition:

The process of increasing the content of micronutrients and their bioavailability in the edible parts of crops during their growth and development, through agronomic measures, plant breeding methods or biotechnological means.

Key difference:

It emphasizes nutrient improvement at the "source" of the crops.

Why Biofortification ?

- **Targeted:** More nutritious staple foods can reach rural communities often missed by other nutrition interventions such as dietary supplementation and food fortification.
- **Cost-effective:** Breeding the nutrient into a crop variety takes an up-front investment, but once the trait is added, it is retained. The crop can be adapted to thrive in a range of agroecological zones at low cost.
- **Sustainable:** This strategy is based on staple foods that people already eat regularly. In most cases, farmers can save the seeds or cuttings to replant, and share them freely with their neighbors.

Contents

1The principle of macronutrients modification

Path redirection

By regulating key genes and altering the metabolic flow, allowing nutrients to accumulate in the desired direction, this is the fundamental logic for quality improvement.

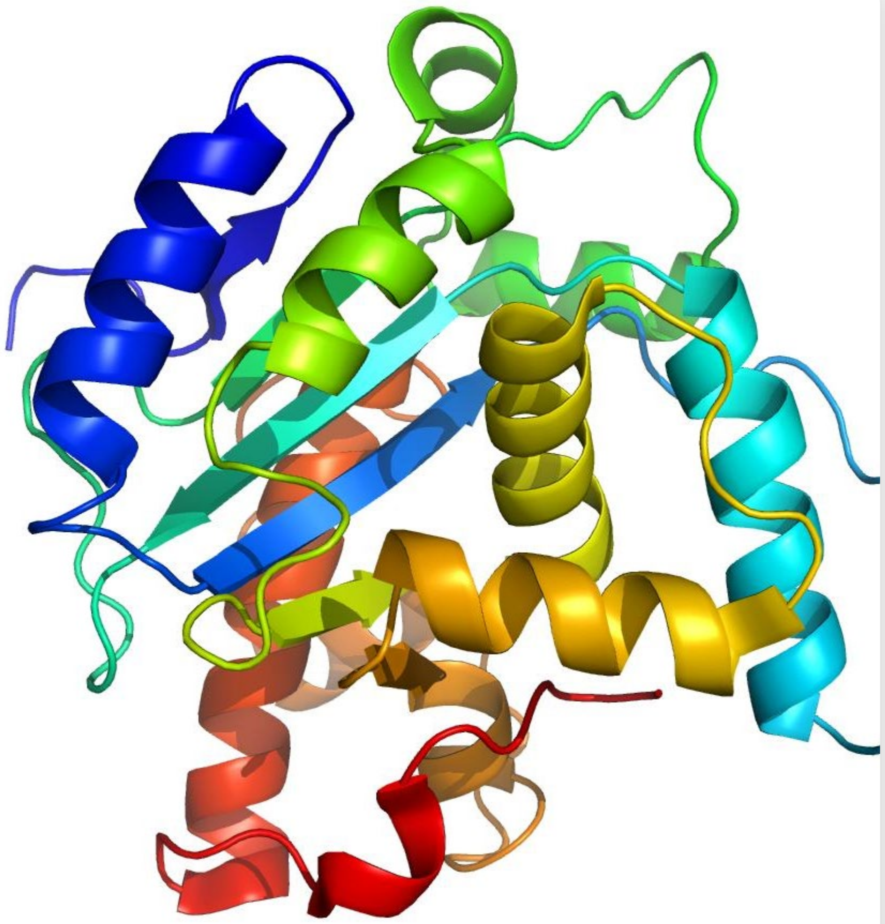
Enzyme activity remodeling

Precisely activate or inhibit key enzymes, modify their catalytic activity or substrate specificity, thereby effectively controlling the synthesis rate and molecular structure of the target product.

Source - Repository Stream Optimization

The system regulates the transportation and distribution efficiency of photosynthetic products, breaks the limitations of natural growth, and ensures that more nutrients are directed towards the edible parts.

Protein Quality Improvement



objective

The focus is on increasing the content of essential amino acids such as lysine and methionine in the seeds, while also knocking out the related genes to reduce the accumulation of anti-nutritional factors such as phytic acid.

Enhance endogenous synthesis

Optimize the translation process

Regulate the carbon-nitrogen ratio

Reduce anti-nutritional factors

Starch Quality Improvement

Objective

By precisely regulating the synthesis ratio of amylose and amylopectin, the processing adaptability and cooking taste quality of crops can be directionally improved.

Mechanism 1: Single gene knockout (Wx)

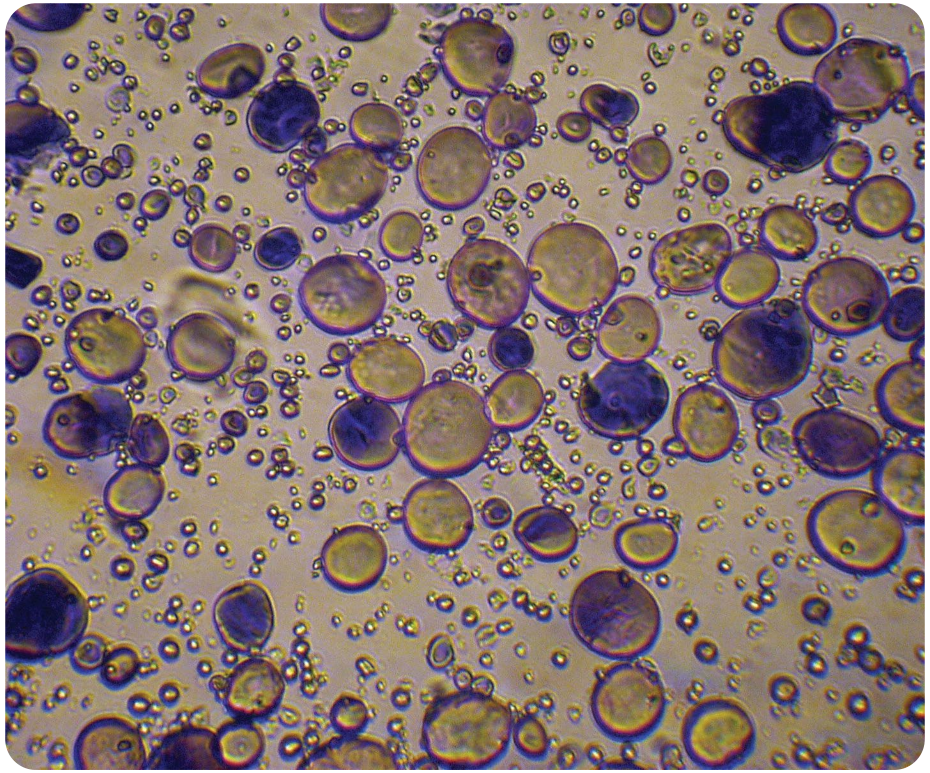
Specific knockout of the Waxy (Wx) gene blocks the amylose synthesis pathway, resulting in the development of superior varieties with high glutinousity and low gelatinization temperature.

Mechanism 2: Polygenic Combined Blockade

Simultaneous knockout of Wx and SSIIa genes synergistically regulates starch biosynthetic flux, creating novel germplasm resources with high resistant starch content.

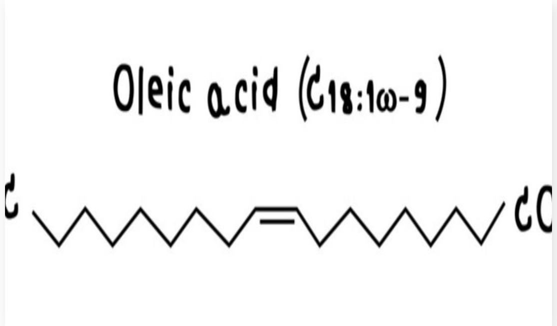
Mechanism 3: Fine-tuned Regulation of Gene Function

Partial retention of Wx gene activity enables precise modification of amylopectin chain length distribution characteristics, significantly improving the palatability and elasticity of rice.



Morphological Differences of Starch Granules in Rice Endosperm Observed Under Optical Microscopy

Oil Quality Improvement



A monounsaturated Omega-9 fatty acid with health benefits of reducing cardiovascular disease risk.

Objective

Through genetic engineering approaches, the oil content of crops can be significantly increased, and the fatty acid composition can be directionally optimized (e.g., high oleic acid content).

Mechanism 1: Increasing oil yield

Overexpression of rate-limiting enzymes in lipid synthesis pathways (e.g., DGAT1) or key transcription factors (e.g., WRI1) can overcome synthetic bottlenecks.

Mechanism 2: Optimization of fatty acid composition

Specific knockout of the FAD2 gene blocks the conversion of oleic acid to linoleic acid, significantly increasing oleic acid content.

Mechanism 3: Creation of novel functional traits

Knockout of DGAT and PDAT genes enables oilseed crops to accumulate healthier diacylglycerol (DAG).

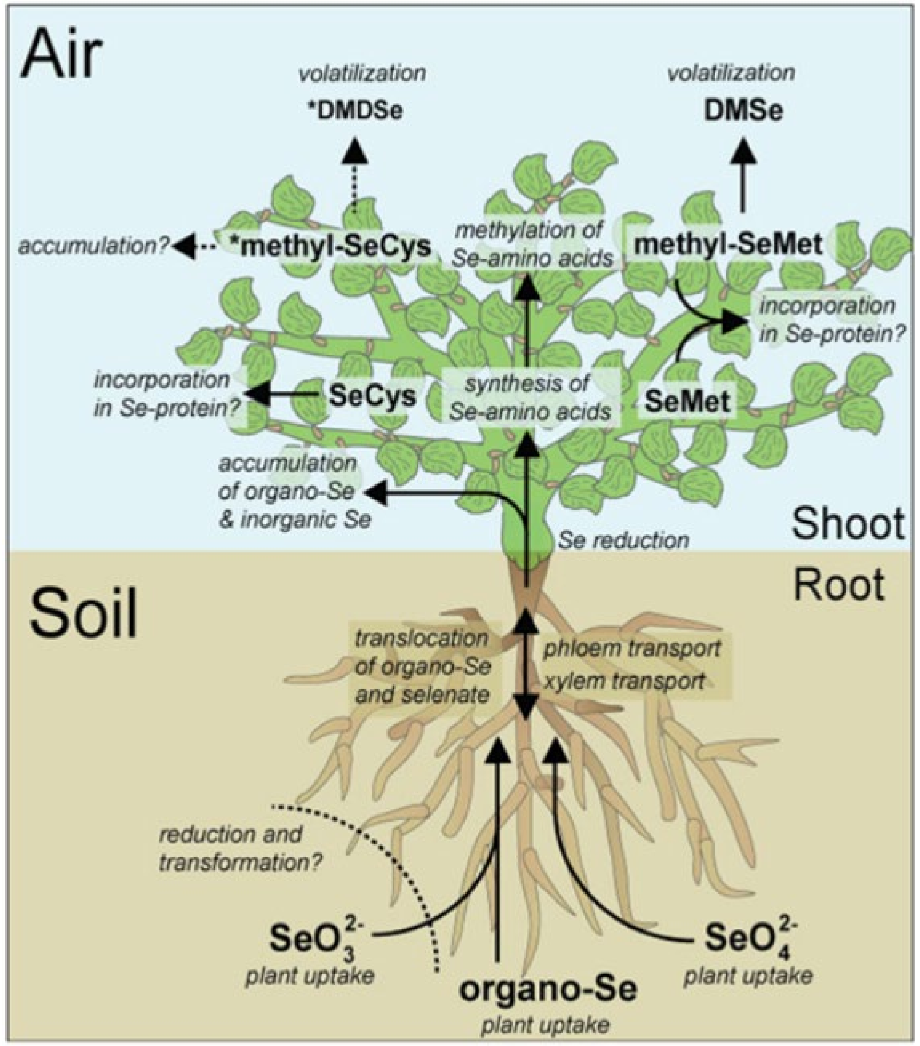
2 Core Molecular Principles of Biofortification

- **Micronutrient Uptake and Transmembrane Transport**
- **Micronutrient Chelation and Targeted Storage**
- **Enhancement of Micronutrient Bioavailability**

2.1 Micronutrient Uptake and Transmembrane Transport

This is the **fundamental step** **of biological enhancement.**

The core lies in the fact that crops absorb minerals from the environment through their roots, leaves, etc., and achieve transmembrane transport and long-distance distribution through specific transporter protein families, which determines the "entry efficiency" of nutrients into the crops.



2.1.1 Whole-Process Molecular Regulatory Pathway

Soil mineral dissolution and activation
(Root zone organic acids / iron carriers)

Absorption by root hair cells
(mediated by transport proteins)

Intracellular short-distance transport
(Intermediate junctions)

Long-distance transportation of wood
(Travels upward through the vessels)

Phloem redistribution
(transverse transfer of sieve tubes)

Granule cell storage
(vesicle/probody)

2.1.2 Core Transporter Protein Families and Functions

Core nutrients	Key transport protein family	Core function	Typical control strategy
iron（Fe）	YSL、NRAMP、IRT1	YSL mediates the chelation of iron by nicotianamine for long-distance transport; NRAMP/IRT1 enables the transmembrane absorption of Fe^{2+} in the root epidermis.	Overexpression of OsYSL2, TaNRAMP5 and AtIRT1 enhances iron absorption and transport
zinc (Zn)	ZIP、HMA	ZIP is responsible for the absorption of Zn^{2+} in the root epidermis and its intracellular transport; HMA mediates the loading of zinc into the seeds.	Overexpression of OsZIP4/5 and ZRT1/2; knockout of HMA2/4 reduces zinc transport to non-edible parts
selenium（Se）	SULTR、APRT	By taking advantage of the similarity in sulfur-seleventh metabolism, SULTR transports selenate; APRT is involved in the synthesis of selenomethionine.	Overexpression of SULTR1;2 enhances the crop's absorption efficiency of selenium
iodine（I）	NIS、SLC家族	NIS mediates the transmembrane absorption of iodide ions, and SLC is involved in the intracellular transport of iodine.	Overexpression of the animal NIS gene enhances the iodine accumulation capacity of crops

2.1.3 Synergistic and Antagonistic Molecular Effects

- **Zinc-cadmium antagonism:** Zinc (Zn) and cadmium (Cd) share the absorption channels of the ZIP transporter protein family. By improving the crop's absorption efficiency of zinc, it can competitively inhibit the absorption of cadmium, effectively reducing the accumulation of heavy metal cadmium in the grains.
- **Iron-arsenic antagonism:** Iron absorption promotes the formation of iron membranes on the root surface to adsorb arsenite; at the same time, the crop preferentially expresses YSL family transporters to absorb iron, blocking the absorption of arsenic at the molecular level.
- **Selenium-metalloid synergistic detoxification:** Selenium combines with cadmium, mercury, etc. to form stable complexes (such as selenium proteins), and segregates them into the vacuoles to reduce the toxicity of heavy metals, while increasing the selenium content in the crop.

2.2 Molecular Regulation of Nutrient Chelation and Storage

Core chelating agent system——The "molecular transporters" of minerals

Targeted storage mechanism——The "directional warehouse" of minerals

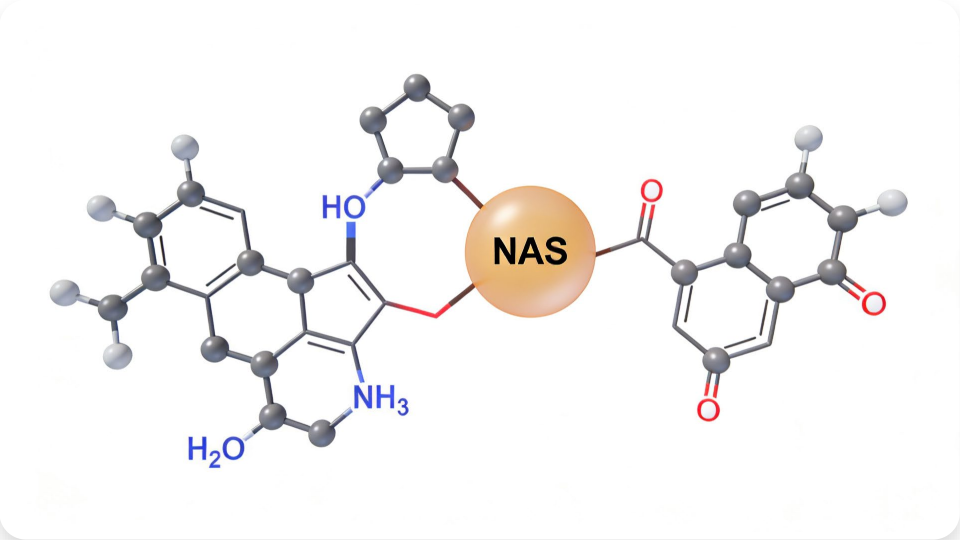
Enrichment strategy——Endosperm-specific promoter

Case Study Analysis——The Application of the AtVIT1 Gene in Cassava

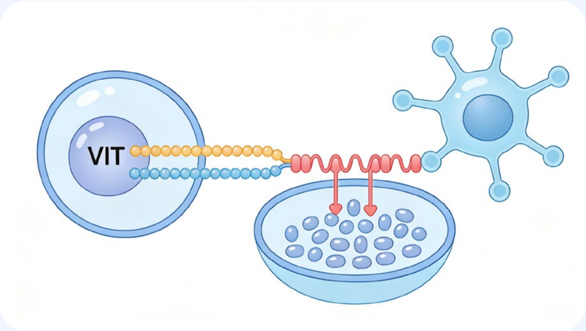
Core chelating agent system

Key molecule：

- **Nicotinamide (NA):** One of the most important metal ion chelators in plants, it is crucial for the transport of elements such as iron and zinc.
- **Plant chelating peptides (PCs):** They mainly participate in the detoxification of heavy metals and the maintenance of their homeostasis, ensuring the safety of cells.
- **Metallothioneins (MTs):** Rich in cysteine, they can efficiently bind various metal ions and maintain balance.
- **Organic acids:** such as citric acid and malic acid, can chelate metal ions in the soil and facilitate the absorption by plants.

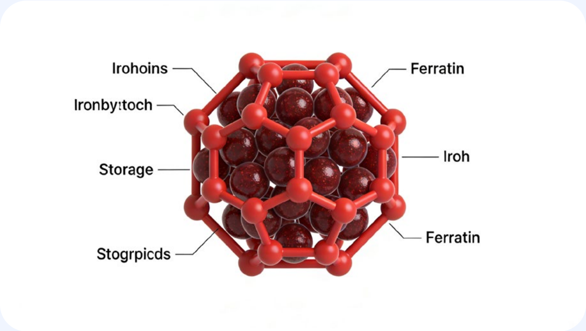


Targeted Storage Mechanism



Vacuole Storage Mechanism

Dependent protein: proteins of the VIT (Vacuolar Iron Transporter) family.
Mechanism of action:VIT proteins are located on the vacuolar membrane and transport metal ions from the cytoplasm into the vacuole for compartmentalized storage, thereby effectively preventing metal ions from exerting toxic effects on cells.



Protein Binding Storage Mechanism

Representative protein: Ferritin.
Mechanism of action:Ferritin forms a hollow spherical structure that can store thousands of iron ions inside, making it one of the main ways for plants to safely store iron.

These two mechanisms work synergistically to construct a "safe storage network" for minerals in plants, ensuring their requirements for growth and development.

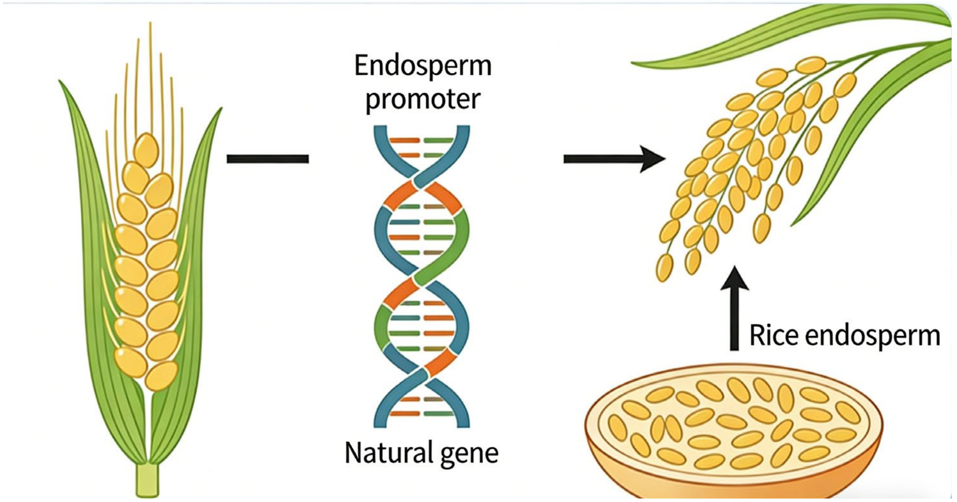
Enrichment Strategy — Endosperm-Specific Promoter

Core Strategy

By using **endosperm-specific promoters** to drive the expression of chelation or storage-related genes, the targeted enrichment of minerals is achieved.

Mechanism of action

Promoters serve as the "switches" for gene expression. Endosperm-specific promoters ensure that target genes are **highly expressed exclusively in the endosperm of seeds**, thereby enabling the precise enrichment of minerals in edible tissues and avoiding nutrient waste.



The process of gene expression driven by endosperm-specific promoters

Key Value: Improve biofortification efficiency and enable "precise delivery" of crop nutrients to the edible parts for humans.

Classic Case Analysis — Application of the AtVIT1 Gene in Cassava

Case Background

As an important staple food crop worldwide, cassava has a naturally low iron content in its storage roots, which can hardly meet human nutritional requirements. Therefore, improvement through biofortification is urgently needed.

Core Strategy

By genetic engineering technology, the Arabidopsis AtVIT1 gene was specifically overexpressed in cassava storage roots to regulate the iron transport pathway.

Improvement Effects

It significantly enhances the capacity of iron storage in the vacuoles of cassava storage roots, increasing the iron content in cassava storage roots by **3-7 folds.**

References: Narayanan, J., Gaitan-Solís, E., Chauhan, R., et al. Biofortification of field-grown cassava by engineering expression of an iron transporter and ferritin. Nature Biotechnology, 2019, 37(2): 142-150.

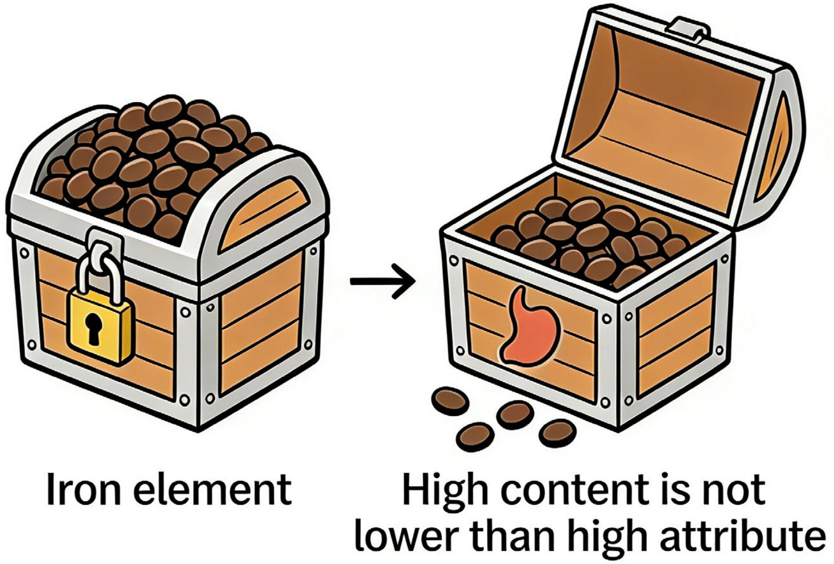
2.3 Improving the Bioavailability of Micronutrients

Core Definition

It refers to the degree to which nutrients in food are **digested**, **absorbed** and **utilized** by the human body. It is the ultimate criterion for measuring the success of biofortification.

Key Insight: Content ≠ Value

Even if the mineral content of crops is high, if their bioavailability is low, the human body still cannot obtain the nutrients. **Improving bioavailability** is a key link in biofortification.



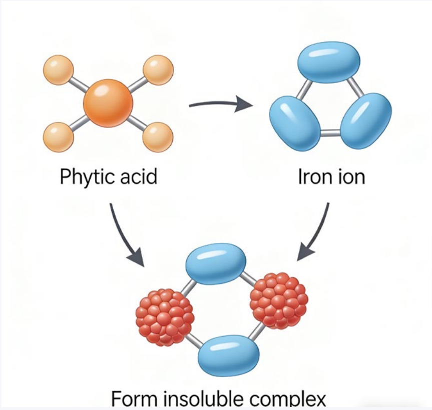
Comparison of bioavailability (difference in nutrient utilization)

Conclusion: Biofortification emphasizes not only the nutrient content, but also its bioavailability.

Strategy 1: Reduce the Synthesis of Anti-Nutritional Factors

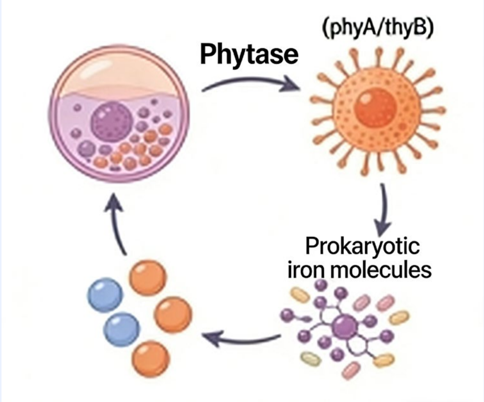
Problem: Obstacles Caused by Anti-Nutritional Factors

Anti-nutritional factors (such as phytic acid and polyphenols) can form insoluble complexes with minerals (iron, zinc), hindering their absorption. For example, phytic acid can form precipitates with Fe^{3+} and Zn^{2+}, making them unavailable for utilization by the human body.



Solution: Gene Editing and Genetic Engineering Technologies

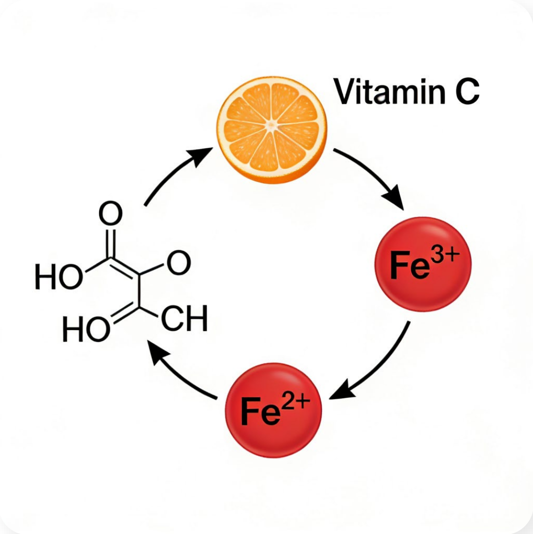
Knockout of key enzyme genes for synthesis (such as IPK1) or heterologous expression of degrading enzyme genes (such as phyA and phyB) can reduce the phytic acid content by more than 60%, releasing more free iron and zinc for human absorption.



Strategy 2: Enhance the Synthesis of Promoting Factors

Core Mechanism: The Role of Vitamin C

Vitamin C is a key promoting factor. It can reduce the poorly absorbable trivalent iron (Fe^{3+}) into the easily absorbable divalent iron (Fe^{2+}) in the human body, thereby significantly improving the bioavailability of iron.



Other Key Promoting Factors

β-carotene

It exerts an antioxidant effect and synergistically promotes the absorption efficiency of minerals.

Specific amino acids (e.g., cysteine)

stabilize mineral ions through chelation, reduce precipitation, and improve intestinal absorption.

Conclusion: Precise Regulation

- **Improve absorption:** Overexpress high-affinity transporter genes to enhance the environmental uptake of minerals by crops;
- **Optimize transport:** Overexpress chelator synthesis genes (e.g., NAS) to achieve stable long-distance transport of minerals;
- **Targeted enrichment:** Use tissue-specific promoters to drive the expression of nutrient genes in edible parts and realize targeted storage;
- **Improve bioavailability:** Reduce anti-nutritional factors + strengthen promoting factors to achieve the dual goal of "high content + high bioavailability".

3 Three Core Technical Strategies

3 Three Core Technical Strategies

Agronomic Practices

Through agronomic measures such as optimized fertilization management and improved soil conditions, the micronutrient content in edible parts of crops can be directly increased.

It is characterized by rapid effects, but the efficacy depends on external inputs, resulting in relatively weak sustainability.

Plant Breeding

Using the natural genetic variation of crops, excellent biofortified varieties are developed through traditional breeding methods such as hybridization and backcrossing, or combined with technologies such as molecular marker-assisted selection (MAS).

It is characterized by a relatively long breeding cycle, but strong sustainability once the varieties are developed.

Genetic Engineering

Through transgenic technology or genome editing technologies such as CRISPR/Cas9, the expression of target genes is precisely regulated to directionally improve the nutritional quality of crops.

It is characterized by accuracy and high efficiency, but faces regulatory challenges.

3.1 Agronomic Practices

Core Principle

Through agronomic measures such as soil fertilization and foliar application, minerals required by crops are directly supplied, thereby increasing their accumulation in edible parts.

Key Technologies

- **Foliar application**: Micronutrient fertilizers are sprayed on leaves at critical stages (flowering / grain-filling stage), with fast absorption and rapid effects.
- **Soil application**: Fertilizers are applied to soil and absorbed by roots; suitable for macronutrients and affected by soil conditions.
- **Microbial assistance**: Microorganisms such as PGPR are used to mobilize soil minerals and promote uptake by crops.

Case and Limitation

Successful Cases

- **Finland Selenium Biofortification Program:** Through national foliar application of selenium fertilizer on wheat, the selenium content in wheat was significantly increased, effectively improving the selenium nutritional status of the population.
- **Iodine Biofortification in Rice (China):** Foliar application of potassium iodide successfully increased iodine content in rice, providing a new strategy for the prevention of iodine deficiency disorders.


Limitation Analysis

- **Economic burden:** Annual repeated investment is required, which imposes great cost pressure on small-scale farmers.
- **Effect stability:** Significantly affected by environmental conditions (such as climate and soil).
- **Environmental risks:** Excessive fertilization may lead to soil or water pollution.

Summary
Agronomic biofortification is an effective approach to improve micronutrient status, but it must be balanced with economic costs and environmental benefits. It is recommended as a short-term auxiliary strategy.

3.2 Plant Breeding

01 Contenvional breeding

Utilizing the naturally occurring genetic variations, through traditional methods such as hybridization, backcrossing and systematic selection, new varieties with enhanced nutrition are cultivated.

02 Mutation breeding

Induce genetic mutations through physical rays or chemical mutagens, artificially create new sources of variation, and rapidly screen for high-nutrition mutants.

Conventional Breeding

Core definition: By leveraging the naturally occurring genetic variations, through traditional techniques such as **hybridization, pedigree selection, and germplasm screening**, without relying on genetic engineering, genes that control the enrichment of trace nutrients are introduced into superior varieties, thereby enhancing the nutritional value of crops.

01 Germplasm screening

Search for donor parents that are rich in the target nutrients from local varieties or wild germplasm.

02 Hybridization recombination

Hybridize high-nutrition donors with high-yield high-quality recipients to recombine excellent genetic traits

03 screening and identification

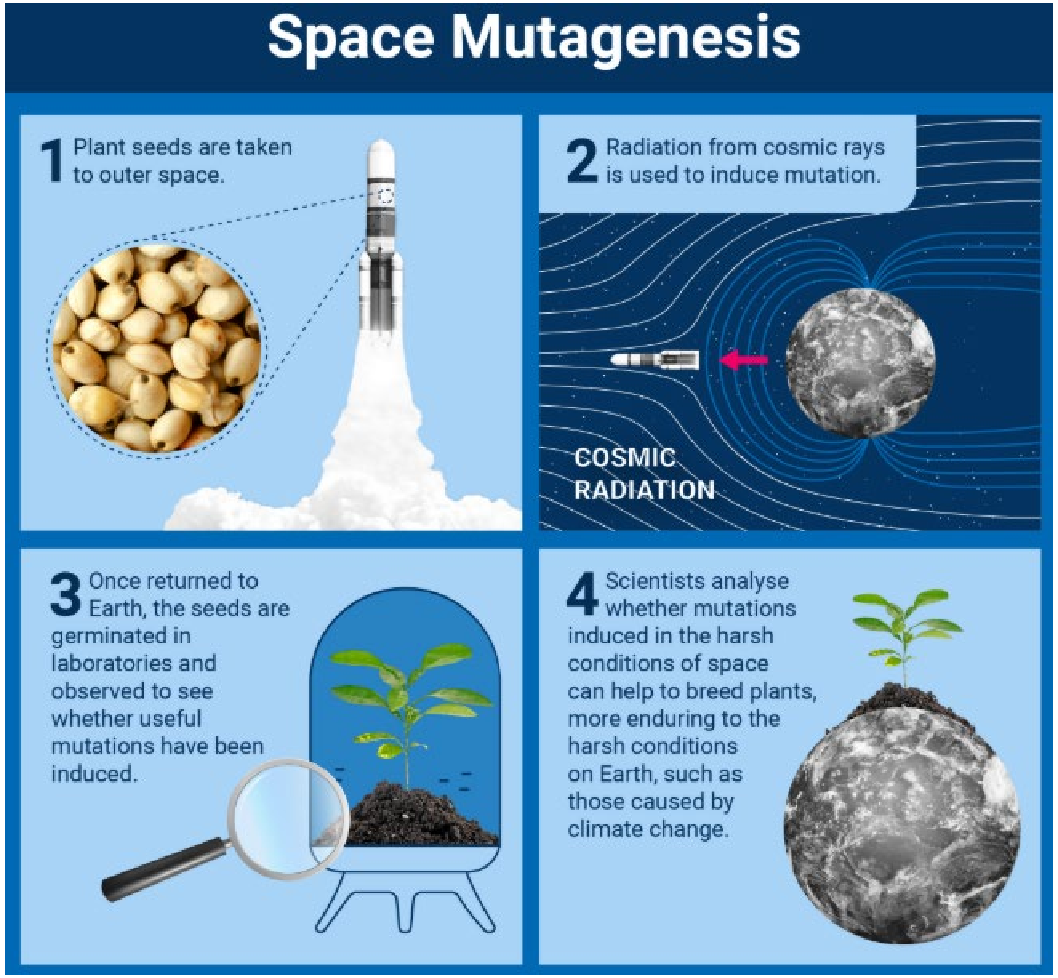
Multi-generation backcrossing and combination of phenotypic or molecular markers for screening of stable and superior strains

04 regional trials

Carry out multi-site trials to verify the adaptability and stability of the new variety, and then officially promote it.

Core advantage: Does not rely on genetic engineering. Utilizes natural genetic resources to safely improve the nutritional quality of crops.

Mutation Breeding



Core principle: Mutagenesis and screening

By using **gamma rays/X-rays (physics) or EMS (chemistry)** to induce random mutations in the genome, and through multiple generations of screening and identification, a stable and heritable nutrition-enhanced new strain was obtained.

Types

- Physical mutagenesis
- Chemical mutagenesis
- Polyploid mutagenesis
- Astronautics-based breeding

Comparison of Two Breeding Techniques

Contenvional breeding

✦ Advantage

Regulatory-friendly, with high consumer acceptance and a mature technical system

✦ limitation

The breeding cycle is long (8-10 years) and relies on natural variation resources.

Mutation breeding

✦ Advantage

Create completely new variant types without the need for genetic modification regulatory procedures

✦ limitation

The mutation direction is random, requires extensive screening, and may be accompanied by negative effects.

Achievement Display

Zincol

Through traditional breeding and MAS technology, the zinc content in grains is significantly increased, showing an obvious effect of biofortification.

Iron-biofortified Rice （IR68144）

Through marker-assisted selection technology, high-iron content varieties have been successfully bred, which effectively addresses iron deficiency.

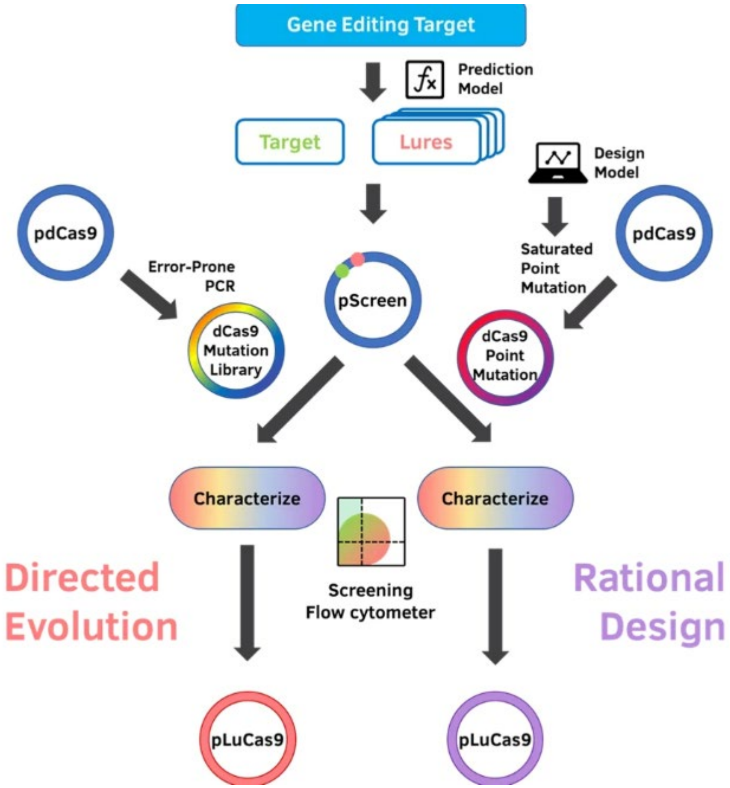
Provitamin A Maize

Aggregate specific alleles to significantly increase the β-carotene content in grains, which can be converted into vitamin A.

Achievement Summary: Biofortified varieties not only increase micronutrient content but also maintain excellent agronomic traits, with great promotion value.

3.3 Genetic Engineering

Technology Type	Core Definition	Key Features
Genetic Engineering	The introduction of exogenous genes into the genome of recipient organisms to make them express new traits	Introduction of exogenous DNA May produce GMOs
Genome Editing	Precise modification of the organism's own genome (knockout/knock-in)	No introduction of exogenous genes Modification of own sequences



Core differences:Genetic engineering focuses on introducing foreign genes to obtain new functions, whereas genome editing emphasizes precise modification of the organism's own genes, with higher accuracy and biosafety.

Main Methods of Genetic Engineering Fortification

single-gene overexpression

Introduce the target gene driven by a strong promoter to directionally increase the content of a single nutrient, such as iron, zinc, etc.

multigene co-expression

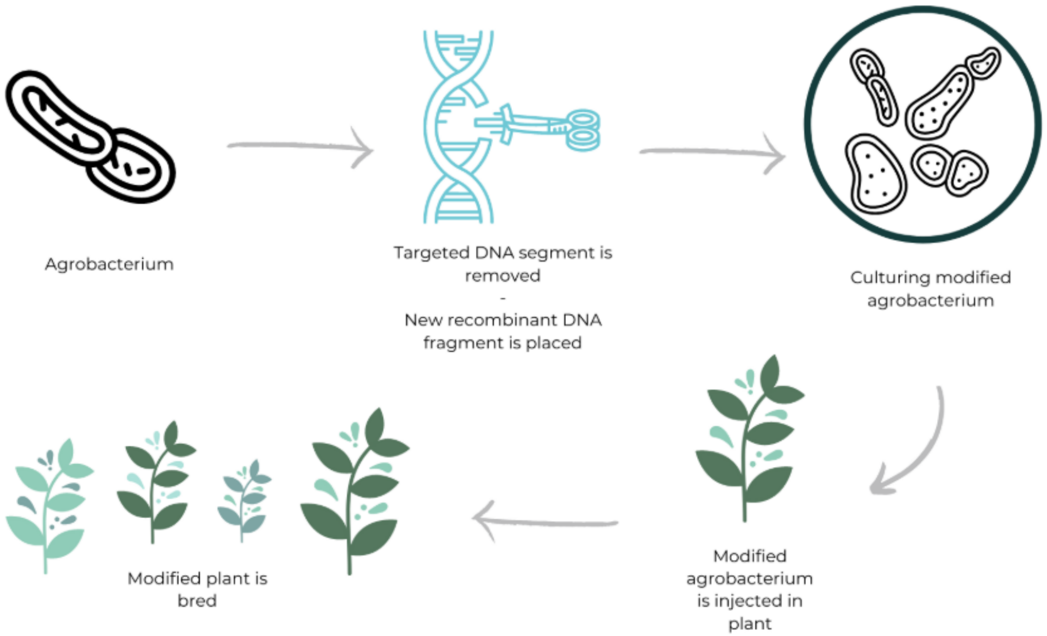
Construct a gene cassette to simultaneously introduce multiple synergistically acting genes, thereby enhancing the efficiency of the overall nutrient metabolic pathway.

gene silencing (RNA interference, RNAi)

Construct a gene cassette to simultaneously introduce multiple synergistically acting genes, thereby enhancing the efficiency of the overall nutrient metabolic pathway.

metabolic pathway reconstruction

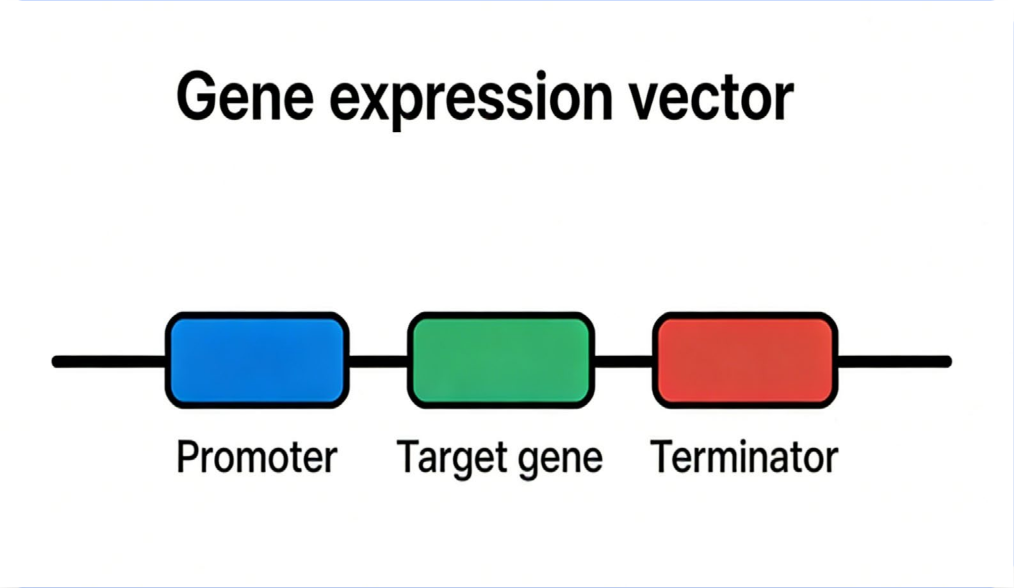
Introduce the complete exogenous metabolic pathway genes to synthesize new nutrients that do not originally exist in crops.



Genetic Engineering Enhancement: Core Principles and Advantages

Core Principle

Through methods such as Agrobacterium-mediated transformation or biolistic bombardment, the expression vector carrying the target gene is introduced into plant cells. After integration into the genome, the gene is stably expressed, thereby enhancing the absorption, transportation and storage of nutrients, or promoting the synthesis of new nutrients.



Schematic diagram of the expression vector structure (promoter–target gene–terminator)

Advantages of Application

- Significant effect
- High specificity
- Cross-species utilization
- polygenic superposition

Genetic Engineering Enhancement: Classic Case – Iron-fortified Rice

Case name: NFP/HIP Rice

Core Principle: A Comprehensive Enhancement System for Iron Metabolism

- **AtIRT1**： Enhance the iron uptake ability of roots
- **AtNAS1**： Promote the chelation and long-distance transport of iron.
- **PvFER**： Enhance the stable storage of iron in grains.

Application Effects

The iron content in polished rice is increased to **6–7 times** that of ordinary rice, and its bioavailability is significantly improved.

Boonyaves K, Wu T-Y, Gruissem W and Bhullar NK (2017) Enhanced Grain Iron Levels in Rice Expressing an IRON-REGULATED METAL TRANSPORTER, NICOTIANAMINE SYNTHASE, and FERRITIN Gene Cassette. Front. Plant Sci. 8:130.

Genetic Engineering Enhancement: Other Cases

Golden Rice

By introducing the bacterial psy and crtI genes, β-carotene (a precursor of vitamin A) is synthesized in rice endosperm, effectively addressing vitamin A deficiency in developing countries.

High-iron Soybean

Introducing the ferritin gene from Phaseolus vulgaris significantly increased the iron content in soybean seeds by approximately **40%**, providing a new food source to alleviate iron deficiency anemia.

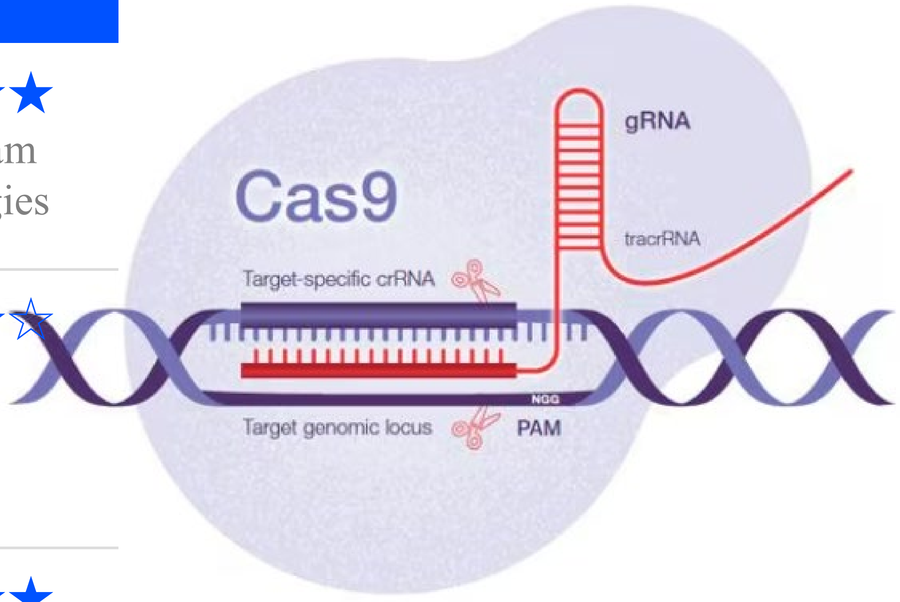
Visual comparison between Golden Rice (right) and ordinary rice (left).

Core value: Genetic engineering technology can precisely improve specific nutritional components of crops, providing a powerful solution for global food security and public health.

1. Ye X, Al-Babili S, Kloti A, et al. (2000) Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287(5451): 303–305.
2. Tang, G., Qin, J., Dolnikowski, G.G., Russell, R.M., Grusak, M.A. (2009) Golden Rice is an effective source for vitamin A. American Journal of Clinical Nutrition. 89:1776-1783.

Genome Editing Enhancement: Major Technical Systems

Technology	Core Components	Mechanism of Action	Precision
CRISPR-Cas9	Cas9 nuclease + sgRNA	Targeted DNA cleavage to induce double-strand breaks (DSBs)	★★★★★ Mainstream Technologies
CRISPR-Cas12a（Cpf1）	Cpf1nuclease+crRNA	Generates sticky ends upon cleavage; high efficiency for **multi-target editing**	★★★★☆
Base Editing(BE)	Catalytic domain-fused Cas9 nickase	Direct base conversion without DSBs	★★★★★ Exogenous DNA-free
Prime Editing(PE)	Cas9 nickase + reverse transcriptase + pegRNA	Precise **insertion and replacement** without donor DNA	★★★★★ Highest precision



Schematic diagram of the principle of CRISPR-Cas9 technology.

Genome Editing Enhancement: Core Editing Strategies

Gene knockout

Targeted cleavage of negative regulatory genes, inducing frameshift mutations via NHEJ, thereby relieving the inhibition of nutrient accumulation.

Gene knock-in / replacement

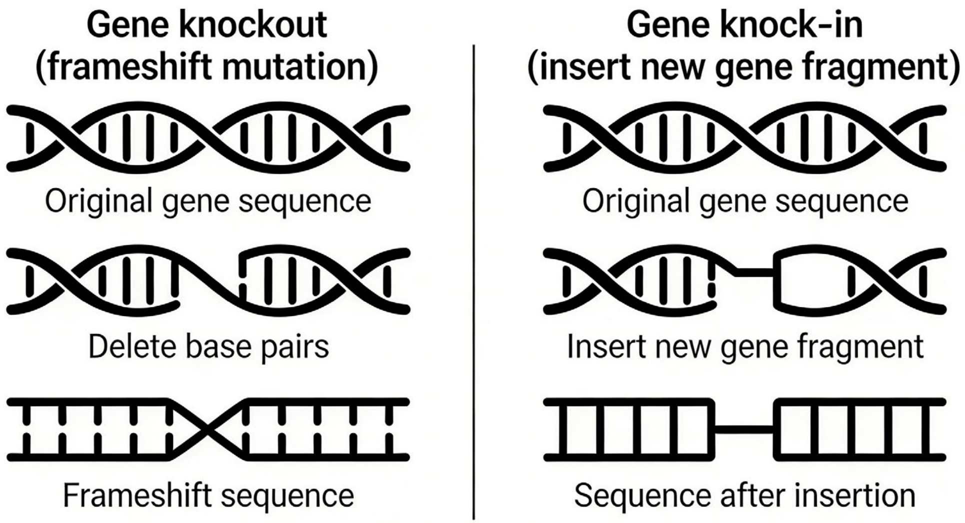
After cleavage, donor DNA is introduced via HDR to replace the target gene, thus improving nutrition-related genes.

Multigene editing

Construct multi-target sgRNA, edit multiple genes simultaneously, and synergistically improve multiple nutrients.

Promoter editing

Modify the gene promoter region, alter expression level, and enhance the expression of endogenous nutrition-related genes.



knock-in.

Technical Application Value

Through these precise editing strategies, crop traits can be improved rapidly and directionally, showing great potential in increasing yield, enhancing stress resistance, and improving nutritional quality.

Genome Editing Enhancement: Application Advantages

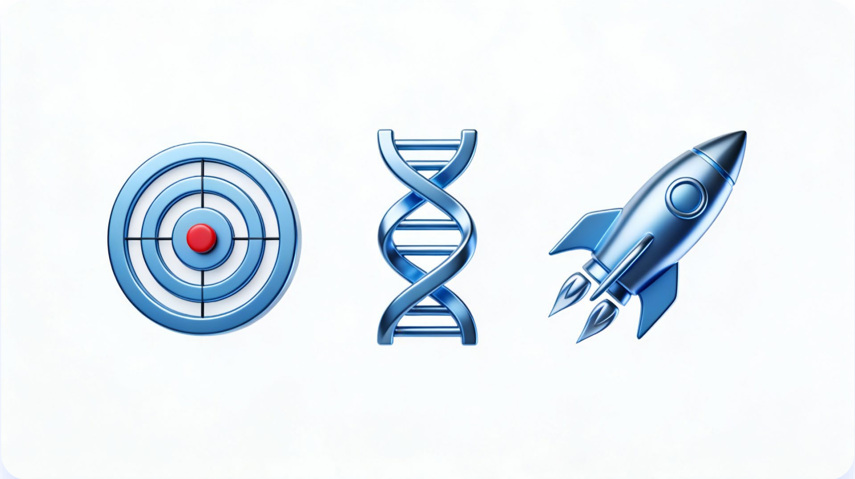
Extremely high precision
Targets specific gene loci with almost no off-target effects, ensuring editing accuracy.

No exogenous gene residue
Plants without exogenous DNA can be obtained, which may evade transgenic regulation and have higher safety.

Modification of endogenous genes
By optimizing the plant's own genetic resources, it is more easily accepted by the market and the public.

Efficient multigene editing
Multiple genes can be edited simultaneously in one step, greatly improving breeding efficiency and accelerating the process.

Shorter cycle
The breeding cycle is significantly shortened from the traditional 5–10 years to 2–3 years, seizing market opportunities.



Core Value

Precision · Safety · Efficiency · Spee
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Genome Editing Enhancement: Classic Case - High-Iron, Low-Cadmium Rice

Core Strategy: Synergistic Editing of Multiple Genes

- **Knockout of OsVIT2:** Knock out the negative regulator of iron storage to reduce iron retention in roots.
- **Knockout of OsNRAMP5**: Block the cadmium uptake and transport protein to significantly reduce cadmium accumulation.
- **Modification of OsNAS2**: Enhance promoter activity to facilitate efficient iron transport into grains.

Improvement Effect: Dual Enhancement of Nutrition and Safety

Grain iron content	Grain zinc content	Cadmium content
3 times higher	2 times higher	90% lower

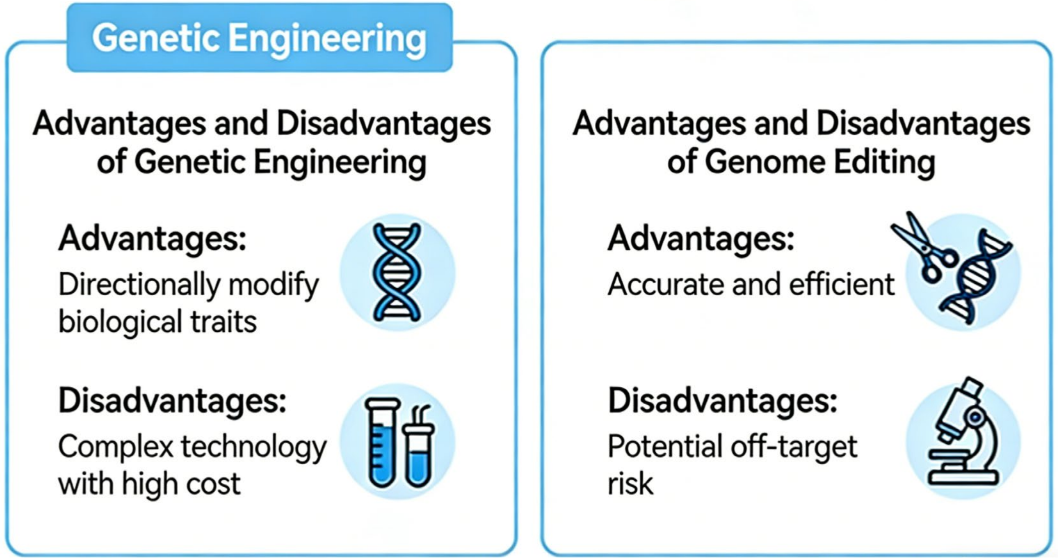
A " kill two birds with one stone" breakthrough:

It not only solves the problem of micronutrient deficiency but also tackles the issue of heavy metal pollution.

1. Tang et al. (2017) CRISPR/Cas9-Mediated Improvement of Major Rice Variety TBR225 for Low Cadmium Accumulation. Plant Breeding and Biotechnology.
2. Wang et al. (2019) Characterization and Evaluation of OsLCT1 and OsNramp5 Mutants Generated Through CRISPR/Cas9-Mediated Mutagenesis for Breeding Low Cd Rice. Rice Science.

Comparison and Selection Guide of the Two Technologies

Comparison Dimension	Genetic Engineering	Genome Editing	Recommended Application Scenarios
Exogenous gene	Must be introduced	Optional (can be avoided)	Synthesis of new substances → Genetic EngineeringTrait improvement → Editing
Accuracy	Low (random integration)	Extremely high (targeted)	Precise regulation requiredPriority: Genome Editing
Regulatory status	Strictly regulated worldwide	Exempt in some countries	Considering market acceptancePriority: Genome Editing
Technical difficulty	Medium (vector construction)	Relatively high (efficiency optimization)	Basic research → Genetic EngineeringCommercialization → Editing
Time cost	Long (5–10 years)	Short (2–3 years)	Rapid breeding demandPriority: Genome Editing



Core Differences Summary

Genetic engineering focuses on gene introduction, suitable for the synthesis of new substances. Genome editing emphasizes gene modification, with the advantages of precision, efficiency and regulatory friendliness, making it the mainstream approach for commercialization at present.

4 Core Challenges, Integrated Strategies and Future Frontiers

- **Core Challenges**

The issue of bioavailability of nutrients, the complexity of genotype by environment interaction (G×E), etc.

- **Integration Strategies**

Technical collaboration, nutrient synergy, and integrated full-chain management, etc.

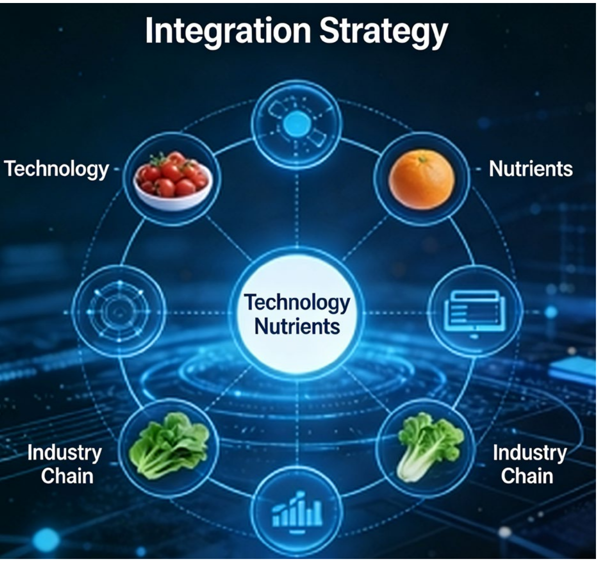
- **Future Frontiers**

Climate-smart biological enhancement, nanobiological enhancement technology, microbial group engineering-assisted biological enhancement, as well as the application of artificial intelligence and multi-omics in precision breeding.

Current Core Challenges

- ❖**Bioavailability bottleneck**: High content does not equal high absorption. How to improve the bioavailability of biofortified nutrients is a key challenge.
- ❖**Genotype-by-environment interaction (G×E)**: Nutritional traits exhibit instability under diverse soil and climatic conditions, which hinders the wide promotion of varieties.
- ❖**Regulation and public acceptance**: Transgenic and genome-edited crops are subject to strict regulation and public skepticism, resulting in slow commercialization.
- ❖**Accessibility for smallholder farmers**: High promotion costs and inadequate supporting services make it difficult for smallholder farmers to access high-quality seeds and technical guidance.

Cross-Technology Integration Strategies



❖ **Technology Synergy**: Combine genotype improvement with optimized agronomic practices (e.g., high-zinc varieties combined with precise zinc fertilization) to achieve a 1+1>2 synergistic effect.

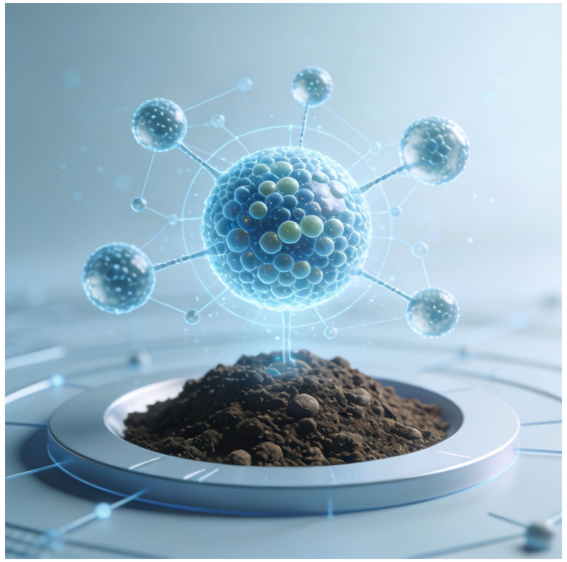
❖ **Nutrient Synergy**: Simultaneously biofortify multiple nutrients, with full consideration of interactions among elements, to effectively address complex malnutrition.

❖ **Whole Industry Chain Integration**: Establish a complete system ranging from soil health, crop biofortification, post-harvest processing to dietary matching, maximizing the benefits of biofortification.

Future Frontiers and Research Directions (I)

Climate-Smart Biofortification

To breed crop varieties with both high nutritional quality and climate change resilience (e.g., drought and high temperature tolerance), ensuring stable expression of nutritional traits under stressful conditions.



Nano-biofortification

By leveraging the high activity and slow-release properties of nano-fertilizers, the efficiency of mineral nutrient uptake by crops is improved, while fertilizer application rates and environmental risks are reduced.

Future Frontiers and Research Directions (II)

Microbiome Engineering

By regulating the microbial community in the plant rhizosphere and designing synthetic communities (SynComs), we can promote the absorption and utilization of minerals by crops, thereby achieving microbe-assisted biofortification.

Artificial Intelligence and Multi-omics

Using machine learning to integrate multi-omics data including genomics, transcriptomics, metabolomics, etc., to predict the interactions among genes, nutrition, and the environment, thereby accelerating the precise design and breeding of nutrient-enhanced crops.

Future Frontiers and Research Directions (III)

Orphan Crop Biofortification

In addition to staple food crops such as rice and wheat, future research should also focus on "orphan crops" including foxtail millet, sorghum, yam, and cassava. These crops possess important economic and cultural value in specific regions, yet often receive insufficient research investment. Biofortification of these crops is of great significance for improving local food security and nutritional status.

Millet

Sorghum

Yam

Conclusion

Biofortification is the most cost-effective and sustainable strategy to address hidden hunger worldwide.

It closely integrates agricultural production with public health and can benefit hundreds of millions of low-income people.

The biofortification technology toolkit has been largely established.

From conventional breeding to CRISPR gene editing, diverse technological approaches each have their own advantages and can be flexibly selected according to crop characteristics and regional needs.

Nutritional biofortification of a full range of crops is feasible.

Mature technical strategies and successful cases have been established for staple cereals, fruits and vegetables, as well as specialty crops.

The key to success lies in interdisciplinary collaboration

requires in-depth cooperation and coordination among plant breeders, molecular biologists, agronomists, nutritionists, policymakers, and farmers.

Crop	Improved method	Enhance metrics	Research Institution	Reference
rice	Overexpression of OsASN3	The total protein content of grains was significantly increased	CAS	Li et al., 2022
rice	Gene editing Waxy	Reduced amylose content and improved glutinous quality	CAAS	Sun et al., 2021
rice	Overexpression of DGAT1 + WRI1	Significant increase in grain oil content	China Rice Research Institute	Fan et al., 2023
corn	Edit the zein gene	Increased levels of lysine and tryptophan	Huazhong Agricultural University	Liu et al., 2022
corn	Overexpression of AGPase	Increase in starch content and thousand-grain weight	Purdue University, USA	Zhang et al., 2020

Crop	Improved method	Enhance metrics	Research Institution	Reference
wheat	CRISPR edit Waxy	Decreased amylose content leads to optimized noodle quality	Hebei Agricultural University	Wang et al., 2023
soybean	edit GmSWEET10a/b	Increased protein content and altered oil distribution	CAS	Chen et al., 2022
soybean	Polygenic knockout of DGAT/PDAT	DAG and lecithin content were significantly increased	Northeast Agricultural University	Zhao et al., 2024
barley	knockout Waxy + SSIIa	Decreased starch content with a significant increase in soluble sugars	Sichuan Agricultural University	Huang et al., 2023
Cassava	genetic engineering technology	Increasing the iron content in cassava storage roots by 3-7 folds	Donald Danforth Plant Science Center, USA & International Institutions	Narayanan J et al., 2019

Thanks!

Technology-based Breeding · Protecting the Future · Creating a New Era of Nutritional Health Together