



IAEA

International Atomic Energy Agency
Atoms for Peace and Development

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

Phenotypic, biochemical, and molecular screening approaches of nutritional mutant

Wang Lihao

**Chinese Academy of Agricultural Sciences
No.12 Zhongguncun south street
Beijing ,100081
Email: wanglihao@caas.cn**

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Workflow & Genetic
Strategies
- Phenotypic screening
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background

The Value of Mutants

- Mutants are individuals in which the genetic material (DNA) of an organism has undergone a heritable change, resulting in traits that differ from the original parent.



Fundamentals of Genetic Variation

Heritable changes at the DNA level



Nutritional composition remodeling

Significant increases in protein, vitamins, and other nutrients



Prospects for Agricultural Application

A New Direction for High Value-Added Crop Breeding



Contributions to Global Food Security

- Quantity Security
- High Yield
- Feeding more people
- Quality Security
- Enhanced Nutrition
- Nourishing people better
- Stability Security
- Stress Resistance
- Ensuring reliable harvests



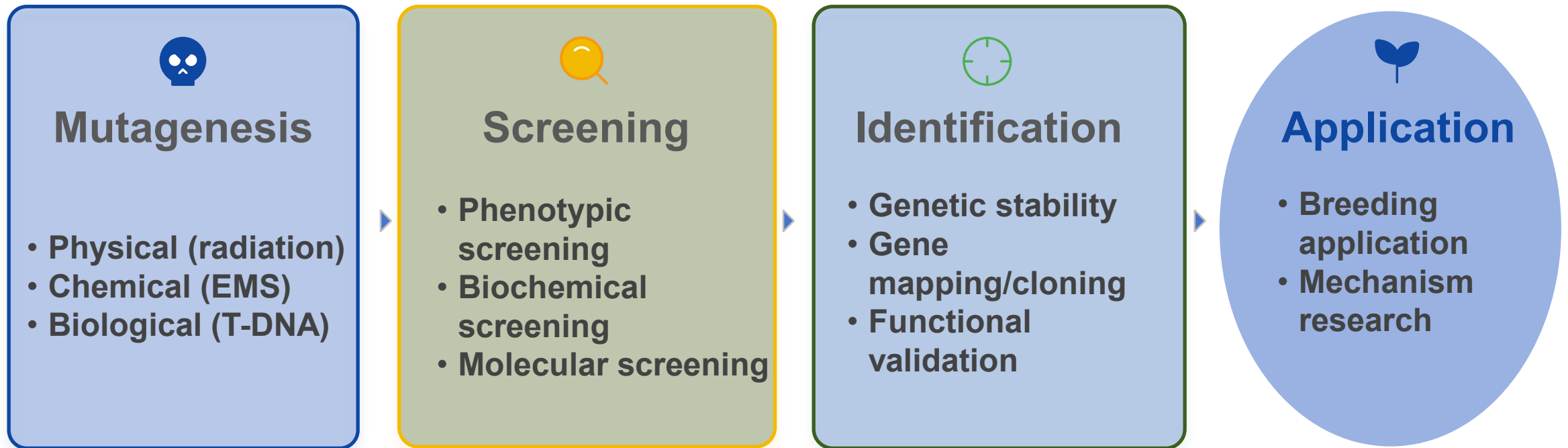
How to Efficiently and Accurately
Screen for Nutritional Mutants?



Appropriate Screening Methods

Mutant Screening Workflow & Genetic Strategies

Basic Workflow of Mutant Screening



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

The Importance of Mutant Screening

Randomness

99% of mutations are useless or deleterious.

Rarity

High-nutrition or stress-resistant mutants?
Perhaps only 0.1%.

Reality

No screening? You're looking for a needle in a haystack.



- **Rapidly and accurately screening through thousands of mutants to pinpoint the few that are truly beneficial.**

Forward Genetics Screening Methods

01. Phenotypic screening

Screening for mutants with target traits through chemical / physical mutagenesis or natural variation

02. Gene Mapping

Using techniques such as map-based cloning, BSA-seq, MutMap, and fine mapping to pinpoint genes to specific chromosomal regions.

03. Gene cloning & validation

Identify candidate genes and validate their functions through transgenic complementation assays or knockout experiments.

Case Study: *ZmYSL2* Gene Mapping

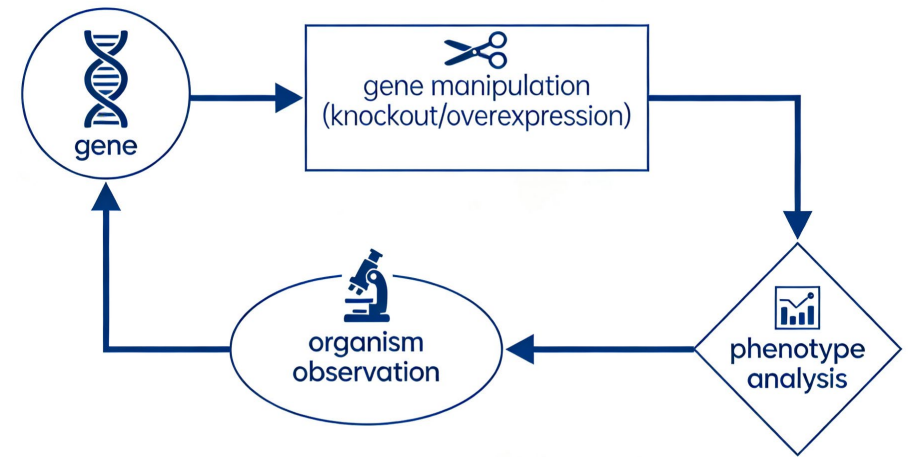
Scientists successfully mapped the key gene *ZmYSL2* from a natural variation mutant *o213*, which controls the lysine and zinc contents in maize grains, using map-based cloning technology, providing a theoretical basis for improving crop nutritional quality.

Reverse Genetics Screening Methods

1. Identify known nutrition-related genes Sequence

2. Perform Gene Editing (Knockout/Overexpression)

3. Conduct Phenotypic Analysis



Case Study: Rice *OsPht* Gene

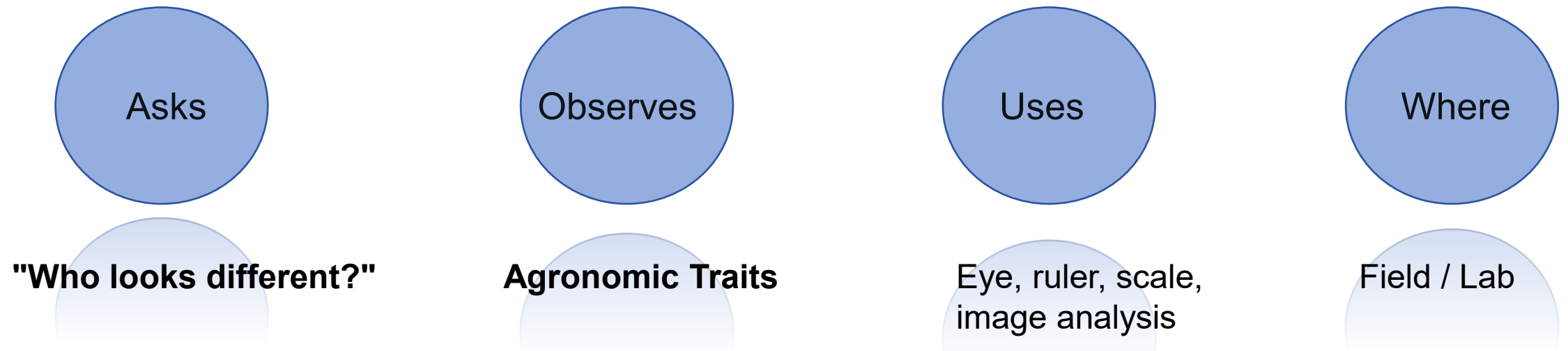
Using CRISPR/Cas9 to knockout the *OsPht* gene in rice successfully obtained phosphorus transport-related mutants, providing materials for studying phosphorus metabolism.



Phenotypic screening

Implication

- Phenotypic screening involves observing, measuring, and evaluating the visible traits of mutant populations — either by eye or with simple tools — to identify individuals with potential changes in target nutritional characteristics.



Phenotypic Screening Based on Morphological Traits



Color Variation

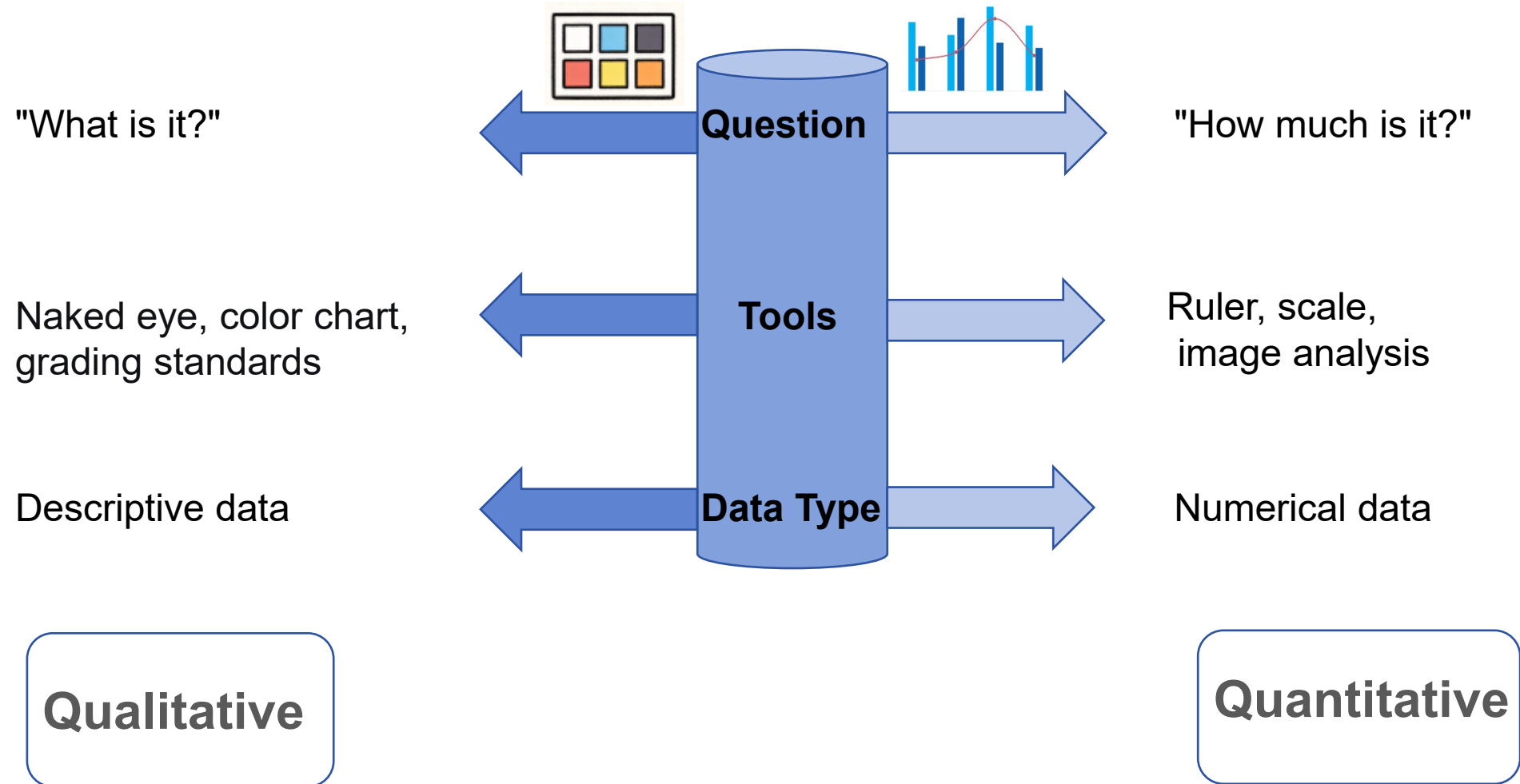
Such as Grain color (indicative of carotenoid and anthocyanin contents), Grain luster and transparency (indicative of amylopectin content, quality protein content and eating quality), and leaf color changes (indicative of nutrient deficiency symptoms) serve as intuitive visual screening markers.



Morphological and Size Variation

Includes qualitative morphological traits such as grain shape (long vs. round grain) and plant architecture (dwarfism, tillering capacity), as well as quantitative size traits including grain dimensions (length/width) and fruit size variations.

Two Classes of Indicators



Main Methods

Field phenotypic measurement

Quantify growth, fruit, yield traits
Accurate, statistical, quantitative

Whole growth period comparative observation

Observe with control (wild type)
Cover seedling to maturity
Intuitive, comprehensive, qualitative

Field measurement & whole-period observation are the core methods of phenotypic screening.

Extended Scope of Phenotypic Screening

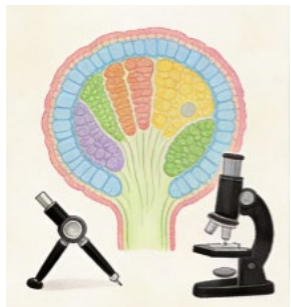


Individual level

Organ level

Tissue level

Cellular level



Pros & Cons



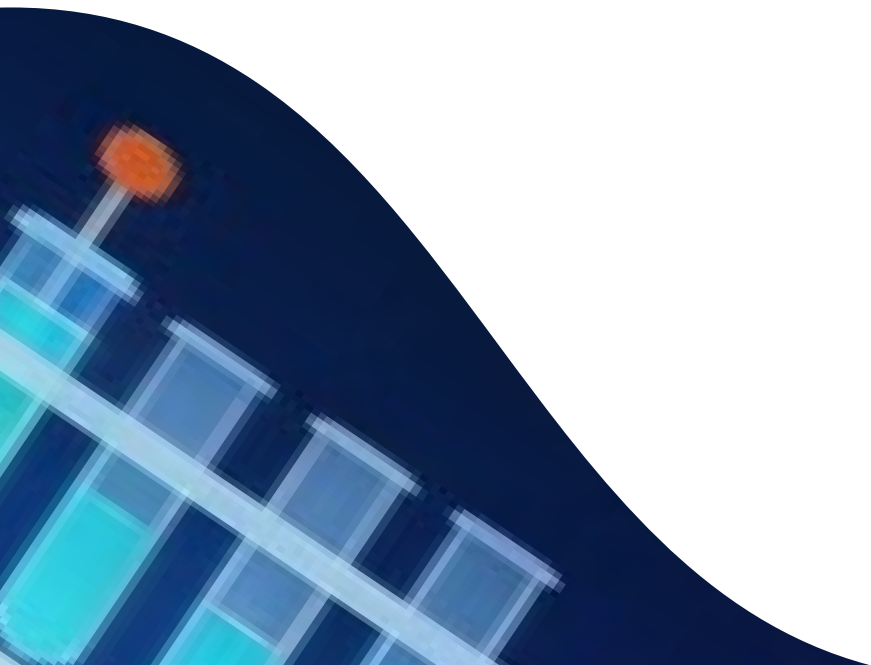
- High-throughput
- Low cost
- Simple & intuitive
- Non-destructive
- Serendipity



- Subjective
- Environmental interference
- Indirect (correlation \neq causation)
- Invisible traits remain unseen
- Low resolution

To explore the metabolic basis of phenotypic variation and uncover the underlying molecular mechanisms, the next step is **biochemical screening**.

Biochemical screening

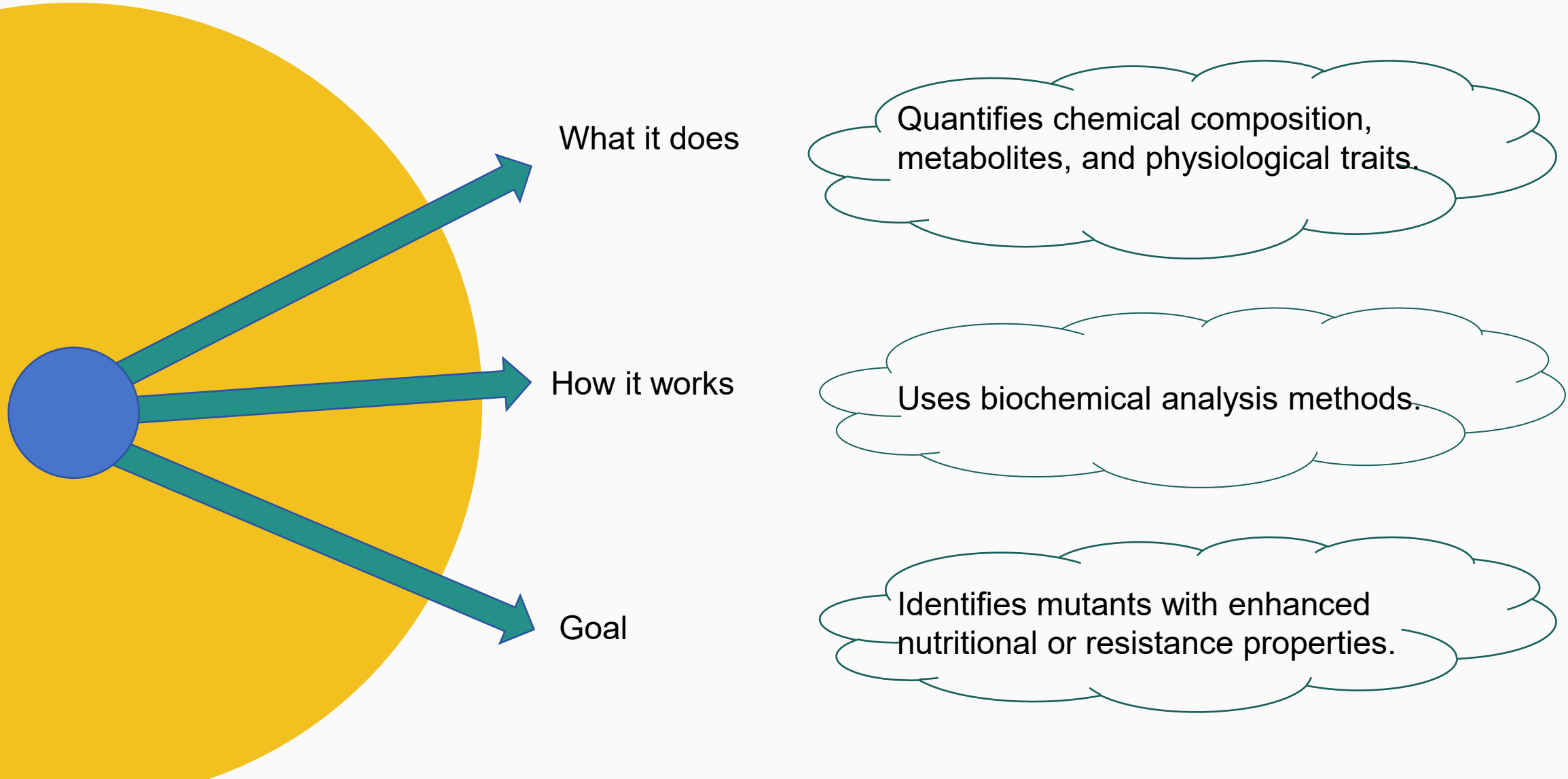


Implication



Biochemical screening uses biochemical analysis to precisely quantify the chemical composition, metabolites, and physiological traits of mutant populations — helping to identify variants with significant changes in target nutritional or resistance characteristics.

Implication



Core Logic: From Phenotype to Metabolism

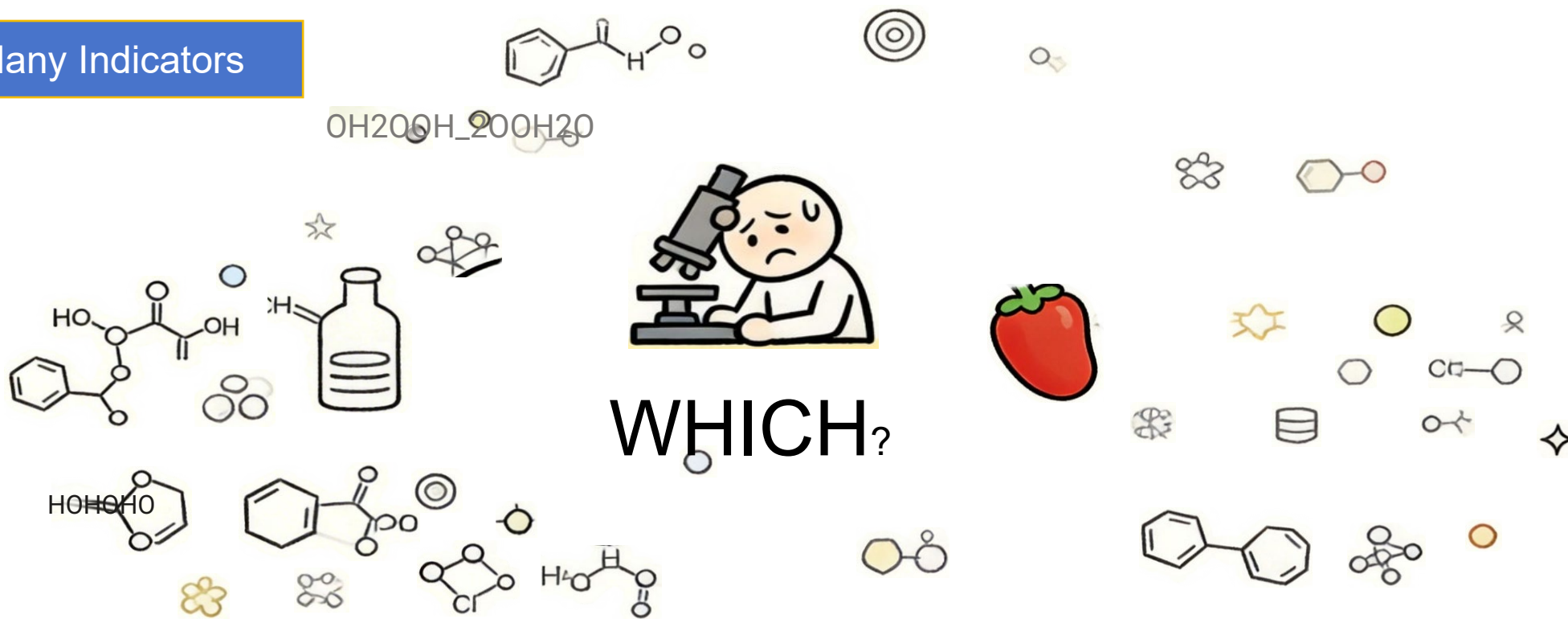
Core Logic Chain: Altered Gene Function → Metabolic Abnormality → Phenotypic Variation

Research Pathway Observe phenotypic variation → Detect metabolic abnormalities (Biochemical screening) → Identify altered gene function

The core task of biochemical screening is to accurately detect metabolic abnormalities, thereby providing key clues and direct evidence for subsequent molecular screening.

Challenges of Biochemical Screening

Too Many Indicators



To uncover metabolic mechanisms and precisely identify mutants, a scientific and systematic indicator framework is essential for measuring and evaluating metabolic changes.

Categories of Physicochemical Indicators

Nutritional Quality

- Nutrients essential for human health
- Biofortification / Nutritional breeding

Processing & Quality

- Taste, storage, processing traits
- Quality improvement breeding

Disease Defense

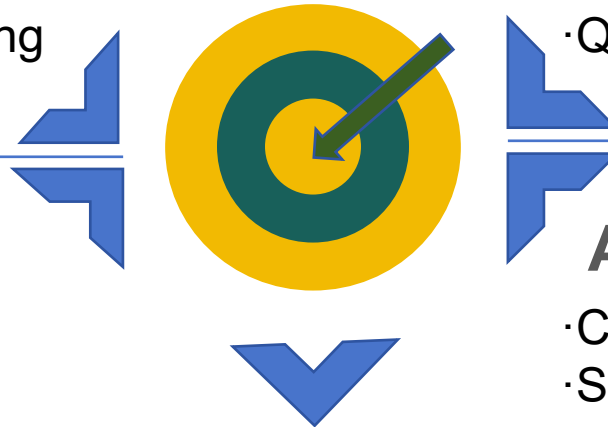
- Defense reactions to pathogens
- Disease resistance breeding

Anti-nutritional & Safety

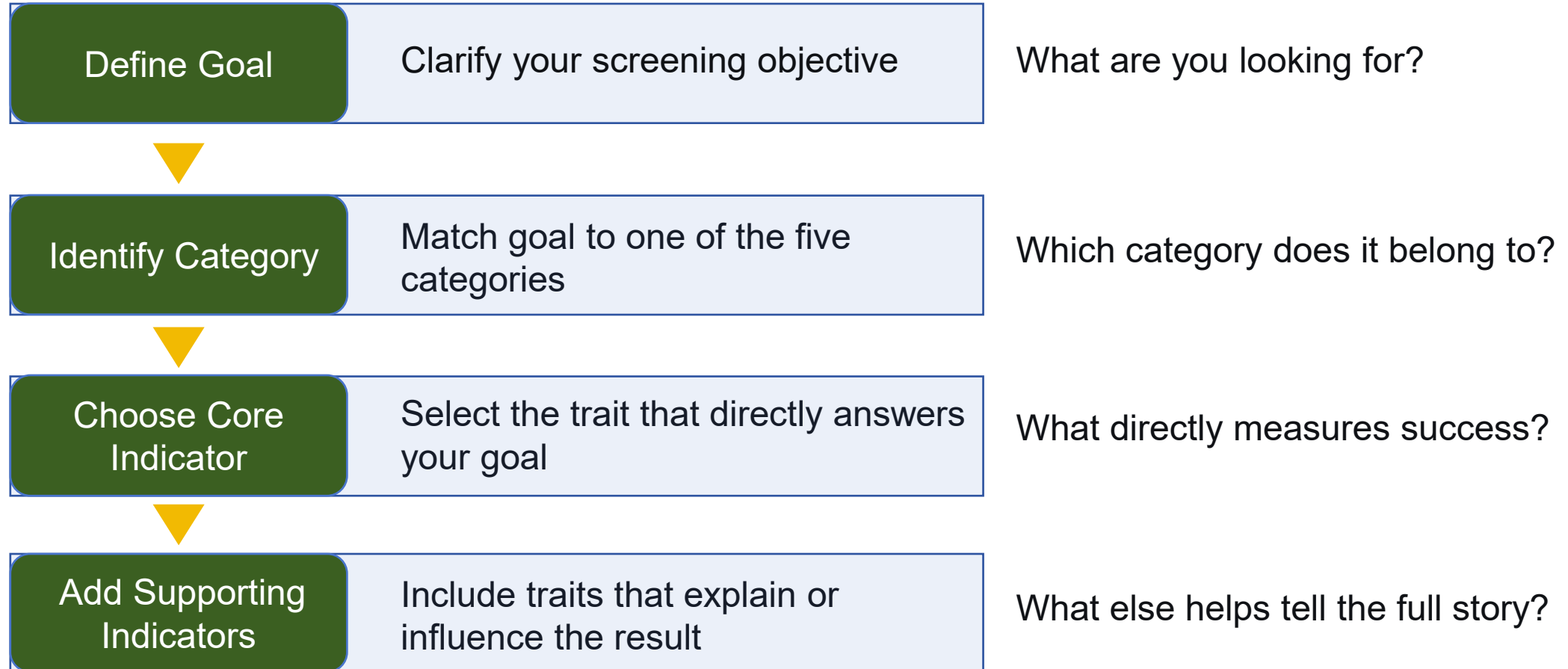
- Compounds affecting absorption or toxic
- Safety evaluation

Stress Physiology

- Physiological responses to stress
- Stress tolerance breeding (salt, drought, cold)



How to Choose the Right Indicators



Main Methods

Metabolite Content Assay

Quantify specific nutrients or intermediates

Sugar content (TSS), Organic acids (total acid), Vitamins (ascorbic acid), etc.

Enzyme Activity Assay

Assess key pathway enzymes

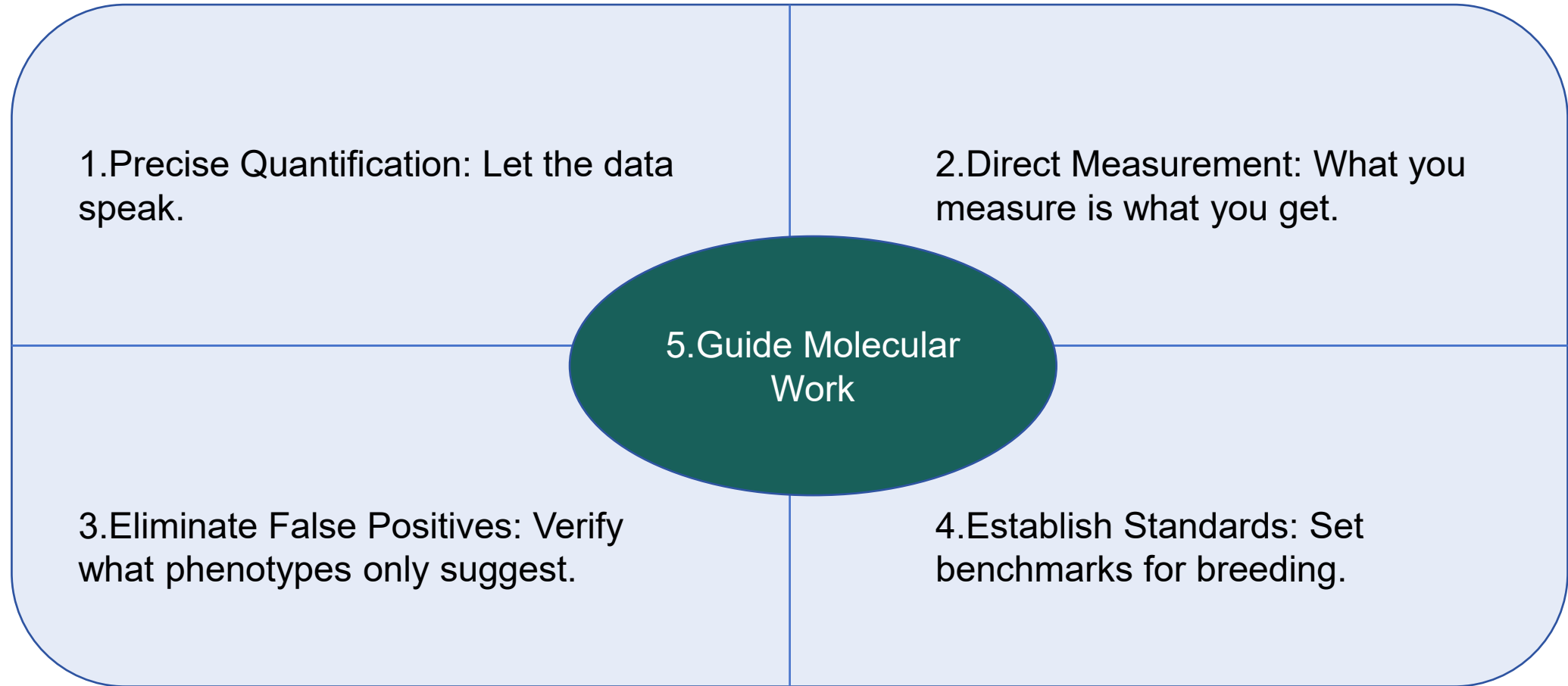
Nitrate reductase (nitrogen metabolism), Sucrose synthase (carbohydrate metabolism), etc.

Metabolomics Analysis

Global, unbiased metabolite profiling

Unbiased; enables discovery of unknown metabolic alterations

Biochemical Screening Value



Bridging Biochemical and Molecular Screening

BIOCHEMICAL SCREENING — Provides Key Clues

Biochemical screening not only identifies mutants with specific metabolic phenotypes but, more importantly, reveals precise metabolic abnormalities (e.g., altered metabolite levels, changes in key enzyme activities).

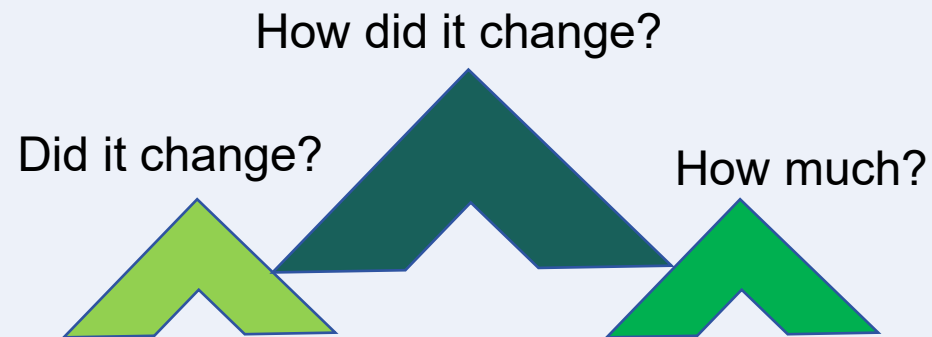
These abnormal metabolic indicators serve as "molecular tags" , pointing us toward the target genes.

Molecular screening



Implication

Molecular screening uses molecular biology techniques to detect genetic variations at the DNA/RNA level — helping to identify mutants with changes in target genes.



Screening mutants is, at its core, screening for the gene that makes the difference.

Why Molecular Screening?

Traditional Screening

Relies on appearance / traits

Highly affected by environment

Long cycle; requires trait expression

Difficult to distinguish heterozygotes from homozygotes

Molecular Marker Screening

Directly detects genotype

Environment-independent

Early-stage screening possible

Enables precise genotyping

Advantages: Efficient, Precise, Reproducible

Two Paths to Molecular Screening

DIRECT DETECTION

INDIRECT DETECTION

● Technology	Sequencing	PCR, Arrays, KASP
● Features	Comprehensive ; Higher cost	High-throughput; Lower cost
● Best for	Mutation discovery; Fine mapping	Known sites ; Breeding apps

Scope of This Presentation

Marker-assisted screening (Indirect detection) — Mainstream for large-scale screening

Molecular Markers-- The "Coordinates" for Screening

Definition

Detectable, specific DNA sequences or loci in the genome that differ between individuals.

Function

Locate genes — Pinpoint gene positions on chromosomes

Track inheritance — Follow gene transmission across generations

Screen target genotypes — Identify desired genetic variants

Core Features

Detectable — Can be read by technical means

Variable — Differ among individuals

Heritable — Follow Mendelian inheritance patterns

Commonly Used Molecular Markers

Technique	What It Detects	Best For	Key Advantage
SNP	Single nucleotide differences	High-throughput genotyping, GWAS, MAS	Most abundant, easy to automate
SSR	Repeat number differences	Genetic mapping, diversity analysis	High polymorphism, multi-allelic
InDel	Insertions or deletions	Genotyping, mutation detection	Easy to detect by PCR

Where Do Molecular Markers Come From?



Mapping Methods for Trait Localization

Method	Population Type	Principle	Output
BSA (Bulked Segregant Analysis)	Extreme phenotype pools	Compare allele frequency differences	Linked interval
QTL Mapping	Biparental progeny	Marker-trait co-segregation analysis	QTL interval
GWAS (Genome-Wide Association Study)	Natural population	SNP-trait association analysis	Associated loci + LD interval

From Marker to Detection

principles

① Base Pairing

Primers/probes specifically recognize target sequences through A-T and C-G pairing.

② Physicochemical Properties

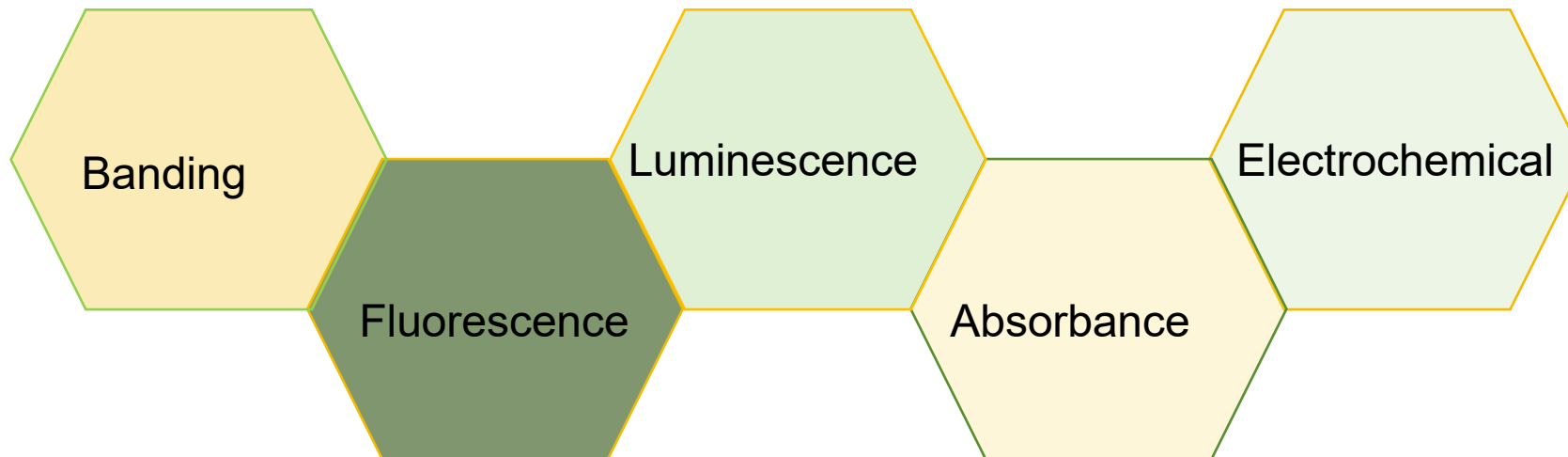
Leveraging DNA's charge (electrophoresis), thermal denaturation (PCR cycling), and enzyme recognition (cleavage/replication) to enable separation and amplification.



These principles form the basis for various detection techniques, which are used to 'read' the signals from molecular markers.

Detection Techniques

- Based on the type of signal, we classify these techniques into the following categories:

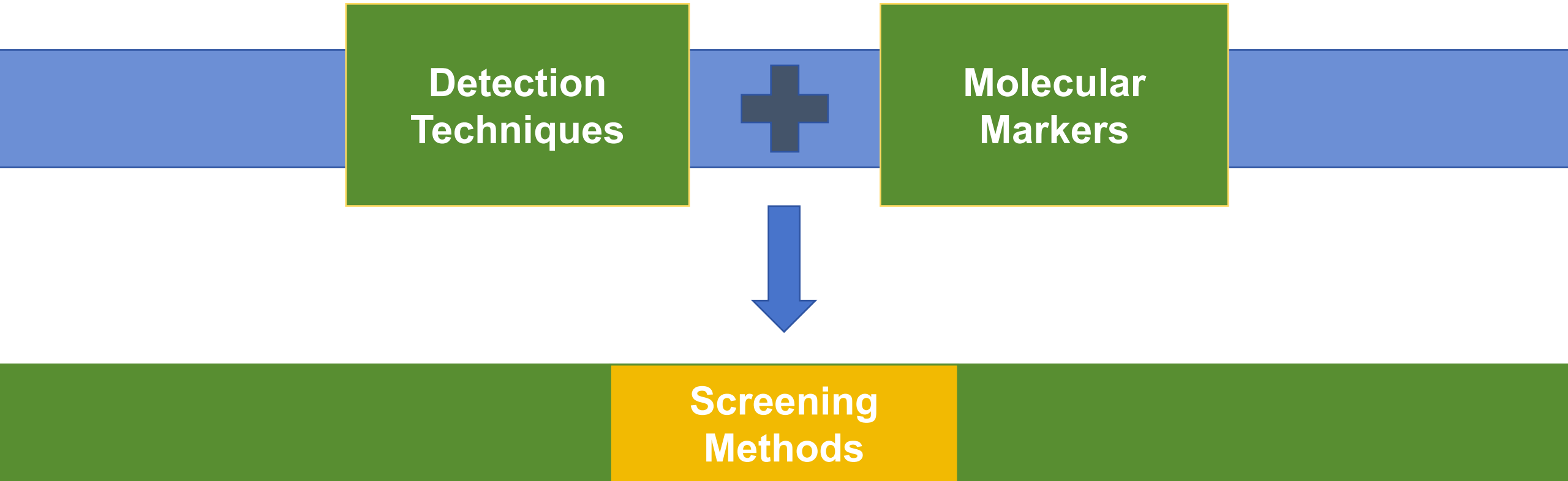


Detection Techniques Classified by Signal Type

Signal Type	Principle	Source	Application
Banding	Molecular sieving	DNA fragment migration	Mapping, Markers, Mutation
Fluorescence	Fluorophore excitation	Dyes, FRET, Quenching	qPCR, SNP, Live imaging
Luminescence	Light emission from chemical reaction	Luciferase, Chemiluminescence	Reporter assays, Bioluminescence imaging
Absorbance	Chromogenic reaction	Chromophores, Turbidity	Reporter assays, Protein quant
Electrochemical	Electron/ion transfer	Current, pH, Potential	Sequencing, Label-free, Field tests

These detection techniques serve as tools to read signals from molecular markers.

Screening Methods



With a variety of detection techniques available, we can now combine them with specific molecular markers to create different screening methods.

Main Methods

Technique	What It Detects	Marker Type	Core Detection Tech	Detection Workflow	Key Advantage
KASP	SNP	SNP	Fluorescence	PCR + Fluorescent probes + qPCR	High-throughput, accurate, automatable
HRM	SNP	SNP	Fluorescence	PCR + Melting curve analysis	Simple, cost-effective, no probes needed
dCAPS	SNP	SNP	Banding (Electrophoresis)	PCR + Digestion + Electrophoresis	Simple, low cost, suitable for small labs
TILLING	SNP / Point mutation	SNP/Indel	Banding/Sequencing	PCR + Digestion + Electrophoresis / Sequencing	Powerful for mutation discovery
RFLP	Any variation affecting restriction sites	Indel	Banding (Electrophoresis)	Digestion + Southern blot + Electrophoresis	Highly specific, reliable

Summary

Molecular Screening	DNA/RNA-based screening	Sequencing, PCR, Arrays	Background
Marker-Assisted Screening	Screening with known markers	PCR, Arrays, KASP	Main focus

Molecular Markers — gene coordinates

Detection Techniques — How signals are produced

Screening Methods — The complete assay workflow



Integrated Case & How to Choose

A New Paradigm for Wheat Mutant Screening Integrating Forward and Reverse Genetics

Integrating forward and reverse genetics, this 'Phenotypic Screening → Gene Association → Editing Validation' closed loop offers an efficient, scalable paradigm for functional gene discovery and precision breeding in complex-genome crops.

01. High-Throughput Phenotypic Screening

Deep learning–based analysis of >20,000 lines (83 traits)
→ confirmed EMS population variation;
spikelet geometry strongly correlates with yield.

02. GH-GLA Molecular Screening (Core Innovation)

Gene-level MAF (integrating intragenic mutations) +
phenotype enrichment
→ identified 175 grain weight & 141 grain number candidate genes.

03. Functional Validation & Breeding Potential

CRISPR/Cas9 validated TaAN-1 etc.;
identified superior haplotypes for high thousand-grain weight
→ direct resources for wheat precision breeding.

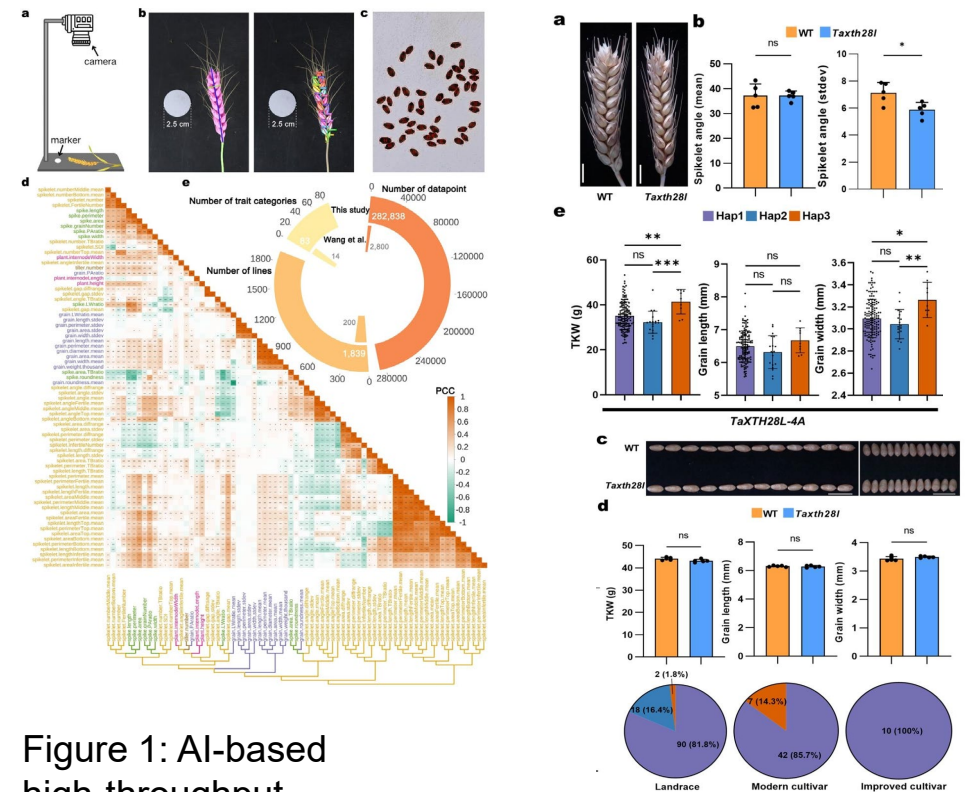


Figure 1: AI-based high-throughput phenotyping and correlation analysis workflow.

Figure 2: CRISPR/Cas9 validation and haplotype analysis of TaXTH28L — confirms spikelet angle regulation and a superior high-TGW haplotype.

Screening & Molecular Mechanisms of High-Yield and Spike-Type Barley Mutants

Multi-dimensional Layered Mutant Screening Pipeline

01. Mutagenesis:

N⁺ ion beam to create M₁ population

02. Stabilization:

M₂-M₃ selfing for heritable, stable lines

03. Phenotypic Screening:

Multi-altitude yield trait evaluation

04. Target Locking:

Isolate E8-38 (high-yield) & D7-67 (spike-type) mutants

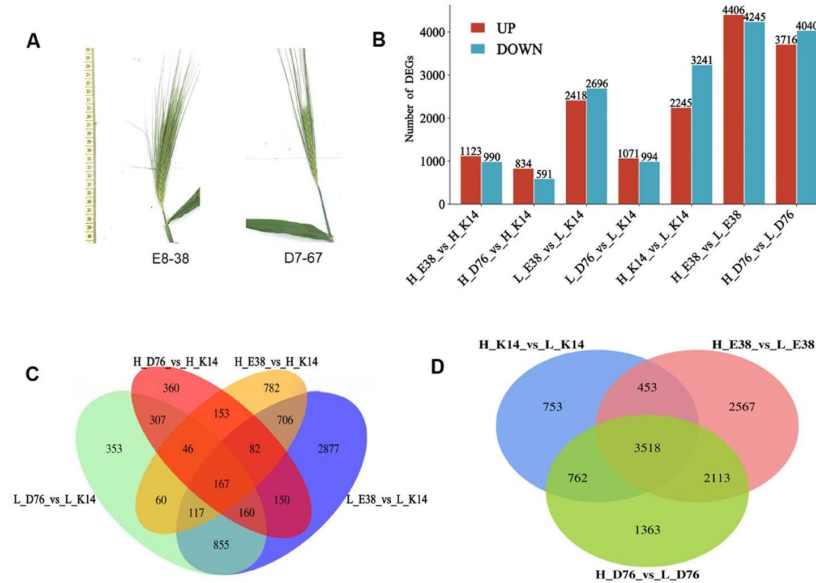


Figure: Phenotypic and Molecular Validation of Screened Key Mutants

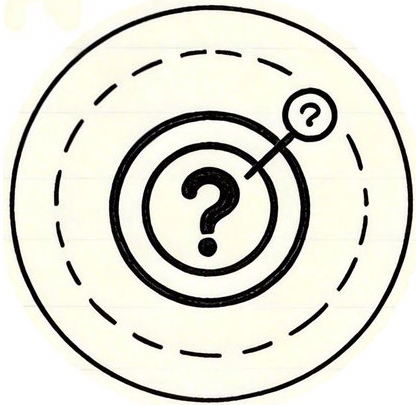
Key Mutants Identified

Through multi-generational and multi-environmental screening, we successfully identified two key mutants with important breeding value: E8-38 (high-yield potential) and D7-67 (spike-type variation).

Molecular Mechanisms:

The high-yield phenotype of E8-38 is driven by hormone balance and metabolic regulation, while the spike-type variation of D7-67 is caused by functional mutations in the VRS1 gene.

How to choose



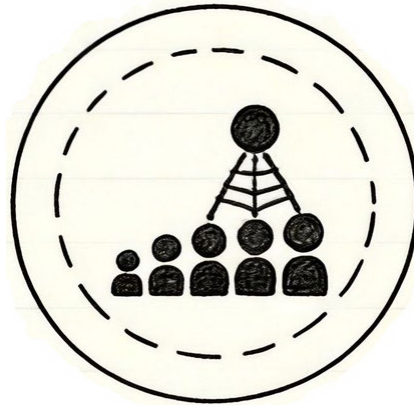
Goal

Known/Unknown
Presence/Absence?



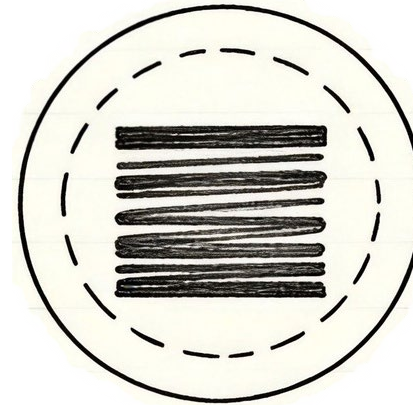
Sample Size

Tens→Hundreds
→Thousands



Scope

Single site?
A few genes?
Whole genome?

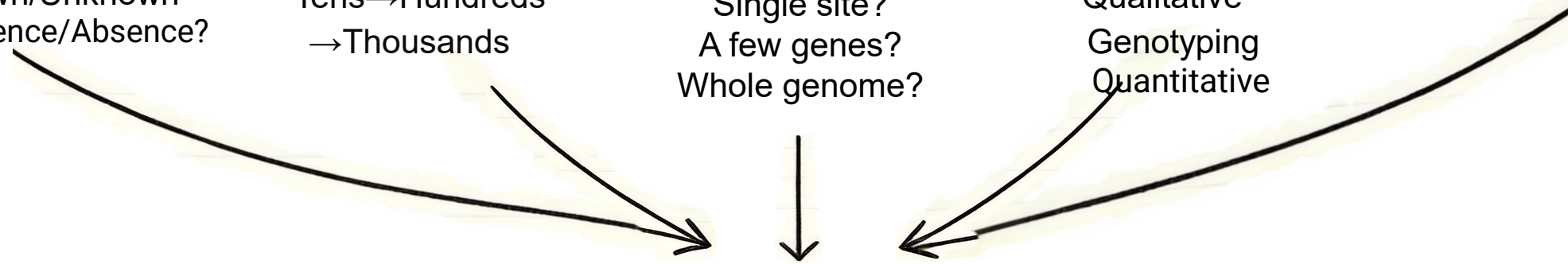


Resolution

Qualitative
Genotyping
Quantitative



Budget



Choose wisely

Data Integration & Analysis

Data Origins: From Traits to Numbers

Screening generates data — the quantification of observable or measurable traits in an organism.

Phenotypic Screening

Morphological traits; Observation, Measurement

Biochemical Screening

Compositional traits; Chemical analysis, Instrument-based detection

Molecular Screening

Genotypic data; PCR, Sequencing, Marker detection

Data Answers Three Core Questions



Who is better?

Provides a basis for comparison

Why is it better?

Provides clues for explanation

How to predict?

Builds association models

Three Core Questions, Three Analyses



Who is better?

➤ Differential Analysis

➤ Identify materials better than control

Why is it better?

➤ Multivariate Analysis

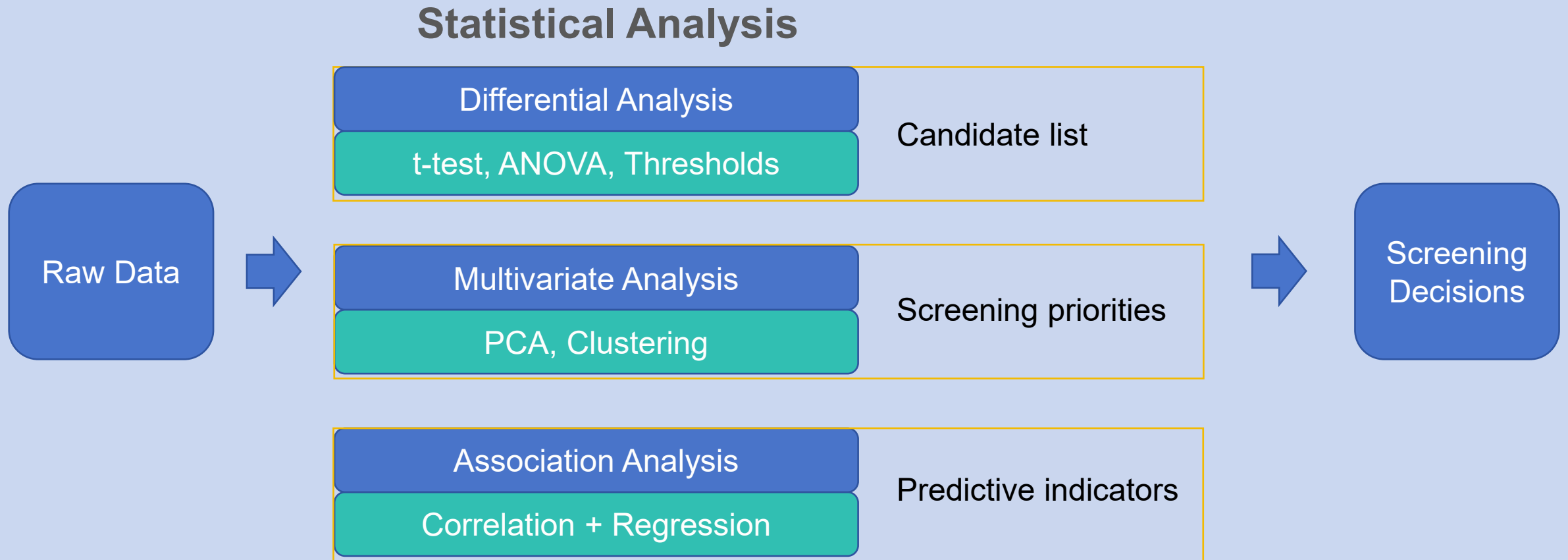
➤ Identify important traits

How to predict?

➤ Association Analysis

➤ Identify correlated traits

Data Analysis for Screening Decisions

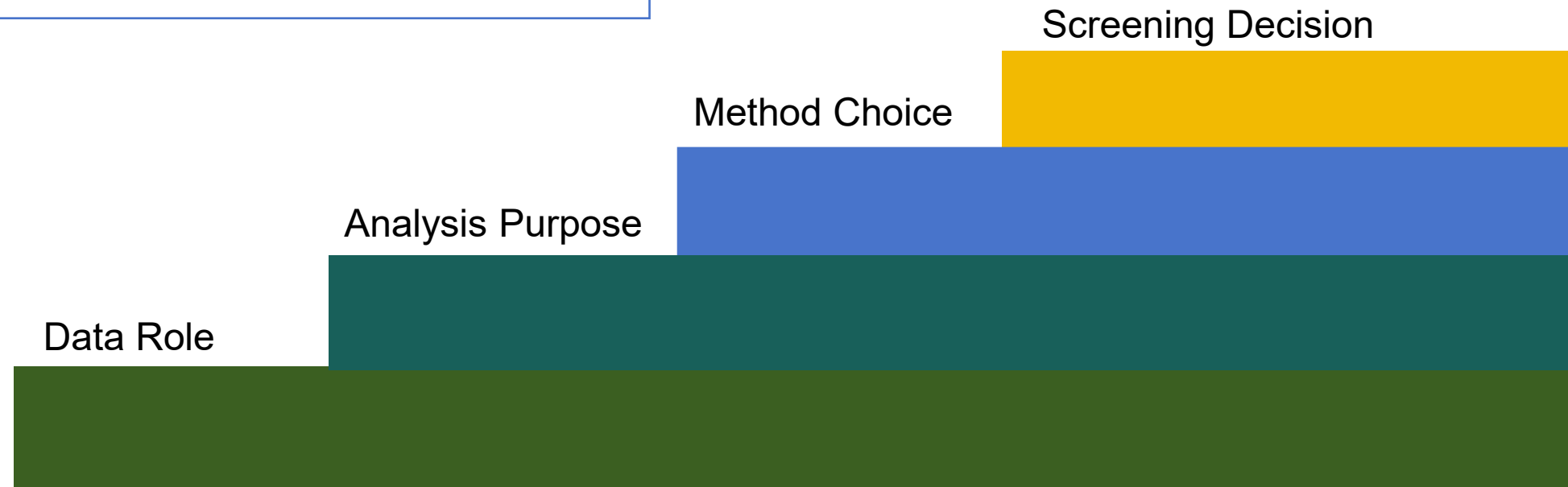


Summary

The role of data determines the purpose of analysis.

The purpose of analysis determines the choice of method.

The results of the method support screening decisions.



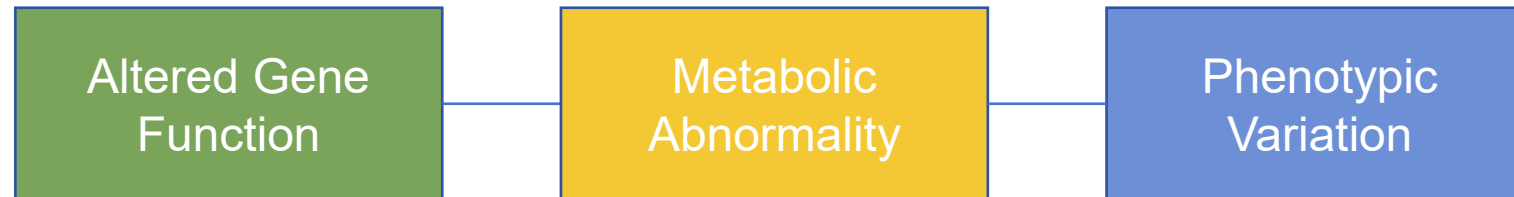
Summary & Outlook

Three Levels of Screening



Overall Research Framework

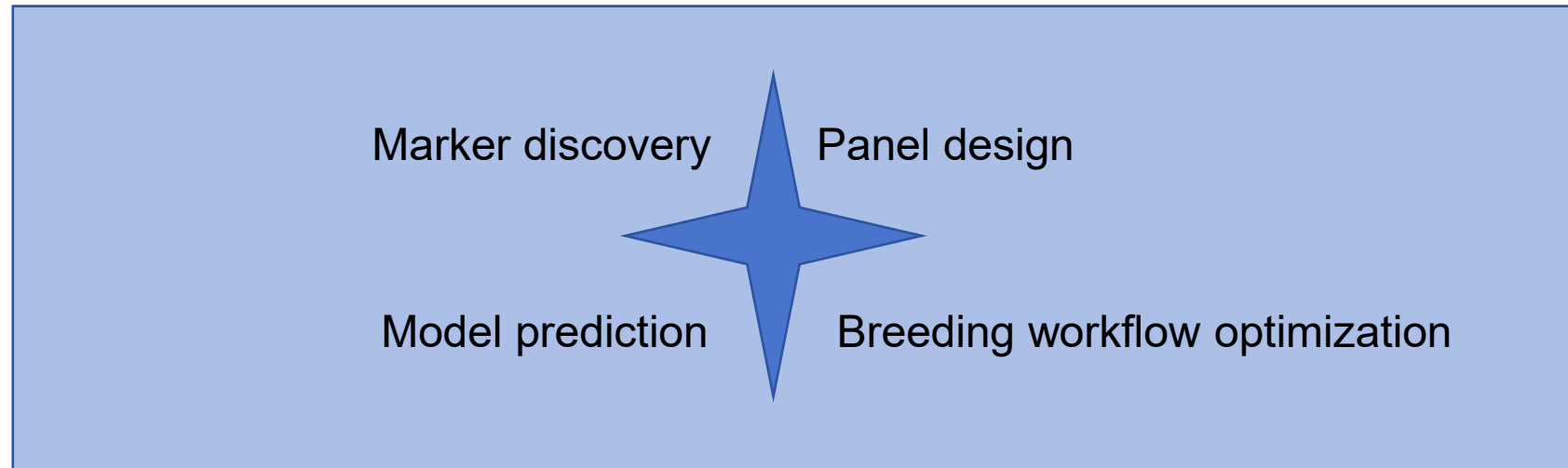
- Core Logic Chain:



- We discover variation through phenotypic screening, reveal metabolic mechanisms through biochemical screening, and ultimately map genes through molecular screening — forming a complete research closed loop from phenomenon to essence.
- These three levels of screening work together as a powerful toolkit for dissecting nutritional mutants, providing a solid methodological foundation for systematically unraveling gene function and metabolic regulatory networks.

Outlook: AI in Breeding

AI Empowers Plant Breeding Across Multiple Dimensions:



Outcome

Smarter, more precise, and more efficient breeding.

Thanks