



Regional Training Course on Efficient Screening Methods for Improved Nutritional  
Quality in Mutant Populations  
Cibinong, Indonesia, 13<sup>rd</sup>-17<sup>th</sup> April, 2026

# Integrating Nutritional/Quality Mutant Screening Results into Breeding Pipelines

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# **01 Introduction: The Importance of Mutant Screening in Breeding**




**Unlocking Genetic Potential for Global Food Security & Nutritional Challenges**




# Food Security & Nutritional Challenges


## Global Hunger Statistics (2024)

 ~673 million people (8.2% of global population) faced hunger, according to the 2025 SOFI report.


## The "Hidden Hunger" Crisis

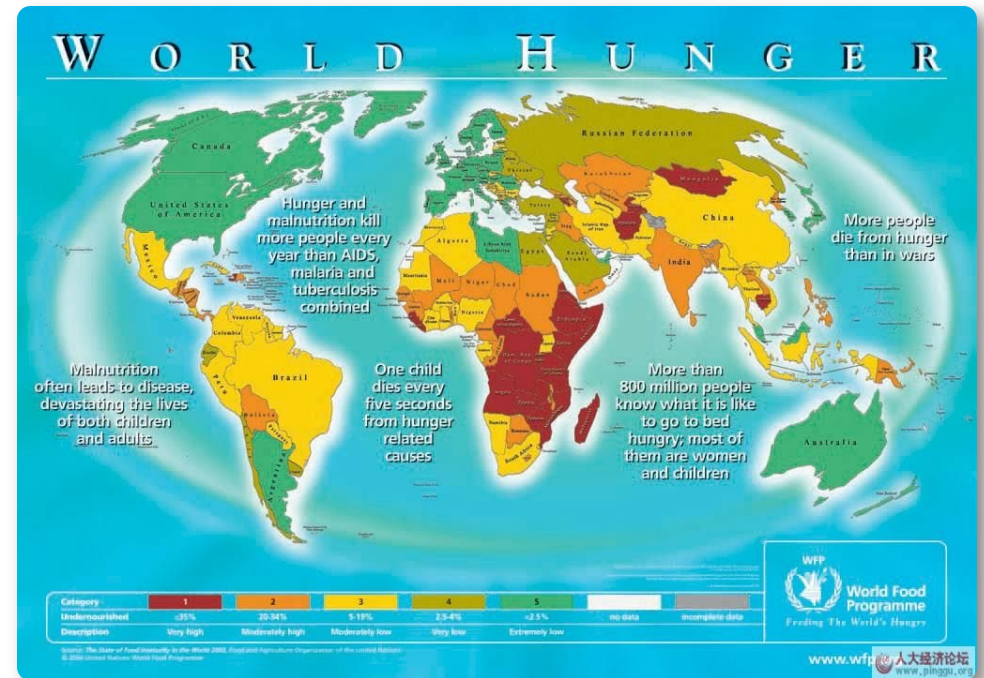
 Billions suffer from micronutrient deficiencies, leading to severe health issues despite caloric intake.

## Limitations of Traditional Breeding

 Slow and inefficient processes struggle to meet the urgent demand for more nutritious food.

## Need for Mutant Screening

 A powerful approach to create novel genetic variations and enhance crop nutritional quality.



World Hunger Distribution Map (Source: WFP)

# What are Nutritional Quality Mutants Screening?

## Definition

Creating new genetic variations (mutations) in crop genomes through physical (e.g.,  $\gamma$ -rays), chemical (e.g., EMS) mutagenesis, or gene editing technologies to improve nutritional components (e.g., proteins, vitamins, minerals, starch).

## Core Logic: Forward Genetics

Starting from an observed desirable phenotype (e.g., higher vitamin content), work backwards to find the gene controlling the trait, laying the foundation for subsequent precision breeding.



## Core Advantage: Creating New Genes

It can create new alleles that do not exist or are rare in nature, providing entirely new genetic resources for nutritional quality improvement.



## Core Advantage: Breaking Bottlenecks

It can break the genetic limitations of traditional breeding and obtain breakthrough desirable traits.



## Summary in One Sentence

Mutant screening is the proactive, large-scale creation of genetic diversity, from which we then select the 'superstar' genes we desire.

# Why Screen for Nutritional Quality Mutants?



## Meet Growing Nutritional Needs

Address global population growth and dietary structure improvement.



## Solve "Hidden Hunger"

Supplement micronutrients such as vitamin A, iron, and zinc.



## Enhance Market Competitiveness

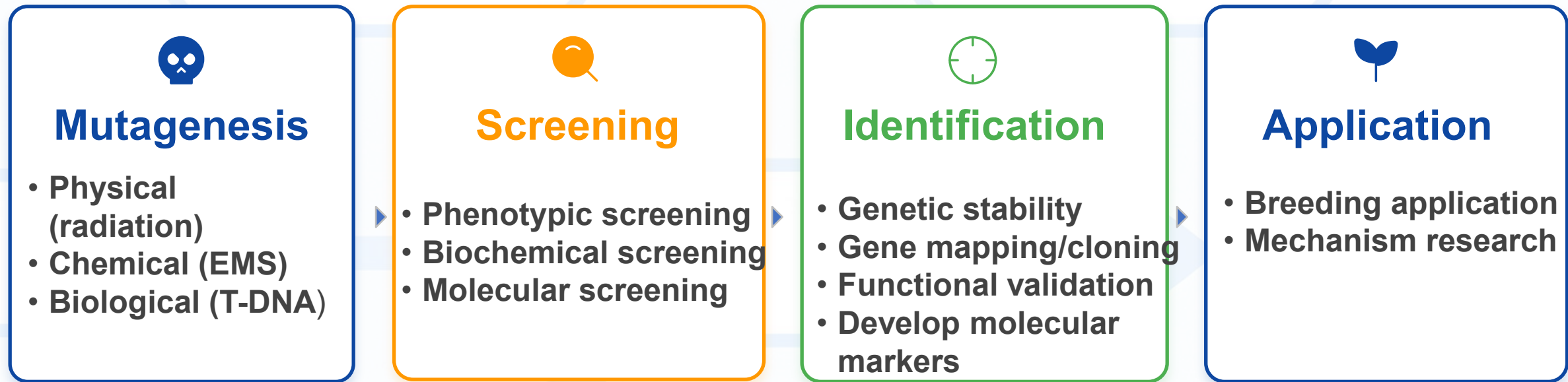
Develop functional foods and increase economic value of agricultural products.



## Fundamental Research Value

Elucidate the molecular mechanisms of nutrient metabolic pathways.

# Basic Workflow of Nutritional/Quality Mutant Screening



Through a systematic mutagenesis and screening process, target genes can be precisely identified from massive variations, supporting agricultural production and scientific research.

# 02 Identification and Evaluation of Mutant Screening Results

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Phenotypic Identification · Molecular Identification · Genetic Stability Evaluation



# Phenotypic Identification

## Morphological Identification

Observing changes in plant architecture, leaf shape, grain color, luster, and other visible structural traits.

## Physiological & Biochemical Identification

Measuring traits like photosynthetic rate, stress resistance, enzyme activity, and nutrient content (protein, vitamins, minerals).

## Field Evaluation

Assessing agronomic performance under different environmental conditions to ensure stability and real-world benefits.

**Modern phenomics platforms allow for high-throughput and precise phenotyping, making this process more efficient than ever.**



Modern Phenomics Platform

# Molecular Identification for mutants with unknown genes

## Linkage Analysis

**For mutants with unknown genes,** construct a genetic population (e.g., F<sub>2</sub>/RIL) and use molecular markers for QTL mapping to locate the trait-linked region.

## Gene Cloning

Isolate and identify the key gene controlling the target trait from the mapped QTL region using strategies like map-based cloning or BSA-seq, MutMap.

## Sequence Verification

Sequence the candidate gene and compare DNA differences between mutant and wild type to pinpoint the mutation site (e.g., SNP, Indel).

## Functional Validation

Validate gene function via complementation tests (rescue phenotype) or knockout/overexpression assays to confirm the causal gene.

# Molecular Identification for mutants with known genes

## PCR Detection

**For mutants with known genes**, PCR amplification is performed based on molecular markers (e.g., SSR, InDel, CAPS, KASP) genetically linked to the mutant genes to rapidly identify the presence of specific mutations or genes.

## DNA Sequencing

Uses Sanger Sequencing or next Generation Sequencing (NGS) to determine the exact mutation site and type (e.g., SNP, Indel).

## Gene Expression Analysis

Uses qRT-PCR or RNA-seq to analyze changes in gene expression levels.

- ✓ **Molecular identification confirms the genetic basis of the mutant phenotype, which is essential for subsequent breeding applications.**



PCR Instrument for Detection

# Genetic Stability Evaluation



## Multi-generational Planting

Mutants are grown for multiple generations to ensure the trait is stably inherited and not a result of epigenetic effects or chimerism.



## Multi-environment Testing

Mutants are grown for multiple environment and years to ensure validate the stability of target quality traits under different environments



## Backcross Verification

Crossing the mutant with the wild-type parent to observe trait segregation and confirm the inheritance pattern (dominant, recessive, etc.).




**Purpose:** Ensures that the selected mutant is a true-breeding line with a stable, heritable trait suitable for further breeding.

# Comprehensive Evaluation and Mutant Classification



## Evaluation Dimensions

When evaluating mutants, we focus on three core aspects to ensure comprehensive assessment:

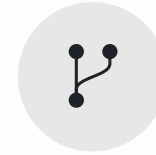
-  Target trait improvement degree
-  Overall agronomic performance
-  Genetic background & pattern



## Direct Use Type

Possesses excellent comprehensive agronomic traits. Can be directly promoted as a new line for regional yield trials.

APPLICATION



## Intermediate Material

Outstanding target trait but with some defective agronomic traits. Used as a donor parent for cross breeding.

BREEDING

# 03 Integrating Mutant Screening Results into Breeding Pipelines



Advanced Crop Improvement Strategy



# Combining Mutants with Marker-Assisted Selection (MAS)



## Principle

MAS uses molecular markers tightly linked to the mutant gene to select for the trait in early generations, bypassing phenotypic observation.



## Advantages

Rapid, accurate, and unaffected by environmental conditions. Enables efficient pyramiding of multiple beneficial genes.



## Application

Significantly shortens the breeding cycle by allowing early and efficient selection of progeny carrying desired mutant genes.

# Strategy1 for Integrating Mutants into Breeding Pipelines-- Direct Use as a New Variety

## Core Definition

When the agronomic traits of a selected nutritional quality mutant basically meet production requirements, it is directly registered and promoted through variety comparison and regional trials. This is the most direct and fastest application method.

## Standardized Implementation Process

- 01 Mutagenesis:** Physically/chemically mutagenize seeds to create genetic variation (M1 generation).
- 02 Progeny Screening:** Large-scale screening for target traits in the M2 generation, observing genetic stability in the M3-M4 generations.
- 03 Line Identification:** Conduct comprehensive evaluation trials such as yield comparison and disease resistance identification.
- 04 Regional Trials:** Conduct multi-year, multi-location production trials and demonstrations in different ecological zones.

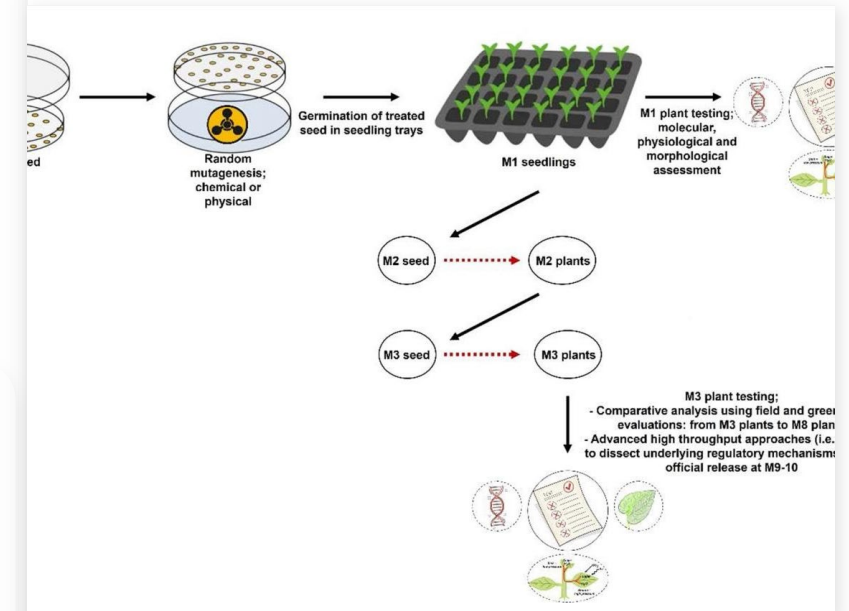


Figure 1: Schematic of the entire process of mutation breeding and mutant screening.

# Strategy 1: Core Advantages and Challenges



## Core Advantages (Pros)



### Significantly Shortened Breeding Cycle

No complex crossing and backcrossing processes, significantly saving time costs compared to traditional methods.



### Low R&D and Breeding Costs

Main investment is concentrated in the early-stage mutagenesis and large-scale screening, with a simple and controllable process.



### Rapid Acquisition of Legal New Varieties

Can quickly transform selected new traits into valuable elite varieties for promotion.



## Limitations and Challenges (Cons)



### High Randomness of Mutations

The direction of mutations from mutagenesis is uncontrollable, making it difficult to simultaneously obtain ideal nutritional quality and excellent agronomic traits.



### Relatively Single Genetic Background

The genetic background of the new variety is limited to the original parent, with low genetic diversity and to-be-improved adaptability.



### Risk of Linkage Drag

The target mutation site may be closely linked to other undesirable genes, making it difficult to effectively separate during the breeding process.

# Strategy2 for Integrating Mutants into Breeding

## Pipelines--directly used as parents Crossing with Elite Lines

### | Core Definition

Use the nutritional quality mutant as a parent to cross with an elite line that is high-yielding and disease-resistant. Through genetic recombination, select new lines from the segregating generations that possess both the target nutritional quality and the excellent agronomic traits of both parents.

### | Key Implementation Steps

01

#### Parent Selection

Identify the mutant (donor) and the elite line (recipient).

02

#### Artificial Crossing & $F_1$

Obtain the first filial generation ( $F_1$ ) through artificial pollination.

03

#### Large-scale $F_2$ Screening

Select individual plants that combine the advantages of both parents from the segregating population.

04

#### Progeny Pedigree Purification

Self-pollinate the elite individual plants for multiple generations to form stable genetic lines.

# Strategy 2: Core Advantages and Challenges



## Core Advantages

### 1. Gene Recombination, Combining Parental Advantages

Effectively breaks undesirable gene linkages, recombining the parents' high-quality nutritional traits with excellent agronomic traits.

### 2. Rich Genetic Basis, Strong Adaptability

The genetic background of the new variety directly comes from two parents, with higher genetic diversity and stronger adaptability to different environments.

### 3. Mature Technical System, Widely Applied

This is the most familiar and commonly used classic breeding method for breeders, with standardized operating procedures and a long history of successful applications.



## Challenges Faced

### 1. Long Breeding Cycle

From the start of artificial crossing to the final acquisition of a stable elite line, the entire selection process typically takes 5-8 years.

### 2. Large F<sub>2</sub> Segregating Population

To ensure sufficient gene recombination types for selection, it is usually necessary to plant thousands of segregating populations.

### 3. Difficulty in Early Trait Selection

Most important quality traits are quantitatively inherited, making field identification in the early breeding generations costly and difficult to achieve with high accuracy.

# Strategy3 for Integrating Mutants into Breeding Pipelines--Backcross Breeding as a Donor Parent

## Core Definition


A targeted improvement strategy. Cross a nutritional/quality mutant (donor parent) with an elite variety (recurrent parent), then repeatedly backcross the progeny with the recurrent parent to precisely introduce the nutritional quality trait into the genetic background of the elite variety.


## Implementation Step Process

- 01 Parent Selection:** Identify the elite recurrent parent (RP) and the target trait donor (DP).
- 02 Initial Cross:** Recurrent Parent  $\times$  Donor Parent  $\rightarrow$  Obtain the first filial generation  $F_1$ .
- 03 Continuous Backcrossing:** Backcross  $F_1$  with the recurrent parent (BC1), and repeat this process for 4-6 generations.
- 04 Screening and Evaluation:** Combine marker-assisted selection (MAS) with field agronomic trait surveys to select stable lines with the nutritional quality mutant loci, high background recovery and excellent agronomic traits.
- 05 Selfing for Homozygosity:** Select individual plants with the target trait for selfing until the trait is stably inherited.


# Strategy 3: Core Advantages and Challenges


## Core Advantages

 **Targeted Improvement, Precise and Efficient:** Precisely improves 'weak' traits while perfectly preserving the superior genetic characteristics of the original variety.

 **Rapid Genetic Background Recovery:** Only 4-6 backcross generations are needed, with a genetic background recovery rate of over 98%.

## Technical Challenges

 **Improves Only a Few Traits:** Mainly suitable for improving simple traits controlled by 1-2 major genes, difficult to address complex traits.

 **Potential Linkage Drag:** The target gene may be closely linked to nearby undesirable genes, making complete elimination difficult.

# Strategy4 for Integrating Mutants into Breeding Pipelines-- Pyramiding Breeding for Multiple Genes



## Core Definition

Combine multiple desirable genes (possibly from different nutritional/quality mutants) into the same genetic background through crossing, backcrossing, and selection, to breed a 'super variety' with multiple desirable traits.

## Pyramiding Backcross

Sequentially introduce different target genes into the same recurrent parent background through repeated backcrossing steps.

## Composite Crossing

Cross parents with different elite genes pairwise, then intercross the hybrids. Select and recombine over generations to stack target genes.

## Core Technology: Marker-Assisted Selection

Utilize molecular markers linked to target genes for precise foreground selection, ensuring all desired genes are retained during breeding.

# Strategy 4: Core Advantages and Challenges



## Technical Advantages and Challenges

**Core Advantages:** Combines multiple traits, breeds breakthrough varieties, and significantly broadens the genetic base.

**Technical Challenges:** Complex technology, long cycle of 8-10 years, high screening difficulty, and risk of trait interactions.



## Application Case: High-Quality Aromatic Rice

By pyramiding aroma, low amylose (soft rice), and high resistant starch genes, a new rice variety combining the three characteristics of 'aroma, softness, and high nutrition' was successfully bred.



Demonstration field of high-quality aromatic rice bred by pyramiding breeding.

# Strategy5 for Integrating Mutants into Breeding Pipelines-- Site-directed Modification via Gene Editing

## ■ Core Definition

Using advanced technologies such as CRISPR/Cas9 to precisely modify (knockout/knock-in/single base substitution) key genes controlling nutritional quality, achieving 'one-step' trait improvement.

## ■ Technical Advantages and Challenges

- ✓ **Precise and Efficient:** No linkage drag, the breeding cycle can be significantly shortened to 2-3 years, with a marked increase in efficiency.
- 💡 **Technical Challenges:** High operational threshold, potential off-target risks, and currently faces certain regulatory and ethical restrictions.

## ■ Typical Breeding Applications

- 🌱 **High-Astaxanthin Maize:** Reconstructing the synthesis pathway to obtain a new high-nutrition germplasm.
- 🌾 **Coenzyme Q10 Rice:** Reconstructing metabolic pathways to breed functional rice.

# 04 Case Studies: Successful Applications

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Examining practical applications of mutant screening integrated into breeding pipelines



# Case Study1 : High-zinc/iron rice Binadhan-20



**F<sub>1</sub> hybrids were obtained by crossing the high-yielding, good-quality, and widely adaptable improved local rice variety Binasail with the high-zinc/iron line IR66946-3R-178-1-1.**



**The resulting F<sub>2</sub> segregating population was irradiated with gamma rays to increase the variation range of zinc/iron content.**



**Targeted selection was conducted in early generations (F<sub>2</sub>–F<sub>4</sub>), including both nutritional screening and field phenotypic selection.**



**Promising lines were further evaluated and tested for stability across multiple years and locations (F<sub>5</sub>–F<sub>7</sub>).**



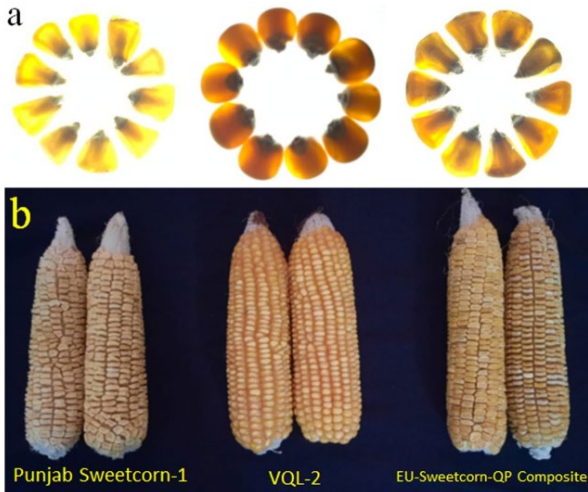
**Official variety registration and release, Binadhan-20.**



Image: Field of Binadhan-20

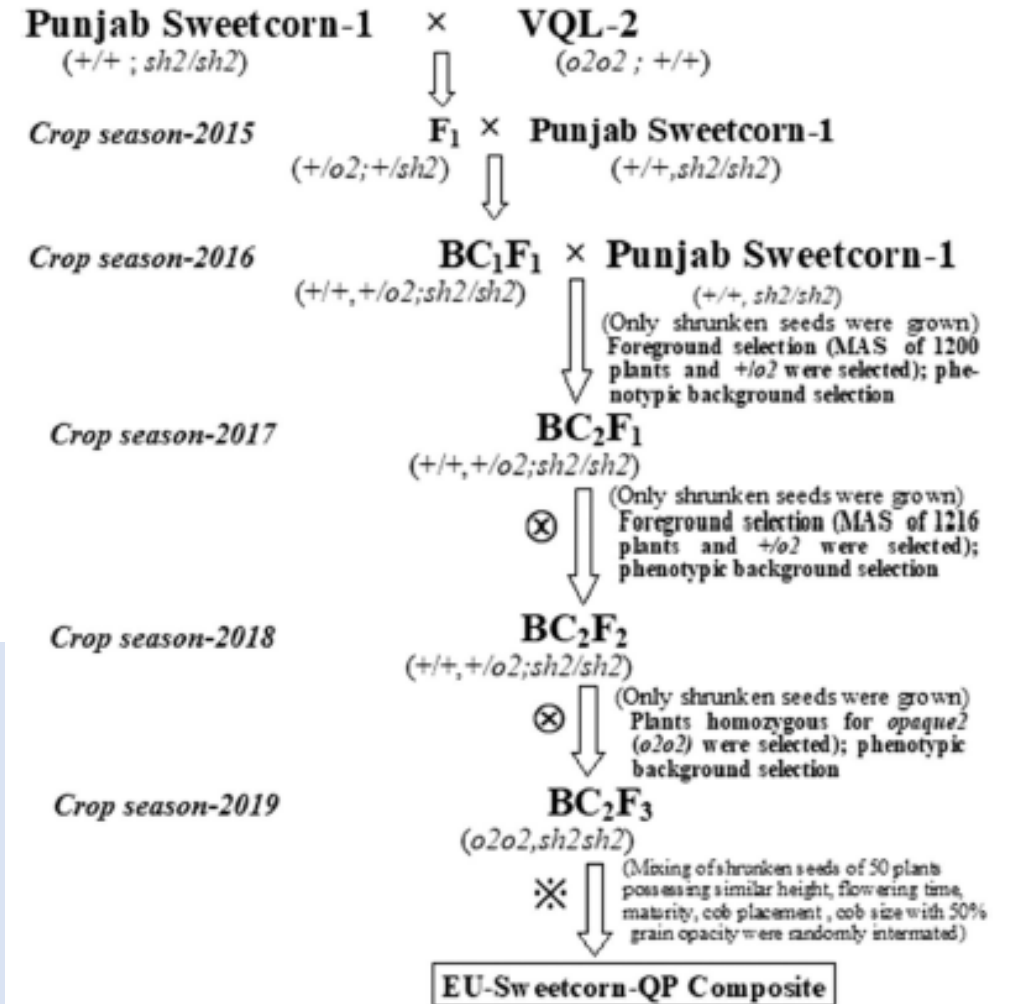
# Case Study2 : Sweet and quality protein maize composite--EU-Sweetcorn QP composite

➤ In this study, via marker-assisted backcross breeding, the *opaque2* (*o2*) allele was pyramided into the sweet corn composite harboring the *Shrunken 2* (*sh2*) allele, thereby developing a sweet and quality protein maize composite named EU-Sweetcorn QP Composite with dual desirable traits.



➤ Specifically, the *umc1066* marker linked to the *o2* mutant was employed for foreground selection, while phenotypical screening at maturity was conducted to select homozygous *sh2sh2* plants.

Fig.1 Breeding strategy for the development of EU-sweetcorn QP composite



# Case Study3: Quality Protein Maize (QPM)



## The Shortcomings of Common Maize



Common maize has poor protein quality, severely lacking the two essential amino acids lysine and tryptophan in its kernels.

## Discovery of the *o2* Mutant



The *opaque-2* (*o2*) mutant has high lysine and tryptophan content in its kernels, but the kernels are soft and susceptible to pests and diseases.

## Modifier Gene Pyramiding and MAS Technology



Using MAS marker-assisted selection, the *o2* gene was pyramided with multiple modifier genes to develop Quality Protein Maize (QPM) with hard endosperm and excellent agronomic traits.

## The Power of Gene Pyramiding



The success of QPM shows that the negative effects of a single mutation can be overcome by pyramiding multiple genes, achieving synergistic improvement of comprehensive traits.

# Case Study4 : Development of High Oleic Acid Peanut via Gene Editing

## Background



High oleic acid benefits human health and extends the shelf life of peanut products.

## Methods

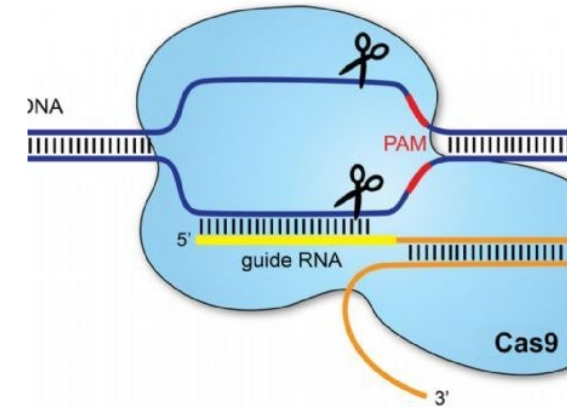


CRISPR-Cas9 editing of the *FAD2* gene to block conversion to linoleic acid.

## Result



Oleic acid content significantly increased from ~37% to over 80%.



Schematic of CRISPR-Cas9-based Gene Editing Technology



High Oleic Acid Peanut Kernels

# 05 Challenges and Future Perspectives



Overcoming Current Obstacles & Exploring Emerging Trends in Breeding Technology



# Current Challenges



## Difficulty in Genetic Dissection of Complex Traits

Many quality traits are controlled by multiple genes with complex genetic bases, making improvement via a single gene difficult.



## Bottleneck in Phenotyping Technology

Accurate, high-throughput, and low-cost methods for quality trait phenotyping are still lacking, limiting screening efficiency.



## Negative Correlation Between Important Traits

Quality improvement may be negatively correlated with other important agronomic traits like yield and resistance, requiring trade-offs.



## Regulatory Oversight and Public Acceptance

New technologies like gene editing face strict regulatory oversight and public skepticism, posing challenges for commercialization.

# Future Perspective



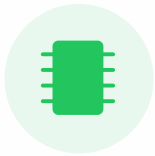
## Automated phenotypic identification

Using robots, drones, and sensors for high-throughput, automated field phenotyping.



## Multi-Omics Data Integration

Build comprehensive biological network models to understand quality formation mechanisms at the systems level, guiding precision breeding.



## Artificial Intelligence (AI) Assisted Decision-Making

Use AI to analyze massive datasets, optimize parent selection, predict breeding values, and accelerate breeding generation turnover.



## Gene Editing Technology Innovation

Based on the continuous upgrading of the CRISPR-Cas system, develop more precise tools like base editing and prime editing to achieve fine regulation of complex metabolic pathways.



Application of Robotics in Modern Agriculture

# 06 Summary



## Efficient Creation and Screening

Obtain valuable mutants using mutagenesis and modern screening technologies.



## Precise Introduction and Pyramiding

Use technologies like marker-assisted selection (MAS/MABC) and gene editing to efficiently integrate desirable genes into elite genetic backgrounds.



## Systematic Evaluation and Validation

Ensure the improved variety performs excellently in both quality and agronomic traits through rigorous field trials.

**Future Outlook:** With the rapid development of various technologies, future crop breeding will move toward greater precision and intelligence. This is not only a deep integration of science and technology but also provides strong technical support and practical solutions for addressing global nutrition challenges and ensuring food security.

# Thank You

Technological Breeding · Creating the Future Together

THANKS FOR LISTENING