Integration of Genomics in Crop Improvement: Molecular Breeding

Chikelu Mba, PhD
Head, Plant Breeding Unit
FAO/IAEA Agriculture & Biotechnology Laboratory
IAEA Laboratories Seibersdorf
International Atomic Energy Agency
Vienna, Austria

Outline

- Establishing a Common Premise: Operative Terms

- Overview of Current Status of Molecular Breeding --- Private & Public sectors

- Perspectives for Molecular Breeding

Operative Terms

Scientific American, Jan. 2009

- **Genomics**

  The study of all components of genetic material in a chromosome set

- **Plant Breeding**

  Science of altering the genetic pattern of plants in order to increase their value

Classical Plant Breeding

- Relies on hybridization
- (Deliberate) interbreeding of closely or distantly related individuals
- Crossbreeding introduces traits/genes from one variety or line into a new genetic background
- Selection

To Facilitate Plant Breeding

Generating diversity

- Mutagenesis
  - chemical & physical mutagens, transposons
- Enhancing hybridization
  - Cell and tissue culture
    - protoplast fusion, embryo rescue, somaclonal variation
    - doubled haploidy
- Recombinant DNA tools
  - Cloning of useful genes and genetic transformation

Hampering Plant Breeding

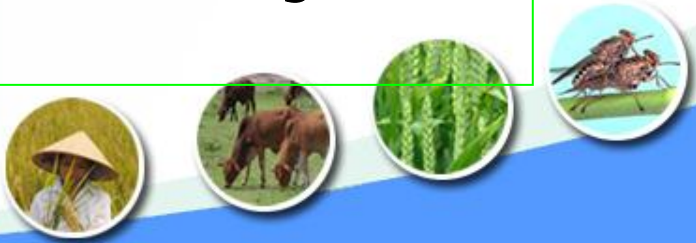
Selection is complicated by

- Genotype by environment interaction
- Polygenic inheritance
- Biology of the plant e.g. growth & development cycles
- Expensive assays
- Volume, bulk, etc.

Molecular Breeding

- Integration of molecular biology techniques into breeding is termed **molecular breeding**

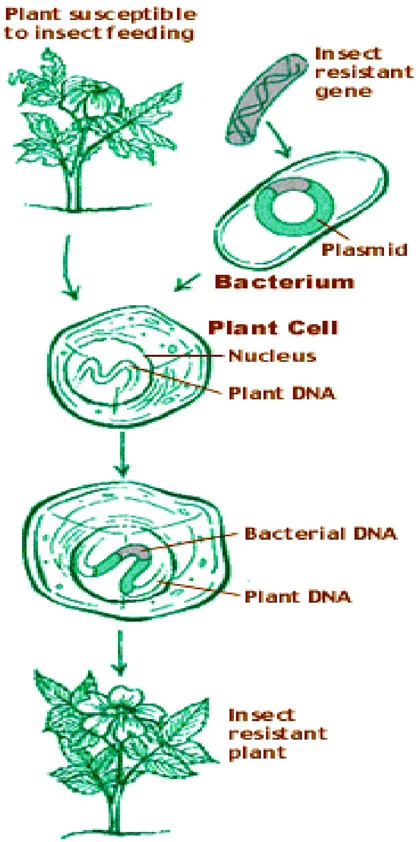
- 2 major components
  - Transgenic approaches
  - Development of associations between DNA sequence variation and trait phenotypic variation
    - High throughput DNA-based prognostics used as a component of a breeding program



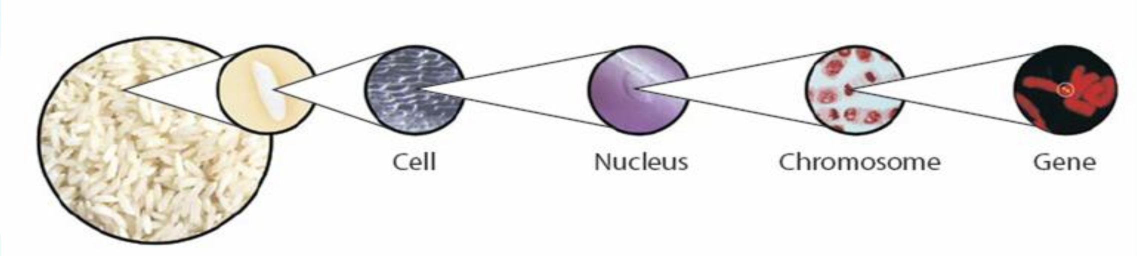
Transgenic approaches

Have worked for traits with a simple gene-to-phenotype relationship

- e.g. Bt for insect resistance and multiple herbicide resistance genes



AgBiotech Infosource (2001)



http://anrcatalog.ucdavis.edu/

Direct querying of the genotype for trait association?

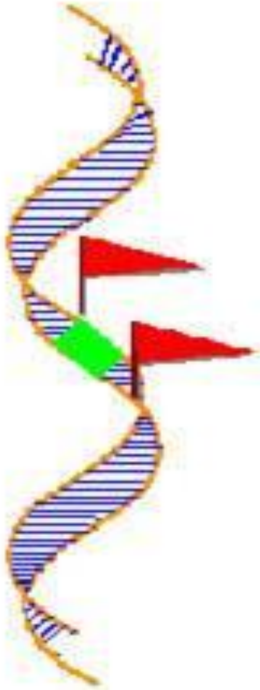
Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Yes, with knowledge of:

- Genes responsible for, or closely linked to, the trait

- Genome location of molecular markers in relation to trait (including quantitative loci – QTL)

- This constitutes Marker-Assisted Breeding (or Marker-Aided Selection, MAS)

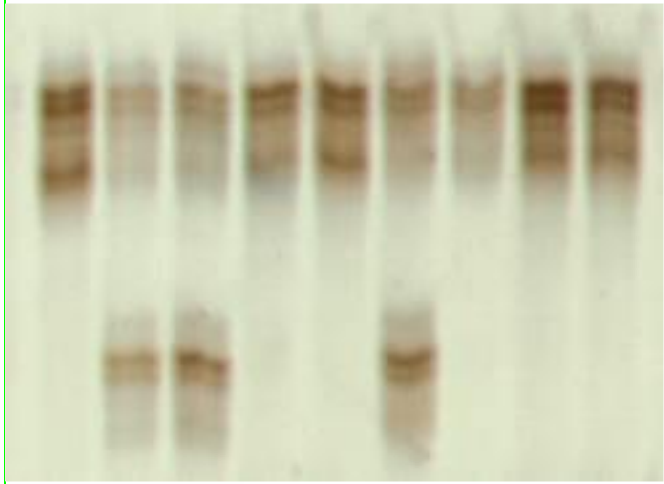


http://www.ars.usda.gov/Research/docs.htm?docid=7203

Direct querying of the genotype for trait association?

Main considerations for the use of DNA markers in MAS

- Reliability
- Levels of polymorphism
- DNA quantity & quality
- Technical procedure
- Cost



Marker Assisted Selection

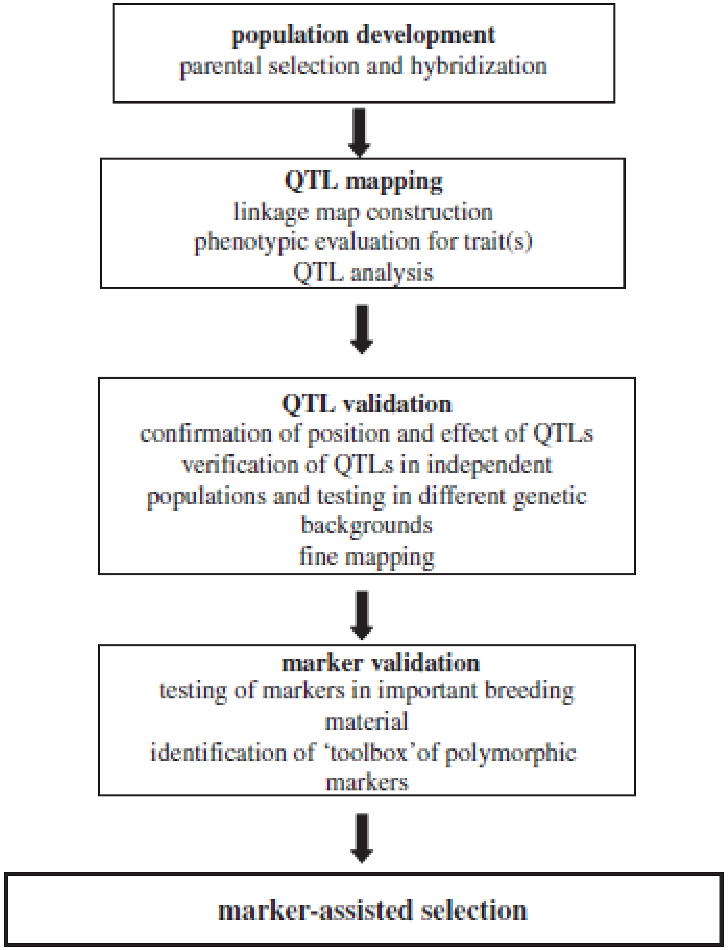
MAS approaches are being applied for:

- simply inherited traits
- quantitative traits

Where:

- marker-trait associations are sufficient
- requisite marker polymorphisms can be identified, and
- a high throughput system for assay is available

Cooper *et al.* (2004)



population development
parental selection and hybridization

QTL mapping
linkage map construction
phenotypic evaluation for trait(s)
QTL analysis

QTL validation
confirmation of position and effect of QTLs
verification of QTLs in independent populations and testing in different genetic backgrounds
fine mapping

marker validation
testing of markers in important breeding material
identification of 'toolbox' of polymorphic markers

marker-assisted selection

Collard and Mackill, 2008

Marker Assisted Selection

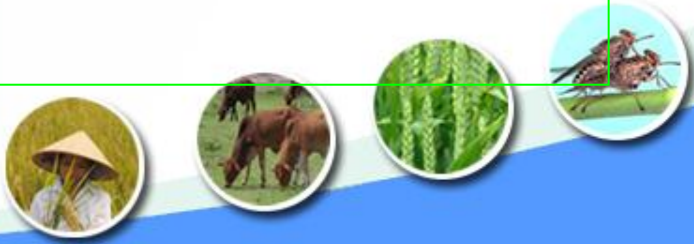
In general, while considering molecular breeding options, are there ---

- Reduction in costs?
- Improvements in precision and accuracy?
- Step-change improvements in phenotypes that were previously not accessible?
- The identification of industry game-changing technologies for complex genotype-environment systems?

Marker Assisted Selection

Common applications of MAS in Plant Breeding

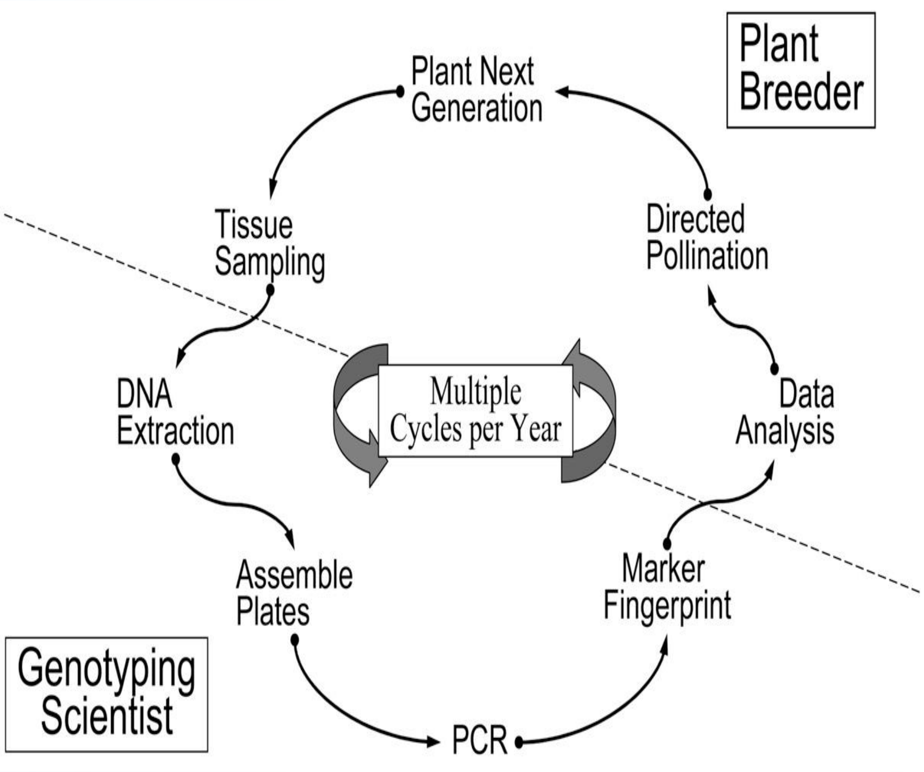
- Marker-assisted evaluation of breeding material
- Marker-assisted backcrossing
- Pyramiding
- Early generation selection
- Combined MAS



MAS: Lessons learned - Private sector, Monsanto

Successful implementation of molecular marker assisted breeding required

- Modification to Breeding Program Structure
- Molecular Markers
- Genotyping Platform
- Phenotypic Information
- Information Technology systems



Plant Breeder

Plant Next Generation

Directed Pollination

Data Analysis

Marker Fingerprint

PCR

Assemble Plates

DNA Extraction

Tissue Sampling

Multiple Cycles per Year

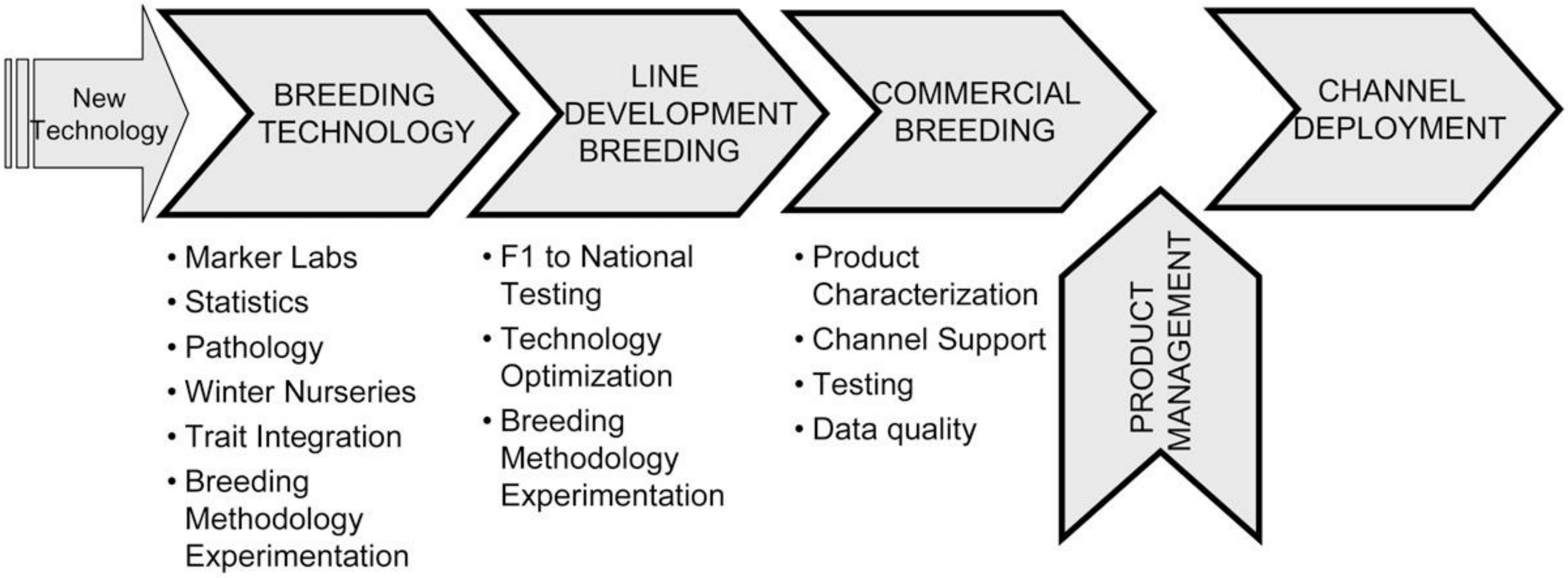
Genotyping Scientist

Eathington *et al. (2007)*

Marker Assisted Selection

Breeding Program Structure

- Molecular marker information increases the complexity of a breeding program (volume of data, frequency of analyses and decisions)



BREEDING TECHNOLOGY
- Marker Labs
- Statistics
- Pathology
- Winter Nurseries
- Trait Integration
- Breeding Methodology Experimentation

LINE DEVELOPMENT BREEDING
- F1 to National Testing
- Technology Optimization
- Breeding Methodology Experimentation

COMMERCIAL BREEDING
- Product Characterization
- Channel Support
- Testing
- Data quality

Partitioned the process into components to enable optimal execution

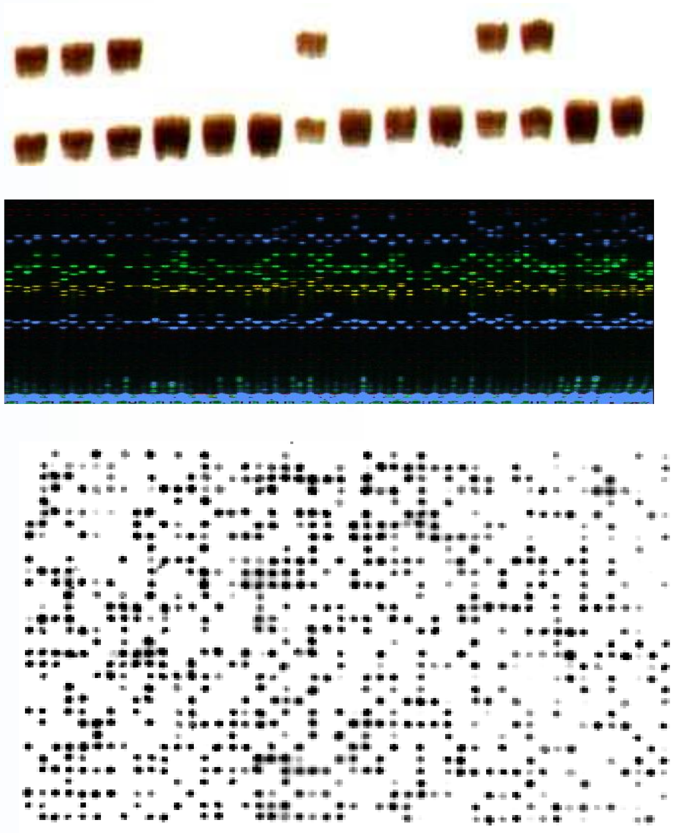
Eathington *et al.* (2007)

Marker Assisted Selection

Molecular Markers and Genotyping Platform

Evolution through cheaper PCR-based markers e.g. SSRs, - made cheaper by automation - to fully automated molecular marker fingerprinting systems from DNA extraction through allele calling based on SNPs

- Identified and developed assays for thousands of corn SNPs
    - large %age in putative genes
    - all SNPs on a consensus linkage map
- From 2000 to 2006
    - total molecular marker data point production grew over 40X
    - cost per data point decreased over 6X



Marker Assisted Selection

Phenotypic Information

- Rule of thumb: Quality of marker phenotype associations is dependent on the quality of the phenotypic information.
- At Monsanto, combination of specialized breeders and equipment enabled an 80% increase in yield trial plot capacity in the last four to five years.
- Phenomics


http://www.cropdesign.com/prod_plant_handling.php


http://www.plantphenomics.org.au/HRPPC

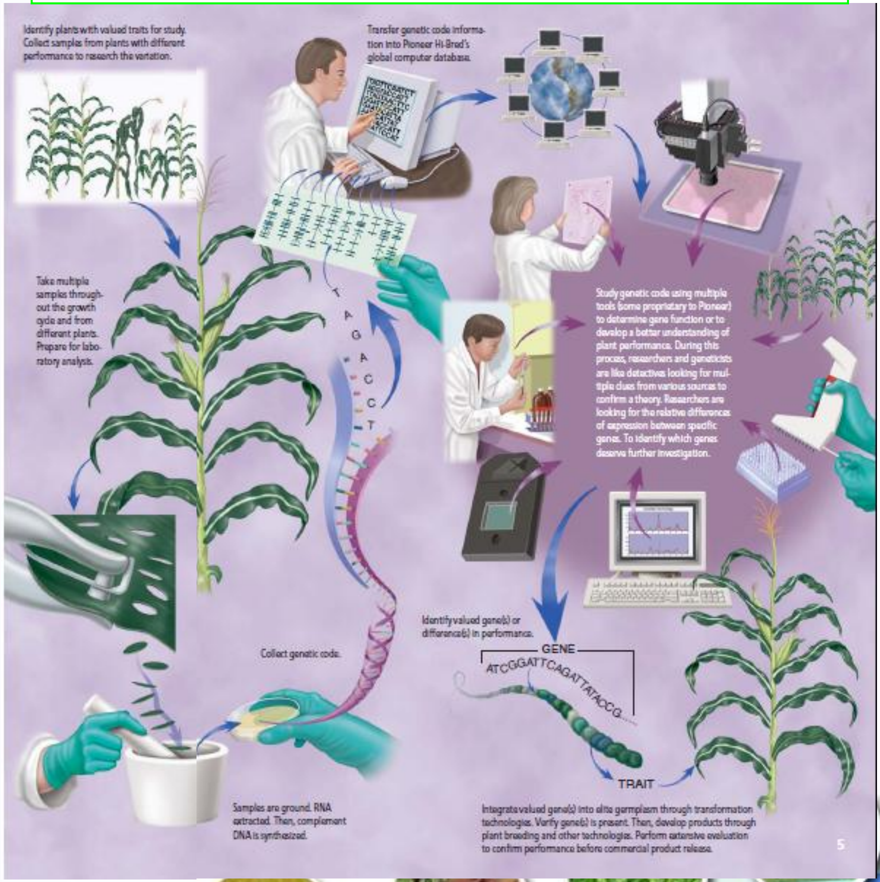
Marker Assisted Selection

Information Technology systems and algorithms

3-tier system at Monsanto

- Global all crop plant breeding system
- Information system for the molecular genotyping laboratories
- An integrated molecular marker decision making system

Pioneer Hi-Bred International, Inc. (1999)



Identify plants with valued traits for study. Collect samples from plants with different performance to research the variation.

Transfer genetic code information into Pioneer Hi-Bred's global computer database.

Take multiple samples throughout the growth cycle and from different plants. Prepare for laboratory analysis.

Study genetic code using multiple tools (some proprietary to Pioneer) to determine gene function or to develop a better understanding of plant performance. During this process, researchers and geneticists are like detectives looking for multiple clues from various sources to confirm a theory. Researchers are looking for the relative differences of expression between specific genes. To identify which genes deserve further investigation.

Collect genetic code.

Identify valued gene(s) or difference(s) in performance.

Samples are ground. RNA extracted. Then, complement DNA is synthesized.

Integrate valued gene(s) into elite germplasm through transformation technologies. Verify gene(s) is present. Then, develop products through plant breeding and other technologies. Perform extensive evaluation to confirm performance before commercial product release.

MAS: Public Sector work on important food crops

Available

- Molecular markers, genetic maps, QTL
- Use of candidate gene-based and association mapping common
- MAS procedures for improvements of biotic and abiotic stresses tolerances and quality traits for crops e.g. rice, maize, wheat, barley, pearl millet, cassava and common bean

Still undone

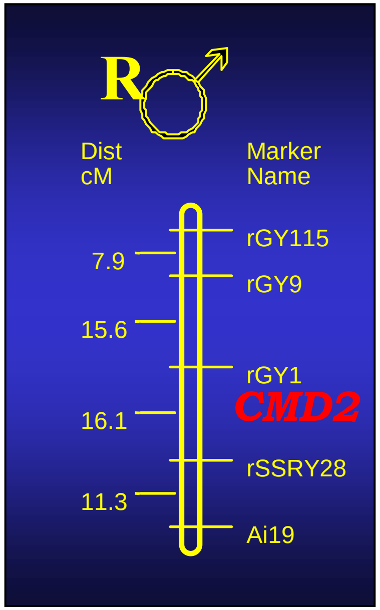
- translate and integrate this knowledge into appropriate tools and methodologies for plant breeding programs

Dwivedi *et al.* (2007)

MAS in Cassava breeding

So far with cassava:

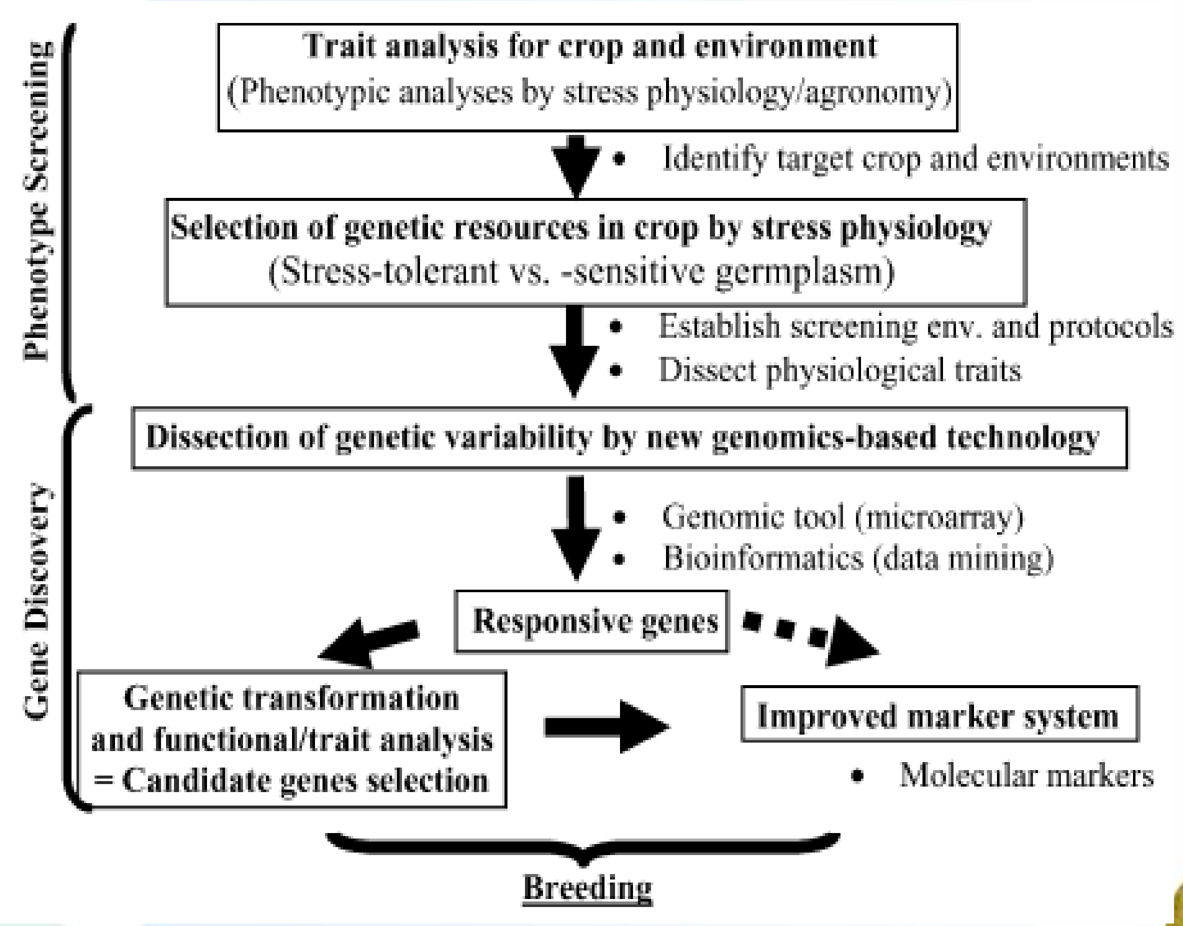
ACMV resistance



Akano *et al.* (2001)

MAS at CIAT

At CIAT --- work on drought tolerance in common bean and aluminum resistance in tropical forage grasses (*Brachiaria* spp.)



Phenotype Screening

Trait analysis for crop and environment
(Phenotypic analyses by stress physiology/agronomy)

- Identify target crop and environments

Selection of genetic resources in crop by stress physiology
(Stress-tolerant vs. -sensitive germplasm)

- Establish screening env. and protocols
- Dissect physiological traits

Gene Discovery

Dissection of genetic variability by new genomics-based technology

- Genomic tool (microarray)
- Bioinformatics (data mining)

Responsive genes

Genetic transformation and functional/trait analysis = Candidate genes selection

Improved marker system

- Molecular markers

Breeding

Ishitani *et al.* (2004)

Strengths of MAS

- Can save time (up to 50%)
- Compared to GM, biosafety concerns obviated
- Unmasks hidden potentials
- Consistent
- Captures gene and interactions rather then single gene action
- With reducing costs, enhanced accessibility

Cahill & Schmidt (2004)

Cahill & Schmidt (2004)

Weaknesses of MAS

- Scope limited by being gene-centred
- Expensive infrastructure
- Minor crops crowded out
- Corporate control and IP
- Confusion & synergy with GM

MAS: Why the low impact

- Still at the early stages of DNA marker technology
- MAS results may not be published
- Reliability and accuracy of quantitative trait loci mapping studies
- Insufficient linkage between marker and gene/ quantitative trait locus
- Limited markers, levels of polymorphism
- Effects of genetic background
- QTL X environment effects
- Relatively high cost of MAS
- ‘Application gap’ between research laboratories and plant breeding institutes
- ‘Knowledge gap’ among molecular biologists, plant breeders and other disciplines

MAS: Cause for optimism?

- Rapid spread of DNA technology, developments in association studies (QTL, MAS)
- Watershed demonstration of advanced BC QTL
- Suited for transgenesis
- Rapid growth in genomics (candidate genes, SNPs)
- High throughput platforms (DNA extraction to genotyping)
- Publicly available markers and user-friendly databases

In the mean time

- A greater level of integration among conventional breeding, QTL mapping/validation and MAS

- Careful planning and execution of QTL mapping studies (especially for complex quantitative traits) and an emphasis on validating results prior to MAS

- Optimization of methods used in MAS such as DNA extraction and marker genotyping, especially in terms of cost reduction and efficiency, and

- Efficient systems for data storage (from in-house laboratory information management systems [LIMS] to publicly available databases)

In the mean time

Exploit advantages of MAS

- Use markers for
  - Selection of parents in breeding programmes
  - MAS for high-priority traits that are difficult, time consuming or expensive to measure
  - Minimizing linkage drag via recombinant selection
  - Pyramiding -- screening of multiple traits per line (i.e. per unit of DNA),

    rapidly eliminating unsuitable lines after early generation selection
- Exploit the time savings for line development for accelerated variety release