Molecular Breeding and Marker Assisted Selection

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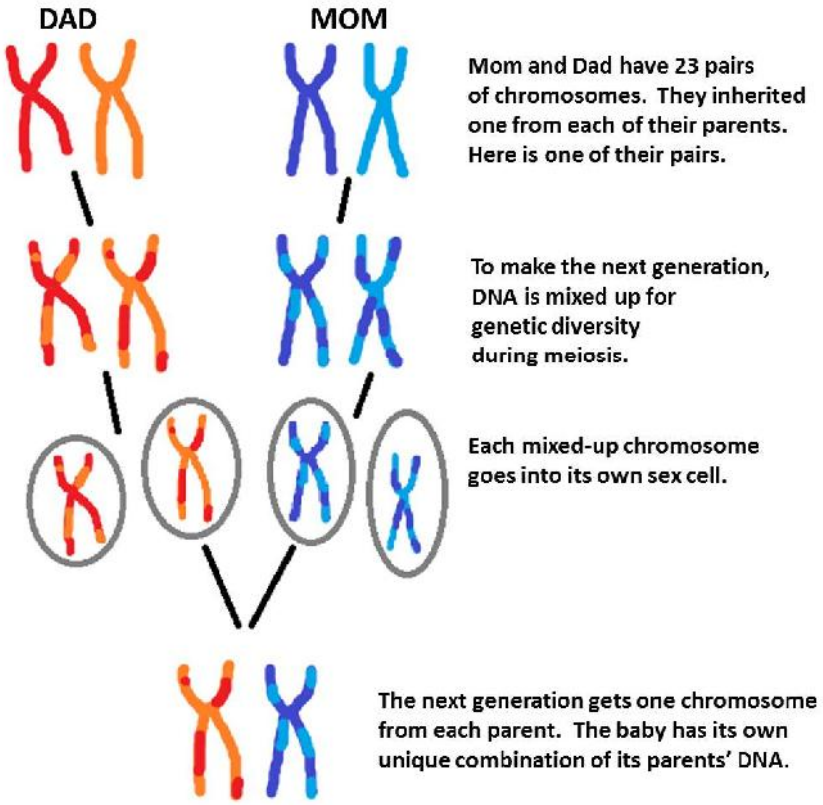
Outline

- DNA Fingerprinting
- Marker Assisted Selection (MAS)
- Marker Assisted Backcross (MABC)
- Marker Assisted Pyramiding
- Quantitative Trait Loci (QTL)
- Marker Assisted Recurrent Selection(MARS)
- Genomic Selection

Introduction

Genetic diversity

The differences that distinguish one plant from another are encoded in the plant's genetic material, the DNA. DNA is packaged in chromosome pairs, one coming from each parent. The genes, which control a plant's characteristics, are located on specific segments of each chromosome.



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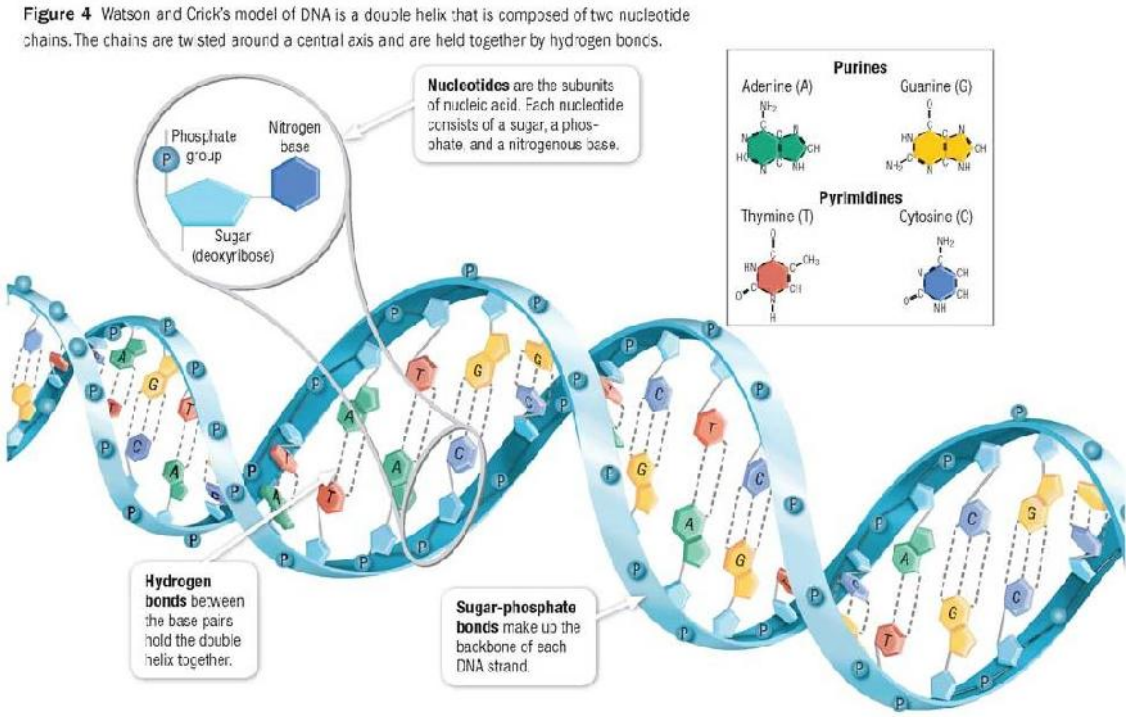
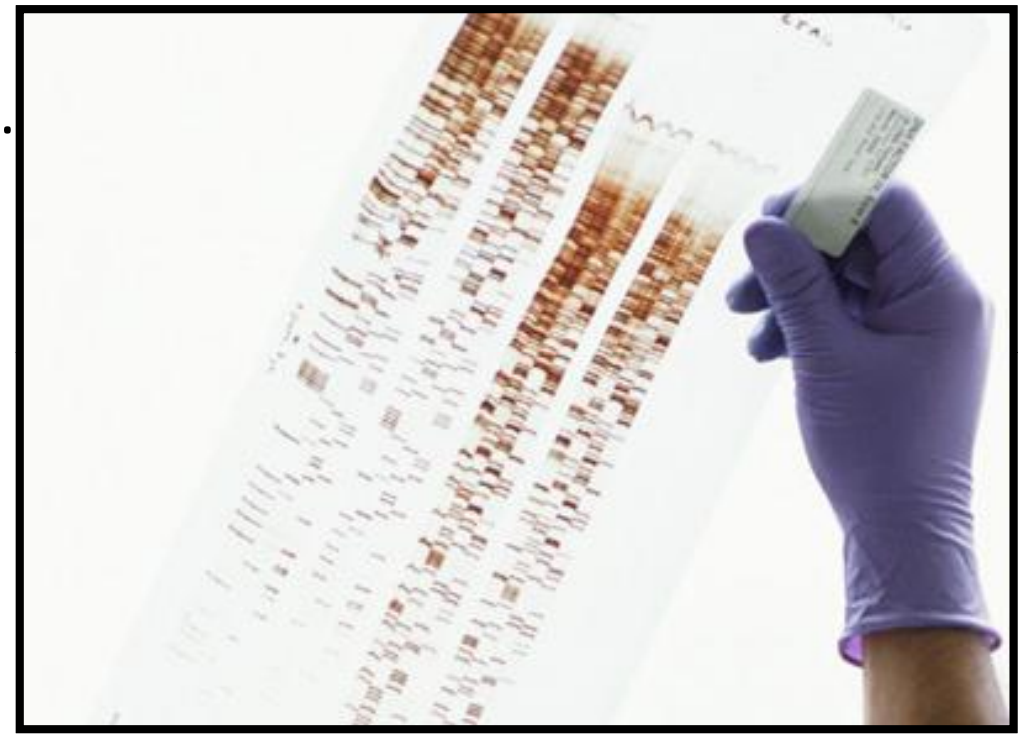


DNA Fingerprinting

DNA fingerprinting, also called DNA typing, DNA profiling, genetic fingerprinting, genotyping, or identity testing, is genetics method used for isolating and identification the base-pair pattern in individual's DNA

DNA fingerprinting is used in several ways.

- Paternity and Maternity test
- Plant Variety Protection
- Genetic purity test
- Studying biodiversity
- Tracking genetically modified crops



DNA Fingerprinting

An Example: Using DNA in Paternity and Maternity test / Plant variety protection and genetic purity test

Each genotype showing unique pattern

Testing can be done on seed or leaf

F = female parent, M = male parent

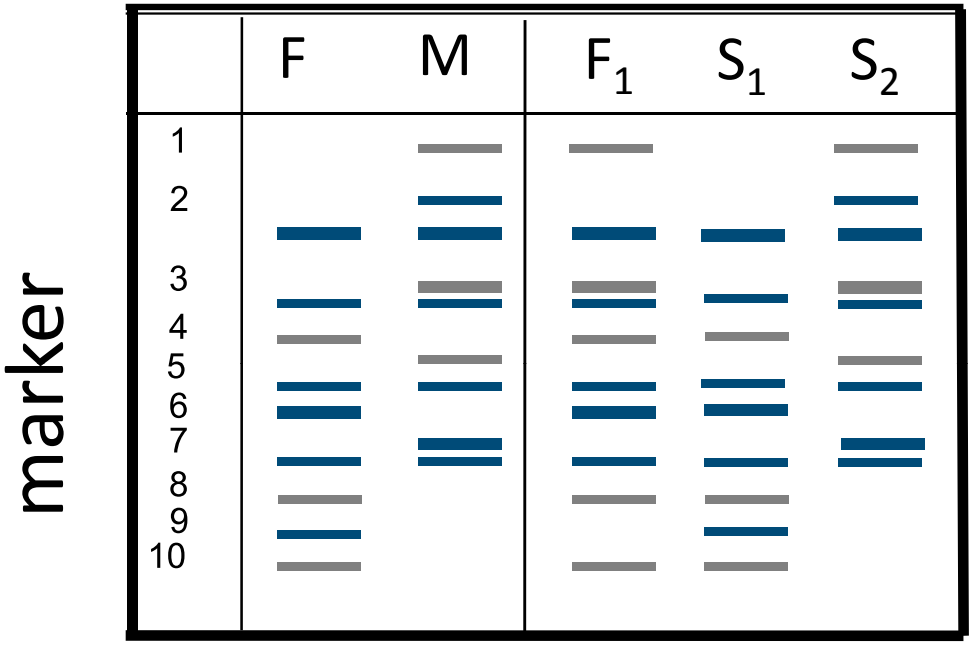
F1 = Hybrid

S1 = Sample#1

: Same female / different male

S2 = Sample#2

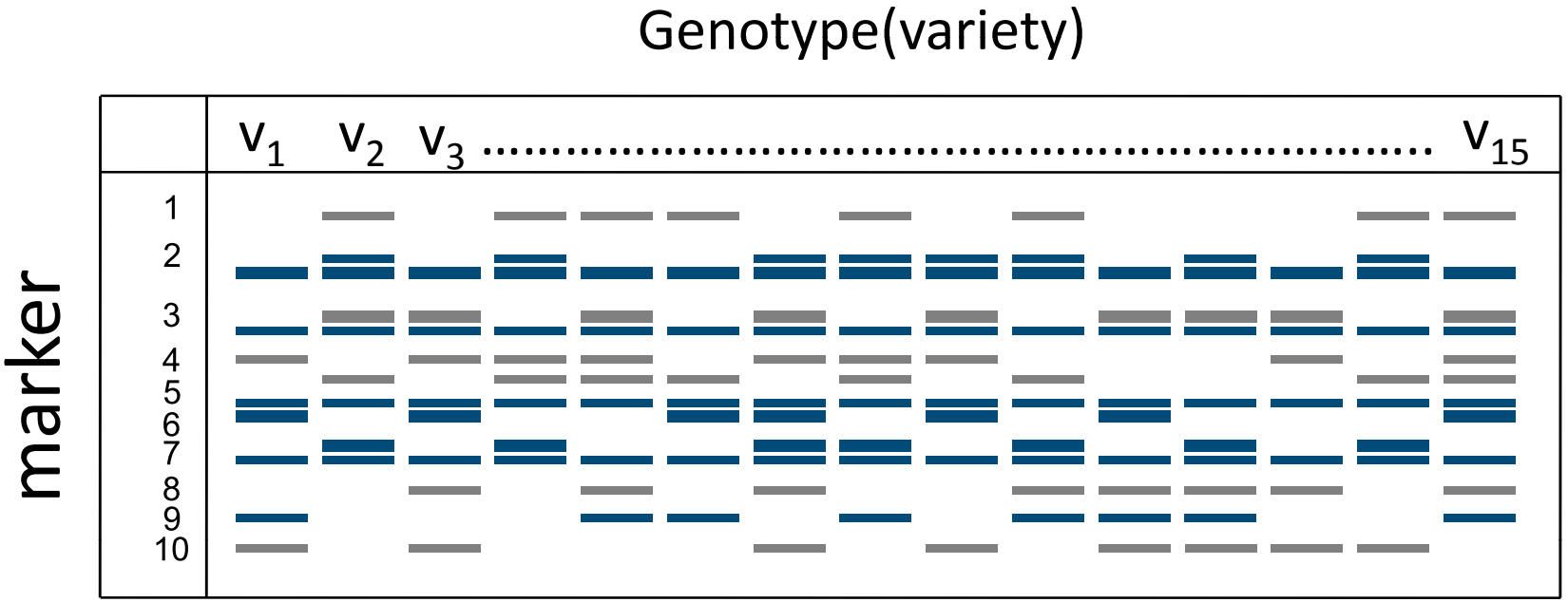
:Different female / Same male



DNA profile using 10 different marker (dominant marker)

DNA Fingerprinting

Studying biodiversity



DNA amplification profile of 15 genotype using 10 different marker (dominant marker)

DNA Fingerprinting

- **Genetic distance**
- **Cluster analysis**

Useful information for Breeder to arrange heterotic group

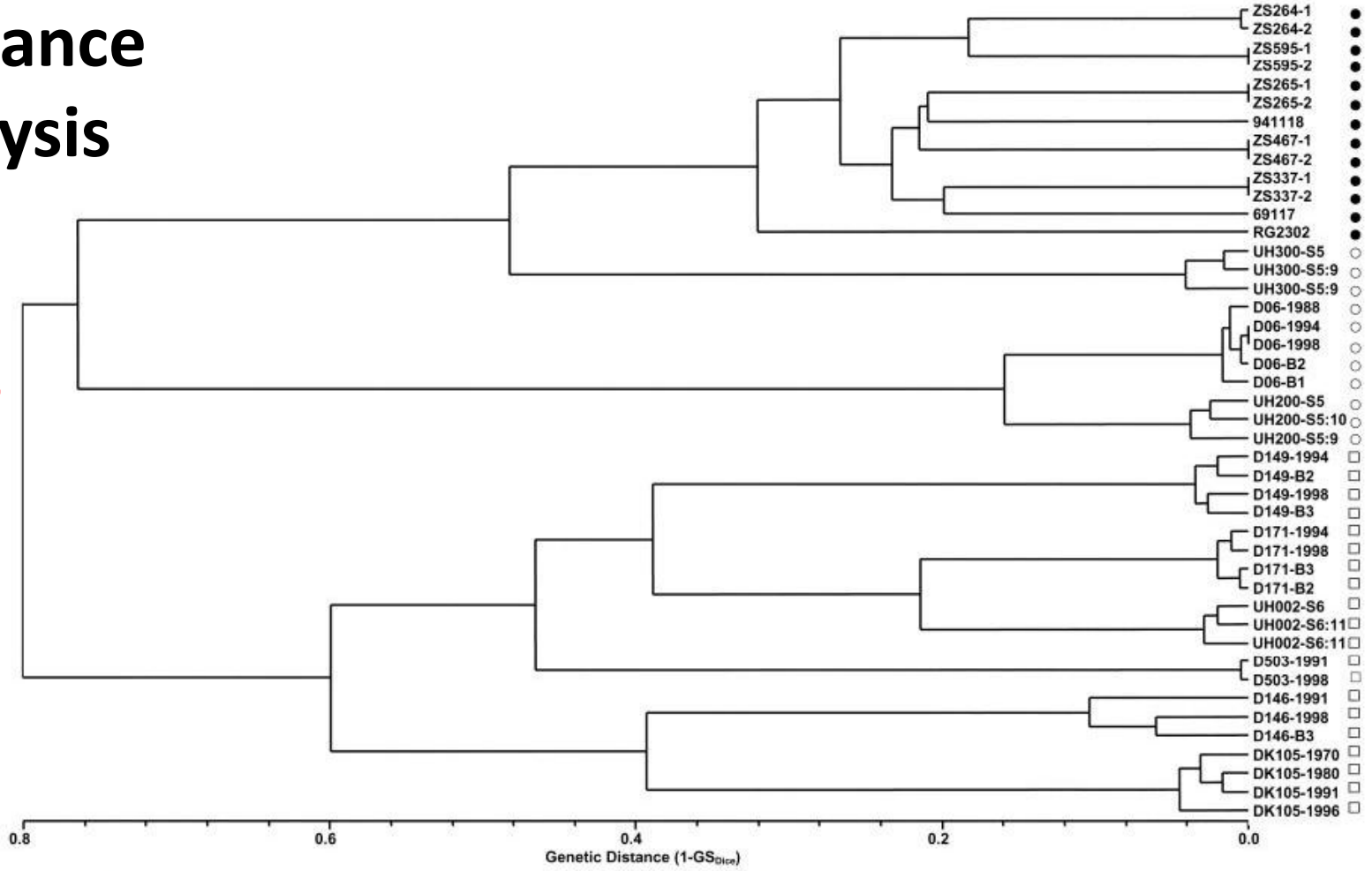


Figure 2. Associations among accessions of maize inbred lines revealed by UPGMA cluster analysis based on genetic distances calculated from SSR data. Asterisks (*) at the forks indicate that the group right of the fork was found for at least 95% of 1000 bootstrap runs. DH lines are marked by filled circles (●). Flint and dent lines are marked with squares (□) and circles (● ○), respectively.

Variation of DNA fingerprints among accessions within maize inbred lines and implications for identification of essentially derived varieties. Molecular Breeding 10: 181–191, 2002

Molecular Breeding

Molecular breeding (MB) may be defined in a broad-sense as the use of genetic manipulation performed at DNA molecular levels to improve characters of interest in plants and animals (MAB+GMO)

Marker-assisted breeding (MAB) and is defined as the application of molecular biotechnologies, specifically molecular markers, in combination with linkage maps and genomics, to improve plant or animal traits on the basis of genotypic assays this term is covered several modern breeding strategies, including marker-assisted selection (MAS), marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), and genome-wide selection (GWS) or genomic selection (GS) (Ribaut et al., 2010)

Guo-Liang Jiang: Molecular Markers and Marker-Assisted Breeding in Plants

Molecular Breeding

Some traits, like flower color, may be controlled by only one gene. Other more complex characteristics like crop yield or starch content, may be influenced by many genes.

Traditionally, plant breeders have selected plants based on their visible or measurable traits, called the **phenotype**. This process can be difficult, slow and influenced by the environment.

http://www.isaaa.org/resources/publications/pocketk/19/default.asp

Molecular Breeding

USING MOLECULAR MARKERS

Some of the advantages of using molecular markers instead of phenotypes to select are:

- o Early selection (at seedling, or even for seeds) → **Chance to select the right plant before flowering**
- o Reduced cost (fewer plants, shorter time)
- o Reduced cycle time (if gene is recessive or measured after flowering) → **Chance to select heterozygous plant**
- o Screening more efficient (if it is a complex trait)

Moreaux, 2011

Molecular Breeding

Molecular Breeding Method

- Marker Assisted Selection (MAS)
- Marker Assisted Backcross (MABC)
- Marker Assisted Pyramiding
- Marker Assisted Recurrent Selection (MARS)
- Quantitative Trait Loci (QTL)
- Genomic Selection

Molecular Breeding Method

Marker Assisted Selection (MAS)

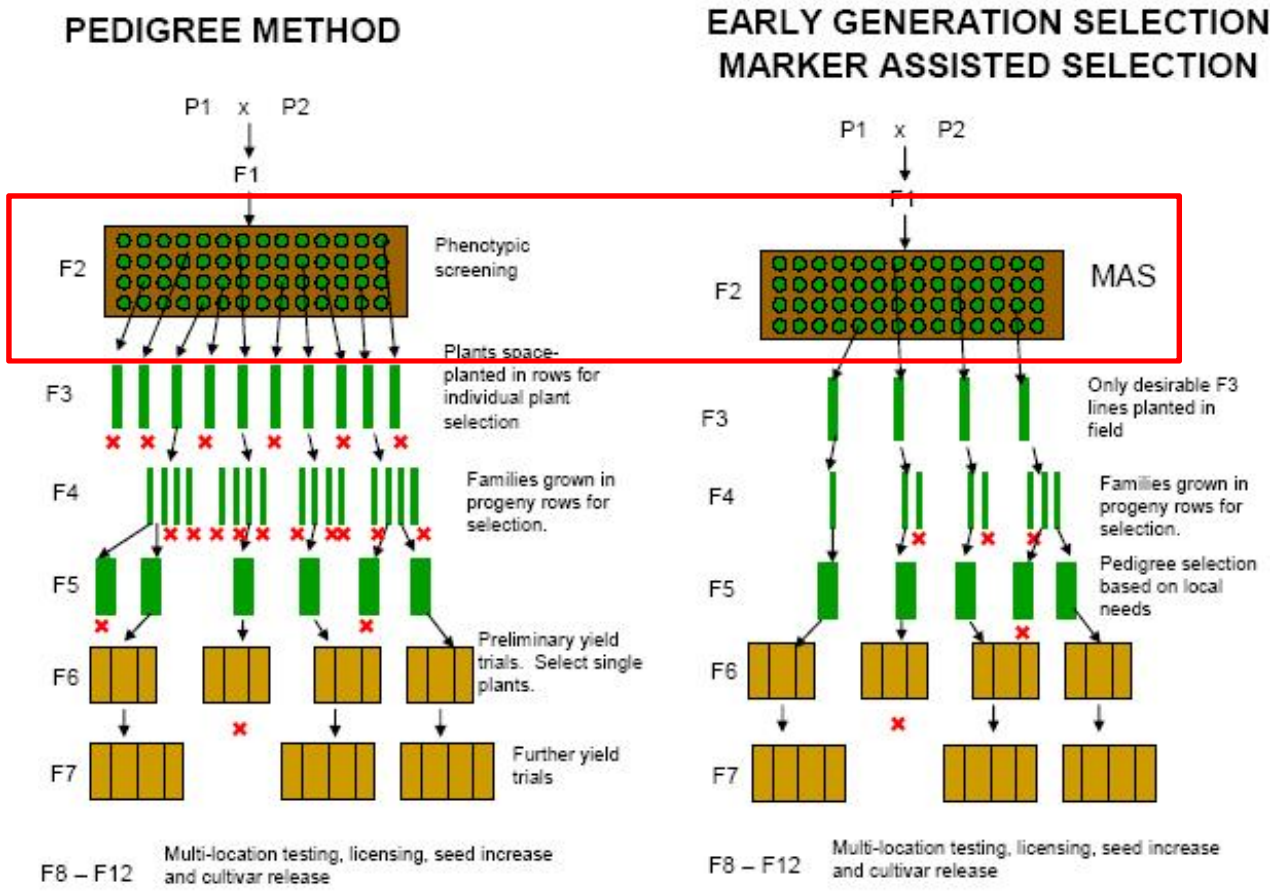
The use of DNA markers that are tightly-linked to target loci as a substitute for or to assist phenotypic screening or selection.

Marker Assisted Selection

Early generation selection

The main advantage is to discard many plant with unwanted gene combinations, especially those that lack essential disease resistance traits .

This has important in the later stages of the breeding program because the evaluation for other traits can be more efficiently and cheaply designed for fewer breeding lines .



http://www.knowledgebank.irri.org/ricebreedingcourse/Marker_assisted_breeding.htm

Marker Assisted Selection: An example with sweet corn

Corn Genes Affecting Carbohydrate Composition of the Kernel

Most well known sweetness gene

Gene[a]	Gene Symbol	Chromosome	Kernel Phenotype[b]
amylose extender1	*ae1*	5	tarnished, translucent, or opaque; sometimes semi-full
brittle1	*bt1*	5	shrunken, opaque to tarnished
brittle-2	*bt2*	4	shrunken, opaque to tarnished
dull1	*du1*	10	opaque to tarnished; S.C.[c] semi-collapsed translucent with some opaque sectors
miniature seed1	*mn1*	2	small, somewhat defective kernel, viable
shrunken1	*sh1*	9	collapsed, opaque
shrunken-2	*sh2*	3	shrunken, opaque to translucent
shrunken-4	*sh4*	5	shrunken, opaque
soft starch1	*h1*	—	opaque
sugary1	*su1*	4	wrinkled, glassy; S.C. not as extreme
sugary-2	*su2*	6	slightly tarnished to tarnished
waxy1	*wx1*	9	opaque
Sugar enhanced	*se*	2	

[a] All gene loci are named and symbolized using the revised rules for genetic nomenclature.[35]

[b] Adapted from Garwood and Creech.[56]

[c] S.C. Sweet corn background differs from dent background.

Source: From Hallauer, A.R., *Specialty Corns*, CRC Press, Boca Raton, FL, 1994. With permission.

Marker Assisted Selection

Important gene controlling endosperm in sweet corn

Category	Gene	Sweetness	Texture	Flavor	Germination /Vigor	Shelf life
Standard sweet	su1	10% sucrose ↓	creamy ↑	good ↑	good ↑	short ↓
Sugar-enhanced	se	2X sucrose	creamy	good	good	longer
Super sweet	sh2,bt1, bt2	3X-8X sucrose ↑	Less creamy ↓	poor ↓	poor ↓	Long ↑

Kamol Lertrat / Taweesak Pulam: Breeding for incresing sweetness in corn

Marker Assisted Selection

In recent years new varieties have been developed that have different combinations of the three major genes (su, se and sh2) 'stacked' together.

Category	Kernels type	Advantage	Variety name
High sugar sweet corn	• 25% sh2 kernels • 25% se kernels • 50% su kernels	• su vigor • higher sugar	• Sweet Chorus • Sweet Rhythm
High sugar sweet corn	• 100% sh2 kernel • se trait in all kernels	• high sugar • long shelf life • tender	• Gourmet Sweet™ • Multisweet™ • Xtra-Tender Brand™

http://www.uvm.edu/vtvegandberry/factsheets/corngenotypes.html

Molecular Breeding Method

Marker Assisted Backcross (MABC)

MABC aims to transfer one or a few genes/QTLs of Interest from one genetic source into a superior cultivar or elite breeding line to improve the targeted trait.

Marker Assisted Backcross

Two levels of selection in which markers may be applied in backcross breeding.

• Select backcross progeny carrying the target gene which tightly-linked to flanking markers (**foreground selection**).

• Select backcross progeny with background markers (**background selection**) to accelerate the recovery of the recurrent parent genome.

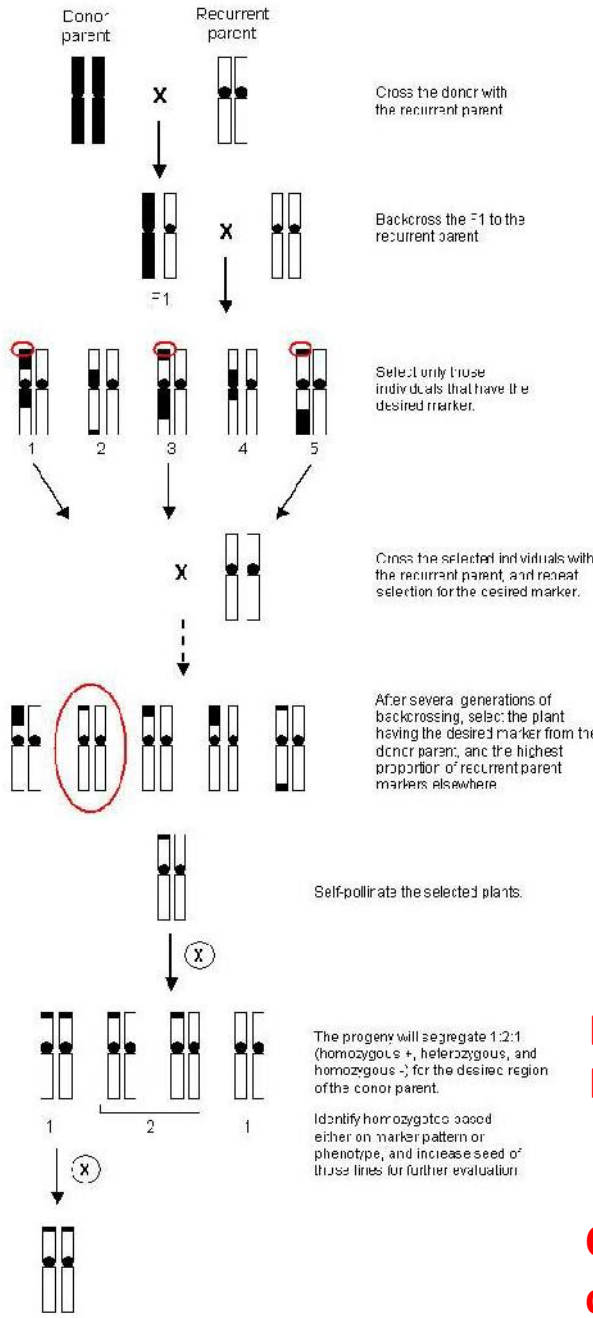
Marker Assisted Backcross (MABC)

FOREGROUND SELECTION

Use markers to transfer genes or QTL of major effects. One or multiple genes may be transferred. Markers should be closely linked to the gene of interest to avoid loosing them by recombination

BACKGROUND SELECTION

Use markers to control for genetic background in a BC cycle. To speed the process of recovery of the elite germplasm, markers may be used along the genome.

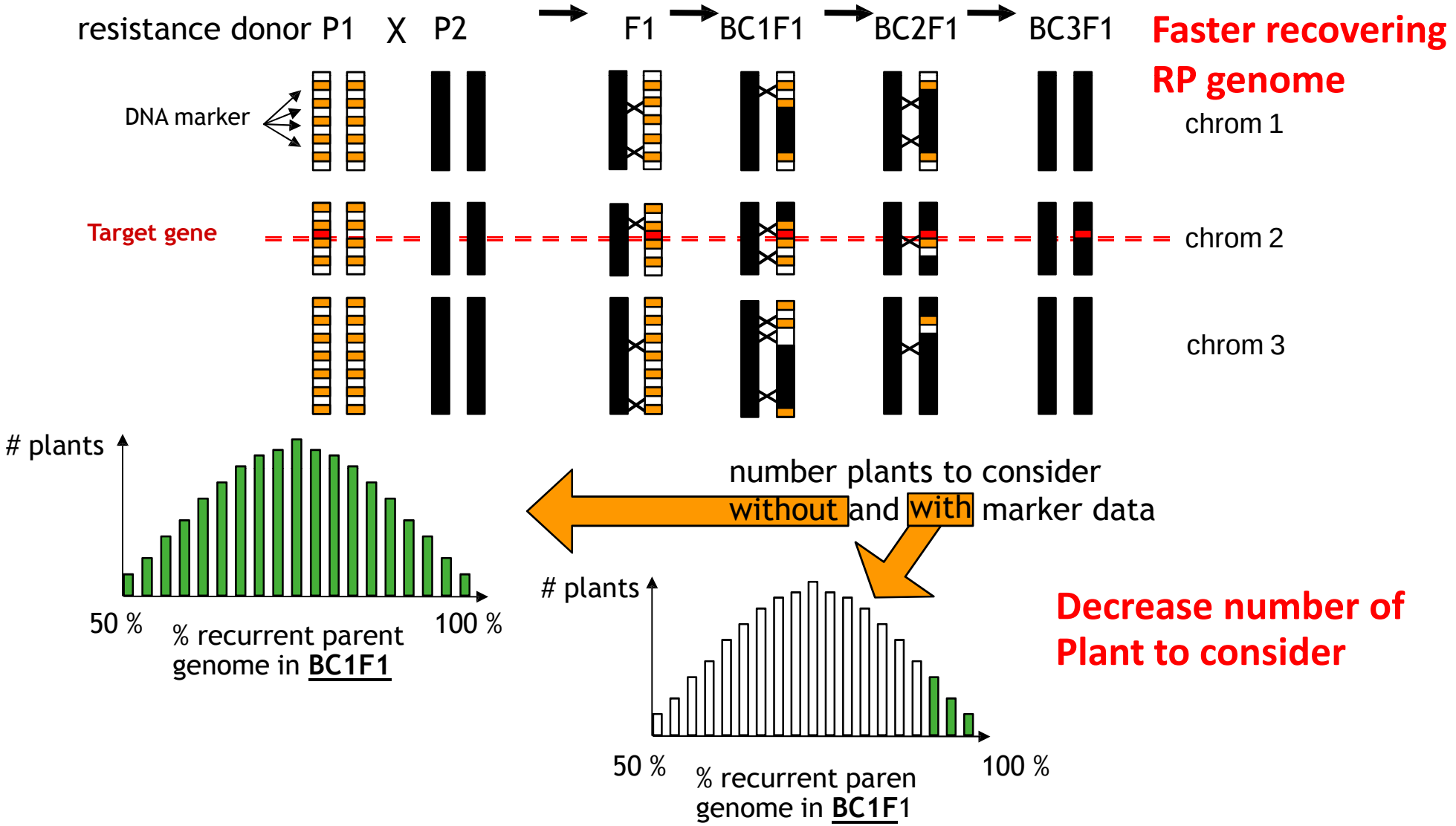


http://passel.unl.edu/pages/informationmodule.php?idinformationmodule=1087

Marker Assisted Backcross (MABC)

<u>Background selection</u>:

Increase the level of recovering recurrent parent genome in BC generation



Molecular Breeding Method

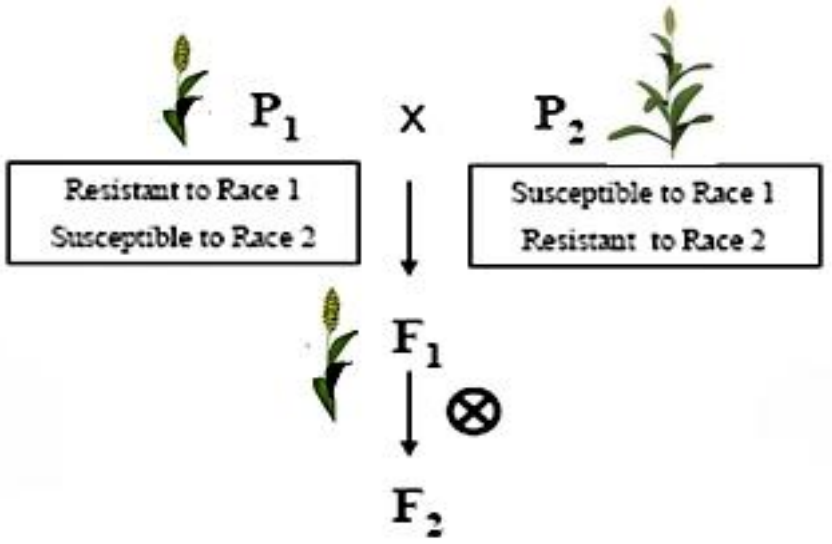
Marker Assisted Pyramiding

Pyramiding is the process of combining multiple genes/QTLs together into a single genotype. This is possible through conventional breeding but extremely difficult or impossible at early generations. DNA markers may facilitate selection because :

- DNA marker assays are non-destructive
- Markers for multiple specific genes/QTLs can be tested without phenotyping.
- The most widespread application for pyramiding has been for combining multiple disease resistance genes in order to develop durable disease resistance.

http://www.knowledgebank.irri.org/ricebreedingcourse/Marker_assisted_breeding.htm

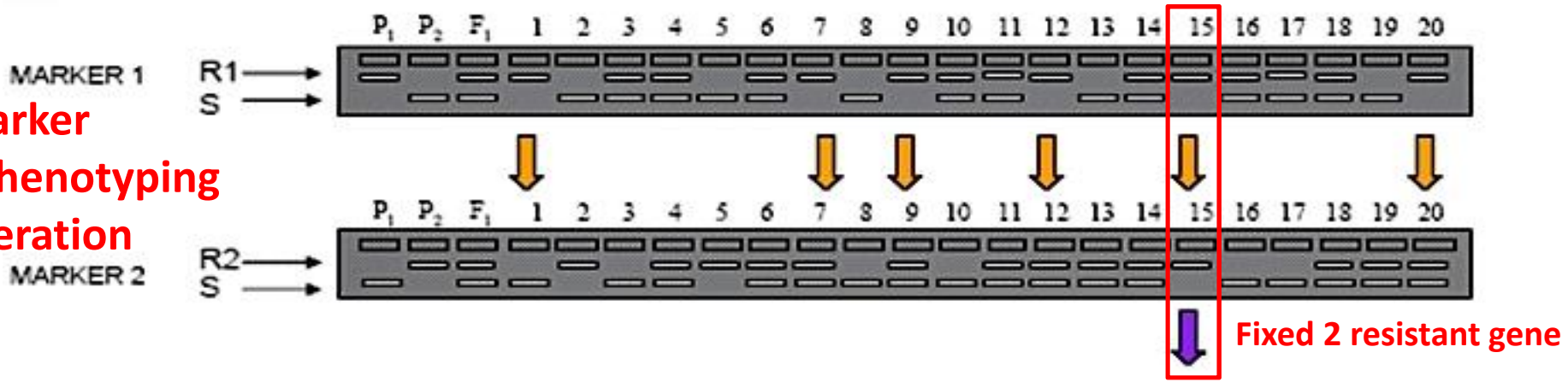
Marker Assisted Pyramiding



Segregating population

Line number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Phenotype Race 1	R	S	R	R	S	R	R	S	R	R	R	R	S	R	R	R	R	R	S	R
Phenotype Race 2	S	R	S	R	R	R	R	S	R	S	R	R	R	R	R	S	S	R	R	R

Marker tightly link to the gene



Select by marker
Instead of phenotyping
In early generation

Marker Assisted Pyramiding

Gene pyramiding in major crop

Table 1. Selected examples of MAS based gene pyramiding for important traits in major crops.

Crop	Trait	Pyramided genes	Reference
Rice	Blight resistance	*Xa4,xa5,xa13,Xa21*	Huang et al., 1997, Singh et al., 2001, Narayanan et al., 2002
	Blast resistance	*Pi(2)t,Piz5,Pi(t)a*	Hittalmani et al., 2000
	Gallmidge resistance	*Gm1,Gm4*	Kumaravadivel et al., 2006
Wheat	Leaf rust resistance	*Lr41, Lr42, Lr43*	Cox et al., 1994
	Powdery mildew resistance	*Pm-1, Pm-2*	Liu et al., 2000
Cotton	Insect pest resistance	*Cry 1Ac, Cry 2Ac*	Jackson et al., 2003, Gahan et al., 2005
Pea	Nodulation ability	*Sym9, Sym10*	Schneider et al., 2002
Barley	Yellow mosaic virus resistance	*rym4, rym5, rym9, rym11*	Werner et al., 2005
Soybean	Soybean mosaic virus resistance	*Rsv1, Rsv3, Rsv4*	Zhu et al., 2006

Marker Assisted Pyramiding

Example: Pyramiding of *xa* gene (blb resistant gene) in rice

Marker-aided selection (MAS)-improved varieties developed by NARES teams from Philippines, Indonesia, India and China, 2002-2003

Country	Background commercial/ Yield standard	Released (R) / Near -release (NR) + Introgressed gene(s)	Yield (t/ha)	Gain over yield std (%)
Philippines	IR64	AR32 -19 -3 -2 (*xa5*/*Xa21*) (NR)	5.1	0
	IR64	AR32 -19 -3 -3 (*xa5*, *Xa21*) (NR)	6.7	31.4
	IR64	AR32 -19 -3 -4 (*xa5*/*Xa21*) (NR)	6.1	19.6
	BPI Ri10	AR32 -4 -3 -1 (*xa5*/*Xa21*) (NR)	6.0	17.6
	BPI Ri10	AR32 -4 -58 -2 (*xa5*/*Xa21*) (NR)	6.5	27.5
	PSB Rc28	Yield standard	5.1	-
Indonesia	IR64	Angke (Bio1) (*Xa4*/*xa5*) (R)	5.4	20.0
	IR64	Conde (Bio 2) (*Xa4*/*Xa7*) (R)	5.4	20.0
	IR64	Yield standard (*Xa4*)	4.5	-
India	PR106	IET17948 (*xa5*/*xa13*/*Xa21*) (NR)	8.2	22.4
	PR106	IET17949 (*xa5*/*xa13*/*Xa21*) (NR)	7.9	17.9
	PR106	Yield standard	6.7	-
China	Zhong 9A/Zhonghui 218	Hybrid Guofeng No. 2 (*Xa21*) (HR, NR)	7.8	11.4
	II-3A/Zhonghu i 218	Hybrid II You 218 (*Xa21*) (HR, R)	8.3	18.6
	Shanyou 46	Yield standard	7.0	-

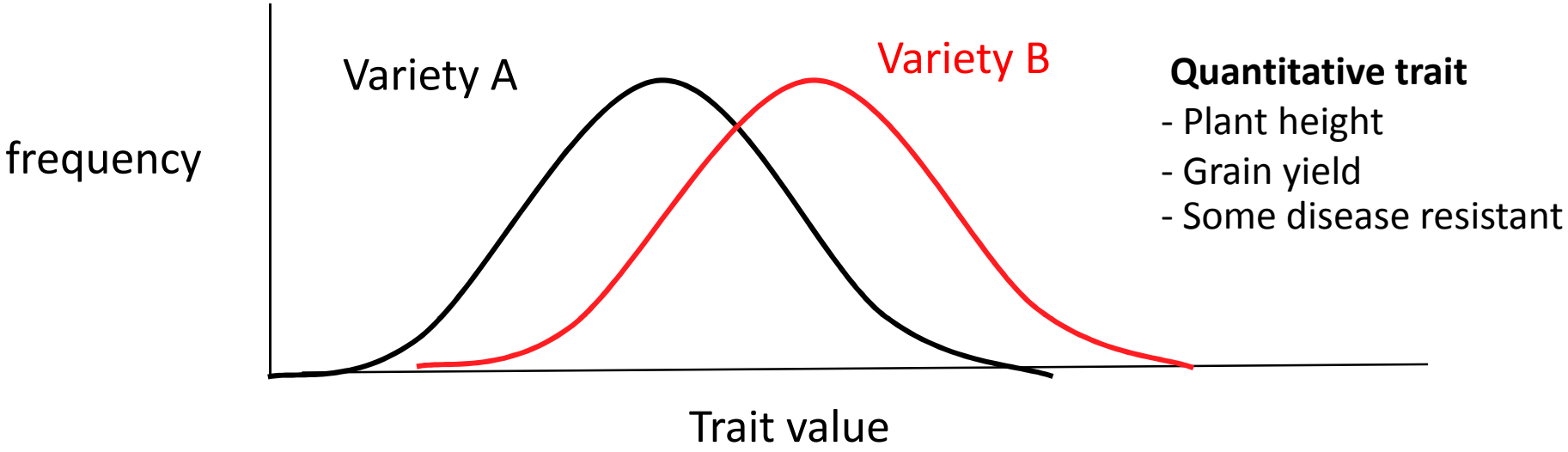
Marker Assisted Pyramiding

MAS-improved pyramided IR64 with xa5, Xa7 and Xa21

Quantitative Trait Loci (QTL)

Quantitative trait

- Trait that show continuous variation in population
- combined effect of several genes
- bell curve distribution of phenotypic values, produces a range of phenotypes



Purpose of QTL study using in plant breeding is

1.To localize chromosomal region that significantly effect the variation of quantitative trait in the population

2. Introgression of favorable QTLs region in to elite variety

QTL mapping

Quantitative Trait Loci (QTL)

A quantitative trait locus/loci (QTL) is the location or region of individual locus or multiple loci in the genome that affects a trait that is measured on a quantitative .

QTLs mapping process

- Develop mapping population (F2, DH, NIL, BC, RIL)
- Genotyping (Polymorphic marker)
- Constructing of linkage maps (linkage between marker)
- Phenotyping (screen in field)
- QTLs analysis
  - Test association between phenotypic trait and marker
  - Identify major /minor QTL

Quantitative Trait Loci (QTL): An example with rice

Linkage maps

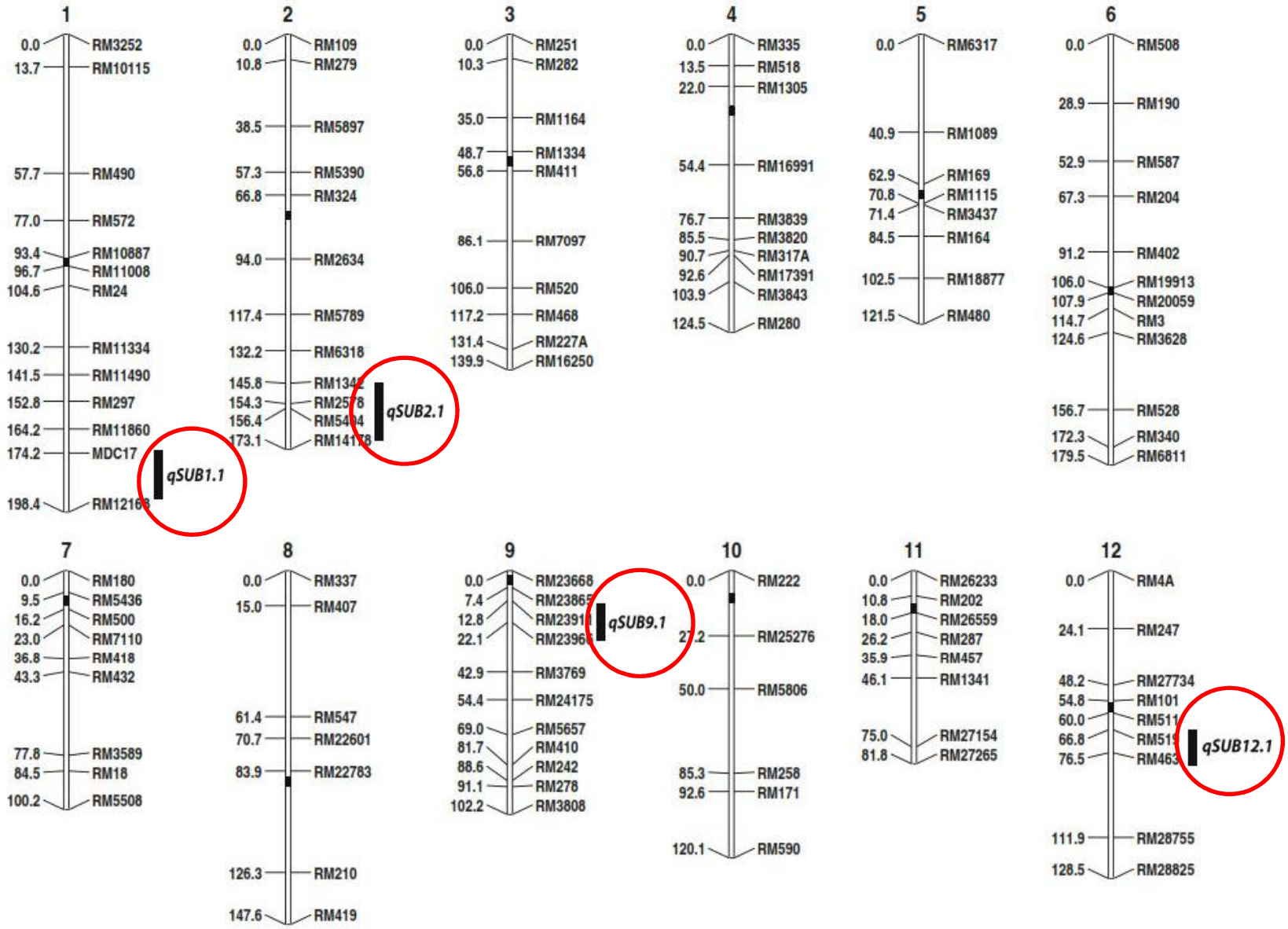


Fig. 2 Mapping of submergence-tolerant QTLs derived from IR72 and Madabaru. Molecular linkage map of an IR72 × Madabaru mapping population constructed with 115 SSR markers. The position of four significant submergence-tolerance QTLs on chromosomes 1, 2, 9, and 12 are illustrated by *black bars* next to the chromosomes. Centromeres are shown as *black boxes*

*Identifying novel QTLs for submergence tolerance in rice cultivars IR72 and Mada:baru:*Theor Appl Genet (2012) 124:867–874 DOI 10.1007/s00122-

Quantitative Trait Loci (QTL): An example with rice

QTL analysis

Table 1 QTLs for submergence tolerance identified from the IR72/Madabaru population

QTL	Chr.	Flanking markers	Source	QGene IM			QGene CIM			QTL Cart. IM			QTL Cart. CIM		
				LOD	R^2 (%)	Add	LOD	R^2 (%)	Add	LOD	R^2 (%)	Add	LOD	R^2 (%)	Add
qSUB1.1	1	MDC17-RM12168	IR72	**9.4**[a]	**41.9**	**30.5**	**9.2**	**40.9**	**28.5**	**11.2**	**52.3**	**24.5**	**11.1**	**37.7**	**21.2**
qSUB2.1	2	RM6318-RM2578	IR72	3.8[b]	19.6	1.2	*3.2*[c]	*16.6*	*2.1*	4.1	36.4	19.3	**4.8**	**19.4**	**15.8**
qSUB9.1	9	RM23911-RM23966	Madabaru	*3.6*	*18.6*	*13.7*	*3.3*	*17.4*	*12.7*	*3.4*	*17.1*	*12.2*	3.6	7.3	8.1
qSUB12.1	12	RM511-RM463	IR72	4.2	21.5	0.0	4.2	21.4	5.3	*3.5*	*16.3*	*13.0*	–	–	–

LOD explain linkage between marker and QTLs

R^2 explain phenotypic variance by QTLs (PVE)

Transfer QTL to elite germplasm

Validate QTLs

*Identifying novel QTLs for submergence tolerance in rice cultivars IR72 and Mada:baru:*Theor Appl Genet (2012) 124:867–874 DOI 10.1007/s00122-

Marker Assisted Recurrent Selection (MARS)

When much of the variation is controlled by many minor QTLs (20-30 QTLs), MABC has limited applicability because estimates of QTL effects are inconsistent and gene pyramiding becomes increasingly difficult as the number of QTLs increases.

A more effective strategy is to deploy MARS to increase the frequency of favorable marker alleles in the population.

Roberto Tuberosa: Dept. of Agroenvironmental Sciences and Technology , University of Bologna, Italy

Marker Assisted Recurrent Selection (MARS)

MARS involves:

- Defining a selection index for F_2 or F_2-derived progenies, use index to weight significant marker for target QTLs (20-30 QTLs)
- Recombining selfed progenies of the selected individuals
- Repeat the procedure for a number of cycles

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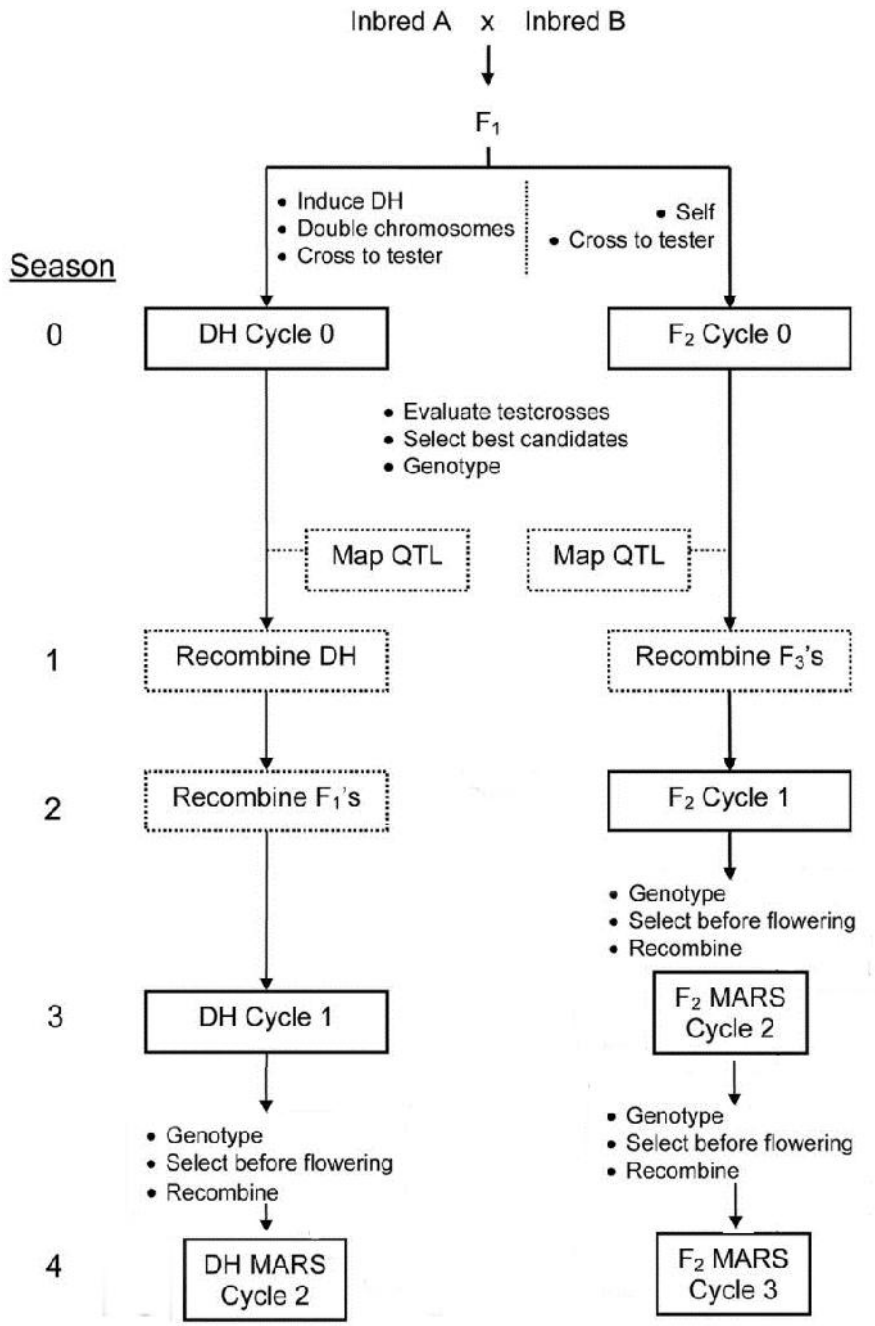
Marker Assisted Recurrent Selection (MARS)

Steps in a MARS in Maize:

1. MAS in Cycle 0

- Create an F2 (Cycle 0)
- Test-cross the F2
- Evaluate progeny in multiple environments
- Identify markers associated with trait of interest
- Create an index weighting significant markers by their effect using multiple linear regression (Lande and Thompson 1990).
- Recombine best progeny (best individuals from Cycle 0)

2. Select in greenhouse or off-season nursery (up to 3 cycles in low h^2 environment).



Patricio J. Mayor and Rex Bernardo:
Genomewide Selection and Marker-Assisted Recurrent Selection in Doubled Haploid versus F_2 Populations

Genomic Selection

Genomic selection (GS) is a new approach for improving quantitative traits in large plant breeding populations that uses whole genome molecular markers and combines marker data with phenotypic data in an attempt to increase the accuracy of the prediction of breeding and genotypic values.

http://genomics.cimmyt.org/

Genomic Selection

Objective of GS is to **predict the breeding value** of each individual instead of identifying QTL for use in a traditional marker-assisted selection (MAS) program

- Requires high-density molecular markers (LD level)
- GS considers the effects of all markers together and captures most of the additive variation
- Marker effects are first estimated based on a so-called "training population" that needs to be sufficiently large (> 300)
- Breeding value is then predicted for each genotype in the "testing population" using the estimated marker effects

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A.M. P?rez-de-Castro, S. Vilanova, J. Ca?izares, L. Pascual, J.M. Blanca, M.J. D?ez, J. Prohens* and B. Pic?

Genomic Selection Scheme

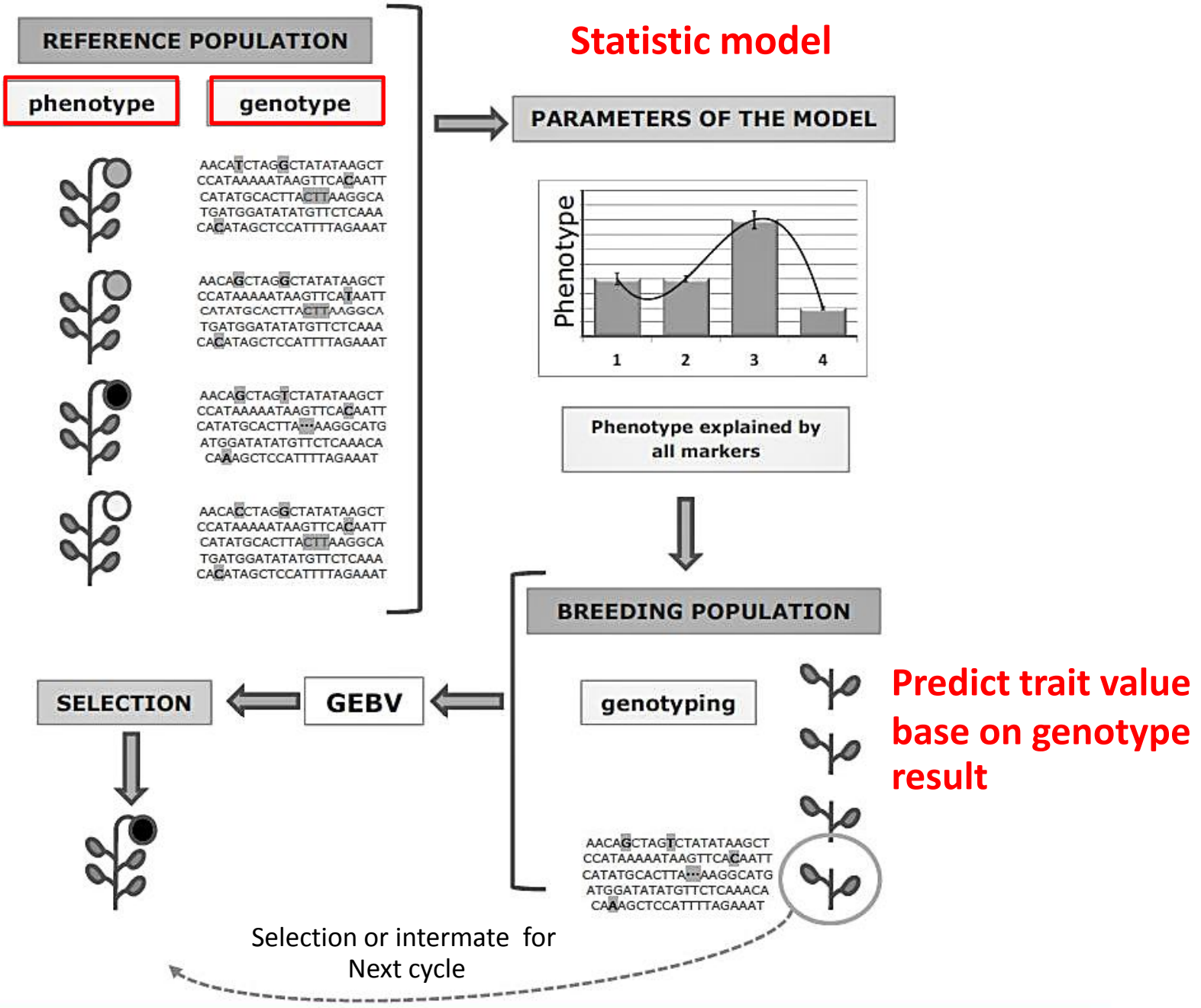

Fig. (1). Genomic selection scheme. Information on phenotype and genotype for a reference population allows estimating parameters for the model. This model explains phenotype based on all markers analyzed. The model predicts the phenotype of plants in a breeding population on the basis of the genotyping results: this is the genomic estimated breeding value (GEBV), used to select the desired phenotypes.

Thanks!