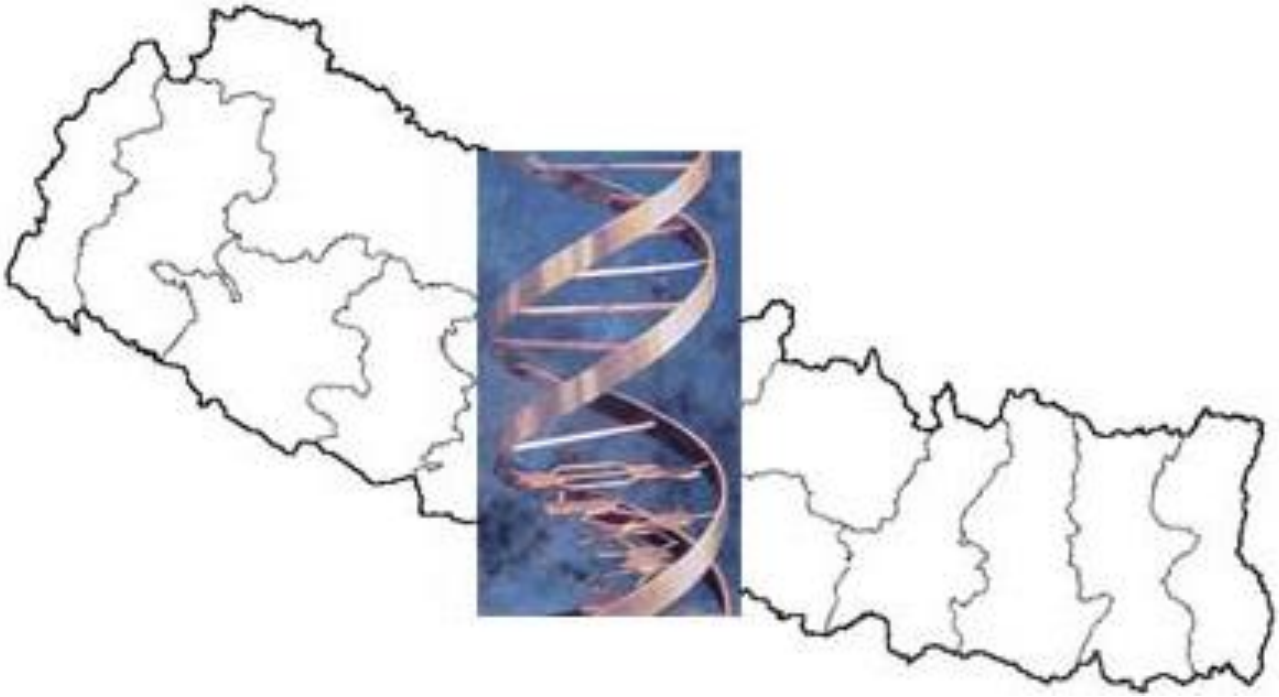
Biochemical and Molecular Markers in Plant Breeding

Progress and Prospect



Bal K. Joshi

Feb 2008

Plant Breeding

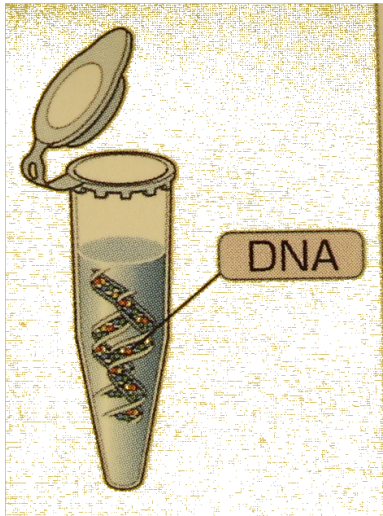
- The science, art and business of improving plants for the human benefit

Plant Breeding Era

Era 1. Domestication and phenotypic mass selection

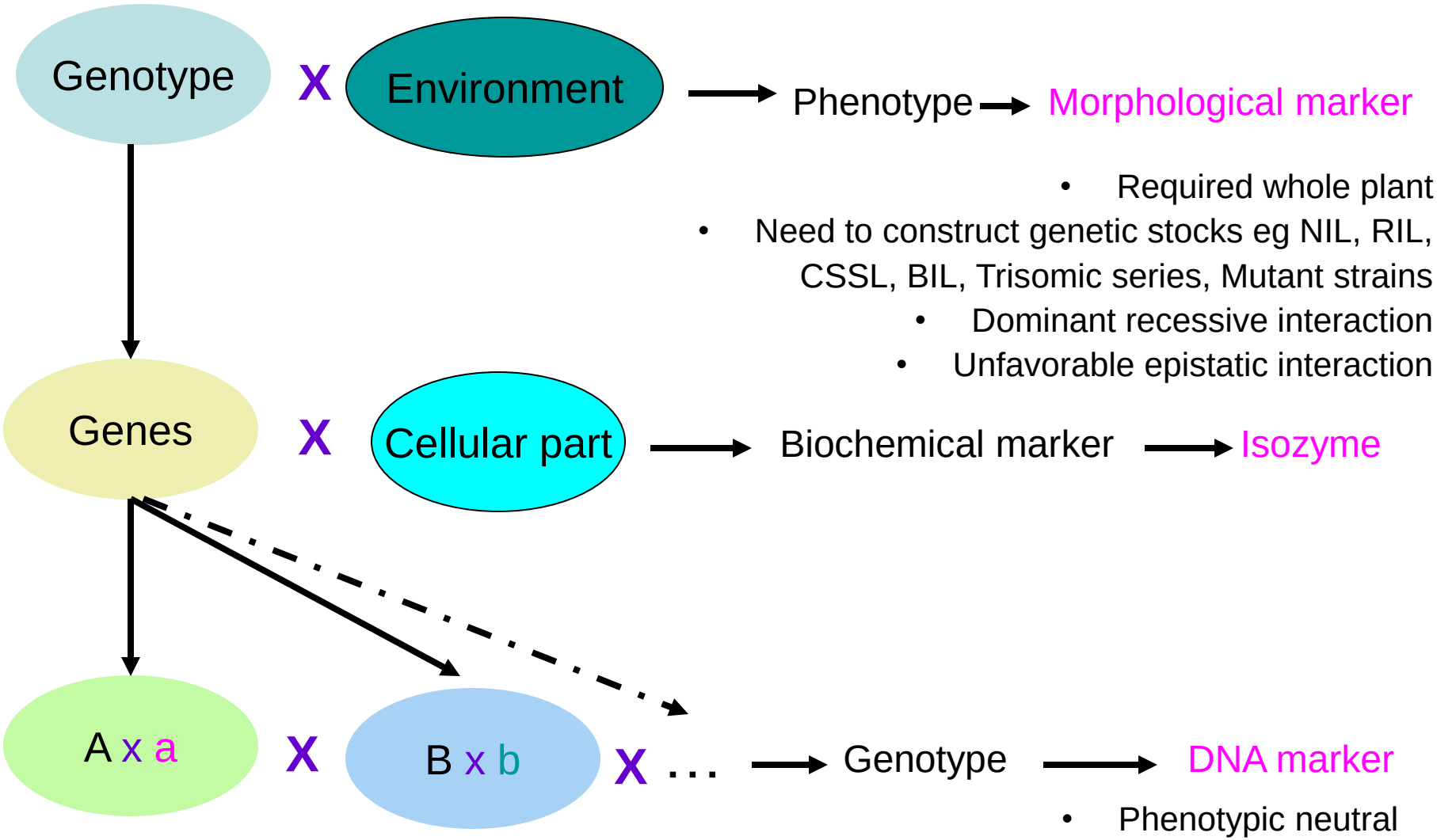
Era 2. Replicated progeny selection

Era 3. Direct genotypic selection



Marker systems

- Gene tagging
- Indirect selection
- Identification etc



- Required whole plant
- Need to construct genetic stocks eg NIL, RIL, CSSL, BIL, Trisomic series, Mutant strains
- Dominant recessive interaction
- Unfavorable epistatic interaction

- Phenotypic neutral
- Codominant manner
- Free of epistatic effect

Dominant marker: RAPD, Morphological…

Co-dominant markers: SSR, EST, Isozymes …

Which one is the best in term of yield &/or disease resistance?

Grow out test

Lab test

Phenotypic
(Conventional plant breeding)

Genotypic
(Molecular based breeding)

- Equally applicable to both mono and polygenic traits
- Equally effective to both recessive and dominant traits
- High resolution mapping

Genotype determined at any stage and time (Without gene expression)

Environment

Time

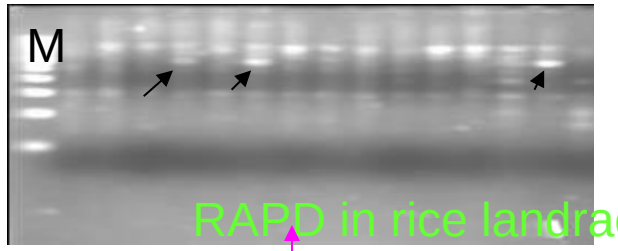
Inputs

- At any stage of crop development
- At early stage, many round of selection
- No need of inoculums
- No need of scoring and testing in hot spot
- From any small part
- Screening of multiple characters

Genotype ascertained only at the whole plant level (Complete gene expression)

Experimental steps in molecular based research

Seedlings
2 wks



RAPD in rice landraces

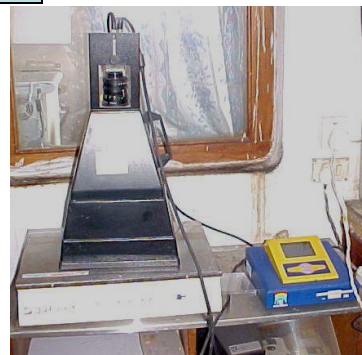
Scoring
And
analysis

DNA

Gel imaging

PCR

Electrophoresis

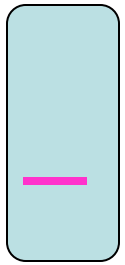


Inheritance of SSR marker

- dominance, recessive
- allelism test, number of alleles

QTL also in Mendelian principle

Parents



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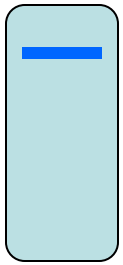

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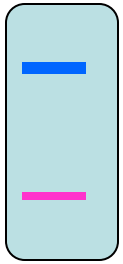
F1



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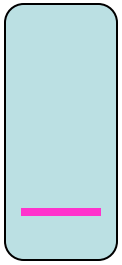
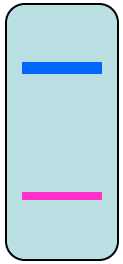
F2



6kb/6kb



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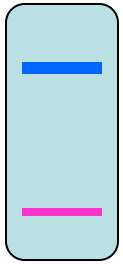

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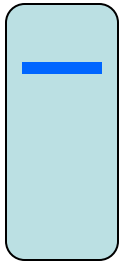

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Achievements

Diversity study

- SSR: rice, barley
- RAPD: wild and cultivated buckwheat, rice
- Isozyme: wild and cultivated rice, barley, mango, pigeon pea, buckwheat, swertia, taro, citrus

Running activities in rice and buckwheat

- Molecular tagging of blast resistance gene
- Marker Aided Selection (MAS) and Marker Aided Backcrossing (MAB)
- Developing NILs
- Linkage map construction
- QTL mapping

Software for analysis of biochemical and molecular data

- NTSYSpc
- PHYLIP
- Tree View
- Free Tree
- WinAMOVA Arelquin
- GGT
- PhotoCapMw

GDA
GenePop
PopGene
GeneStat

QTL Cartographer
MapMaker/EXP
MapMaker/QTL
Scion Image

Prospects

- Information based on the DNA is considered the most reliable
- Opportunity to select desirable lines based on the genotypes rather than phenotypic- extremely attractive to plant breeders
- Must identify marker linked to target traits

1. Characterization
2. Identification
3. Screening
4. Diversity and biosystematics study
5. Population structure
6. Linkage map construction and gene tagging
7. MAS and MAB
8. Comparative mapping

A. Characterization

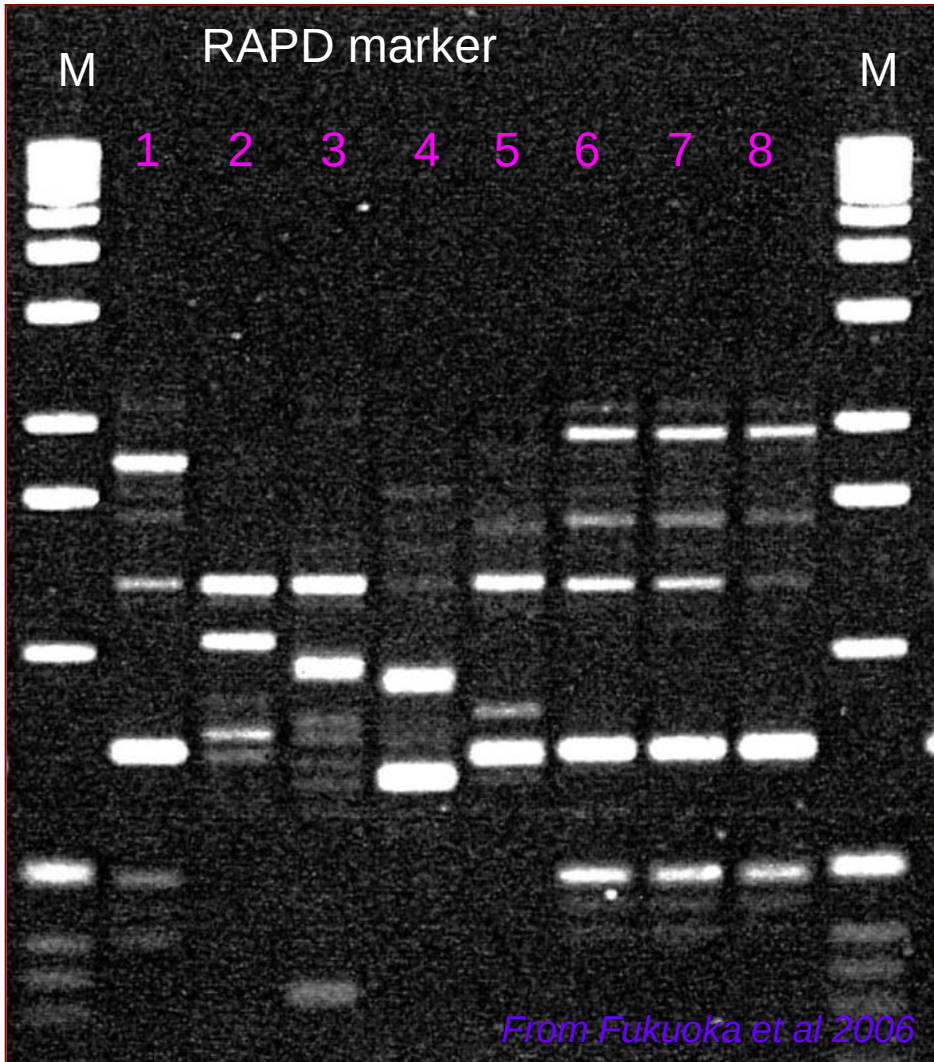
A. Characterization of accessions
B. Characterization of markers

Descriptive statistics

- No. of bands, Size, Homo/ heterozygous, Private allele

Diversity indices

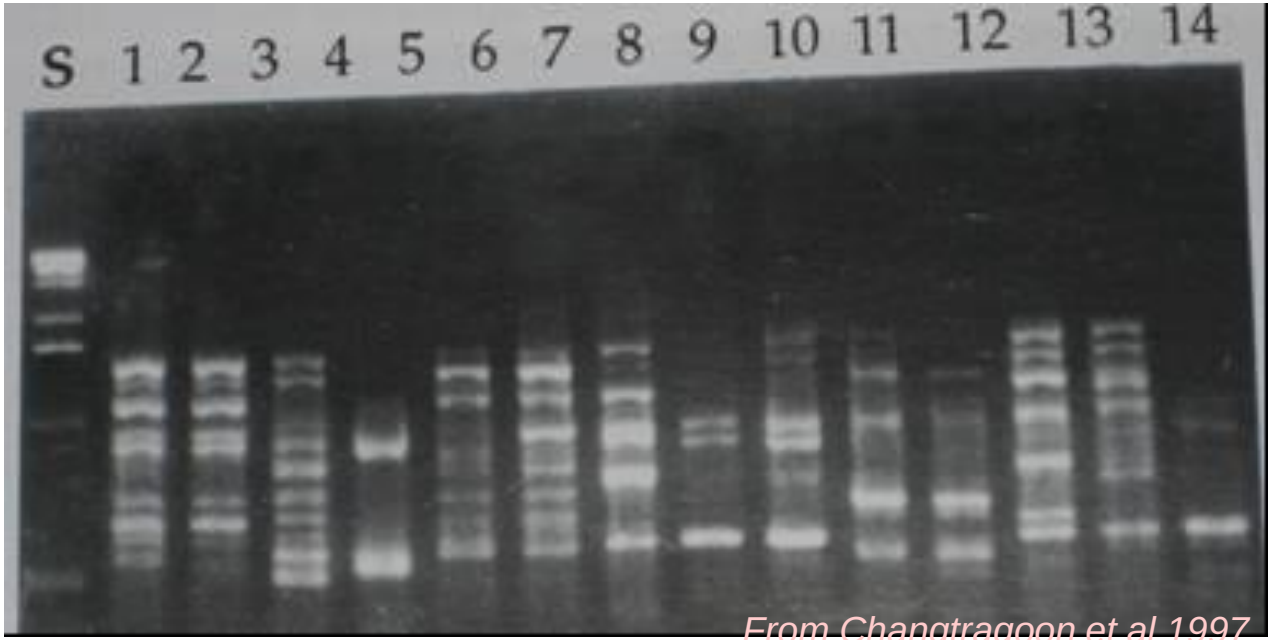
- Observed/ Expected heterozygosity
- Effective/ Number of alleles/locus
- Percent polymorphic loci
- Shannon's gene diveristy
- Gene/genotype frequency
- PIC



B. Identification

- Similarity/ dissimilarity in bands
- Private allele/s
- Fingerprinting
- Standard

- **Uses:**
- Plant variety/ cultivar protection, plant patenting
- Hybrid testing
- PGR management (duplicate identification)
- Breeding works (parent selection, introgression etc)



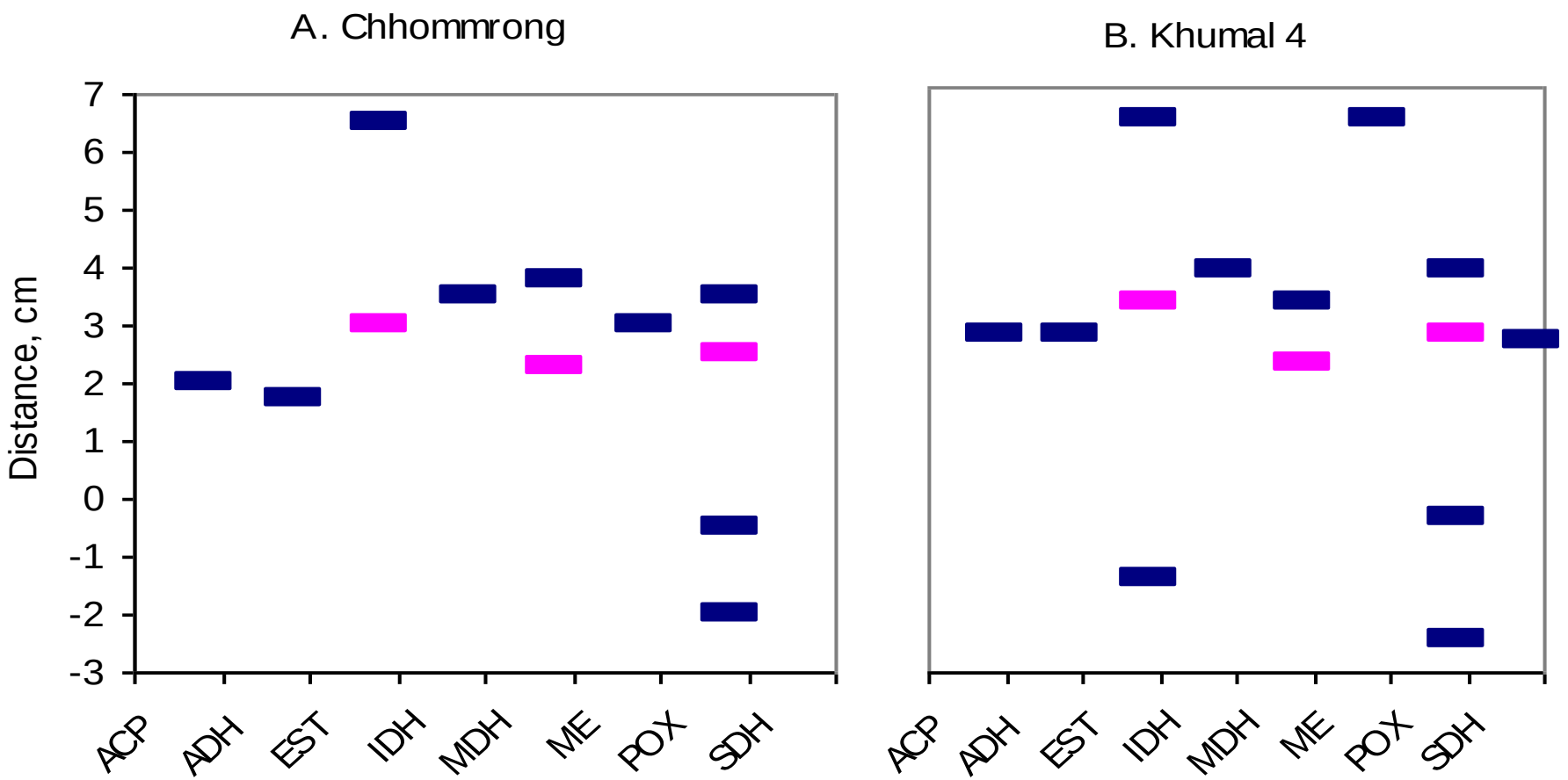
RAPD fragments amplified by OPA 13 primer in 10 spp of rattan showing species specific polymorphism (1=2, 3, 4, 5=6, 7, 8=9, 10=11, 12=13, 14, S= 1kb ladder

From Changtragoon et al 1997

Fingerprinting (Genotyping)

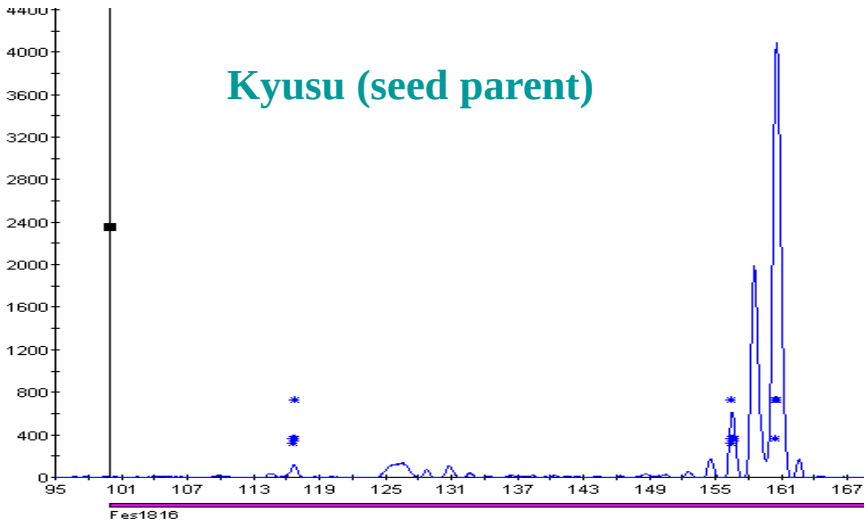
- 15 individual samples per variety in cross pollinated crop species
- 1 individual sample per variety in self pollinated crop species

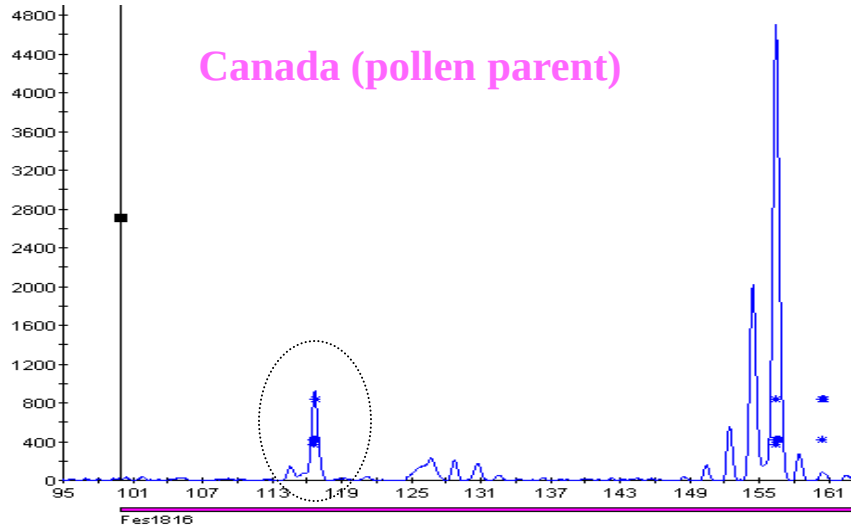
Isozyme profiles of rice cultivars (Multiple markers band)



Joshi and Bimb 2004

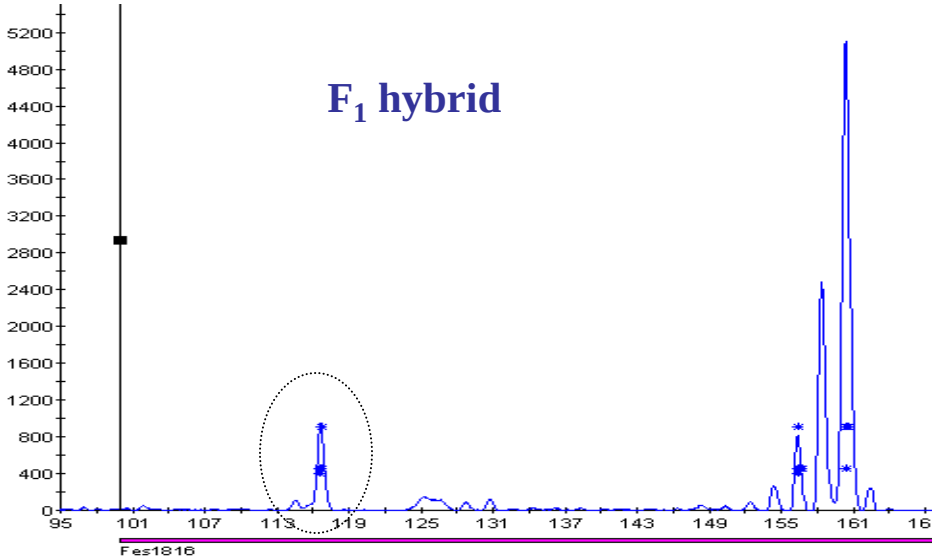
DNA sequence based F_1 testing

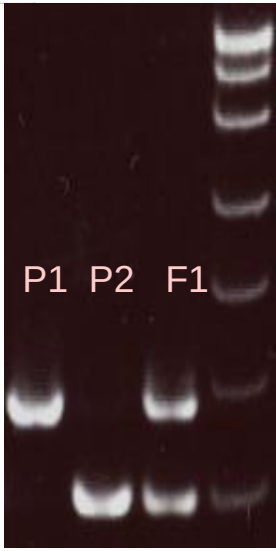




Primer: Fes 1816

SSR electropherogram clearly distinguished F1 hybrid



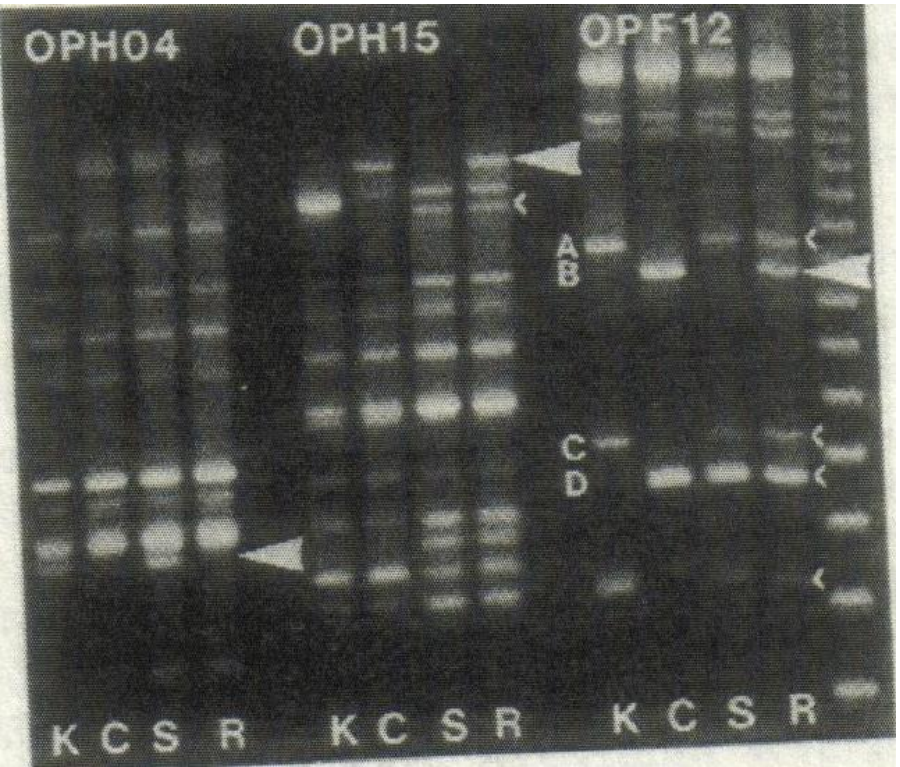


Joshi et al 2007

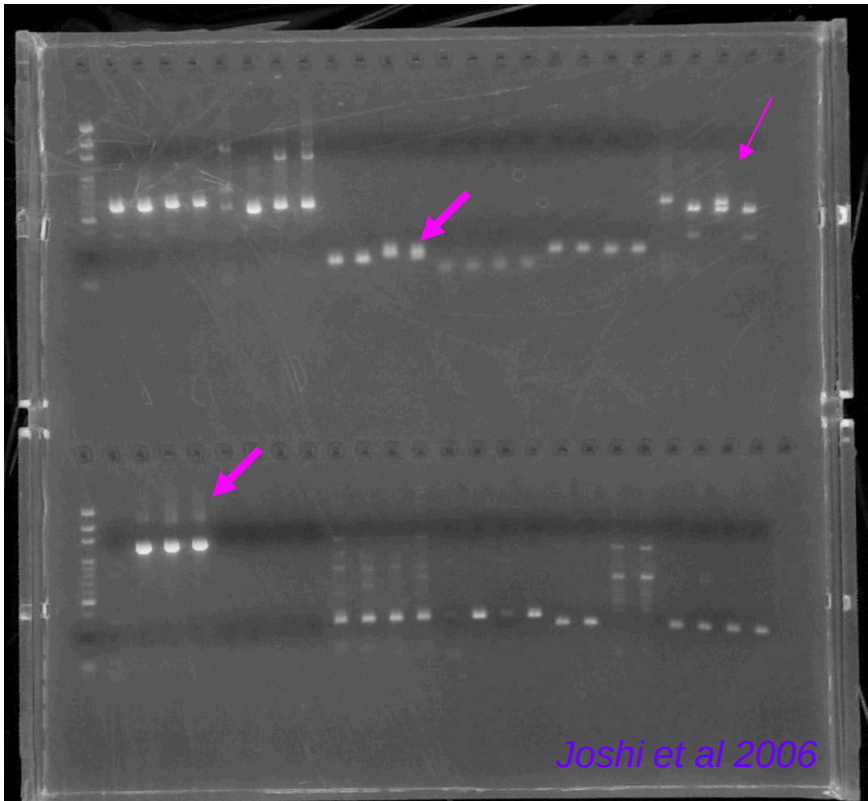
C. Screening

- Resistance and susceptible
- Cold tolerance
- Drought tolerance
- High protein content

RAPD markers detecting downy mildew resistance gene in lettuce parent and progeny bulk



Different EST markers in 4 lines of common buckwheat



D. Diversity and biosystematics study

Diversity indices

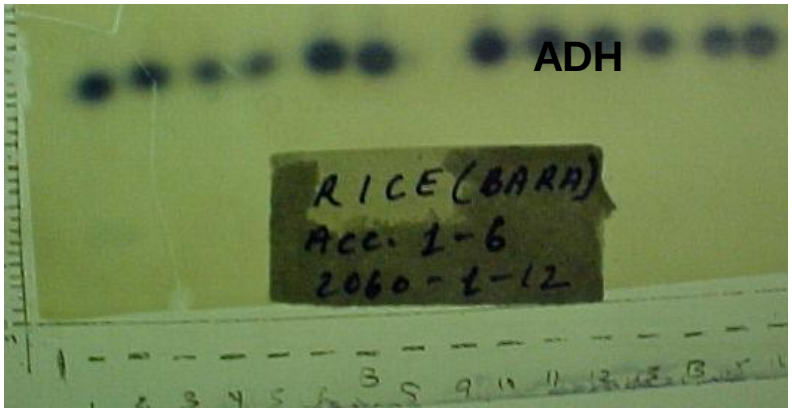
Genetic distance

- Nei's
- Roger's

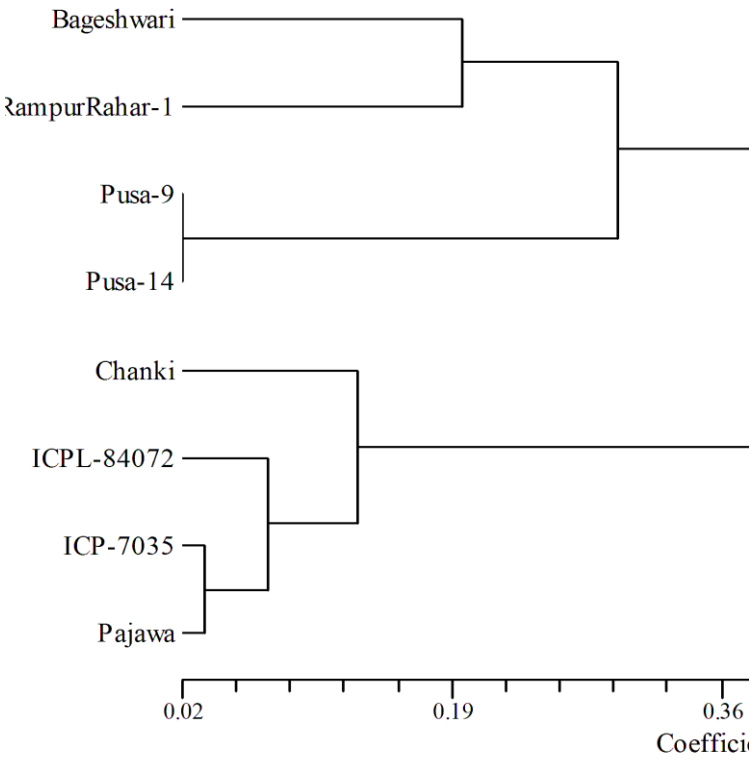
Multivariate analysis

- Cluster
- PCA

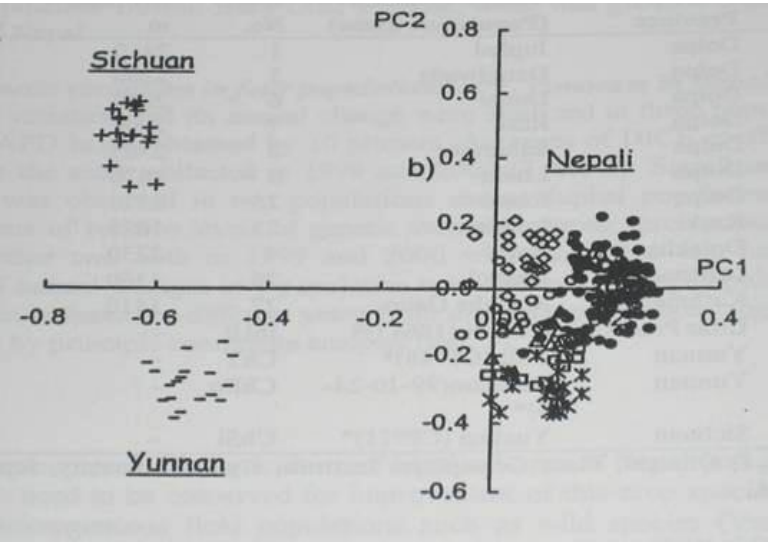
Isozyme bands in Bara rice landraces



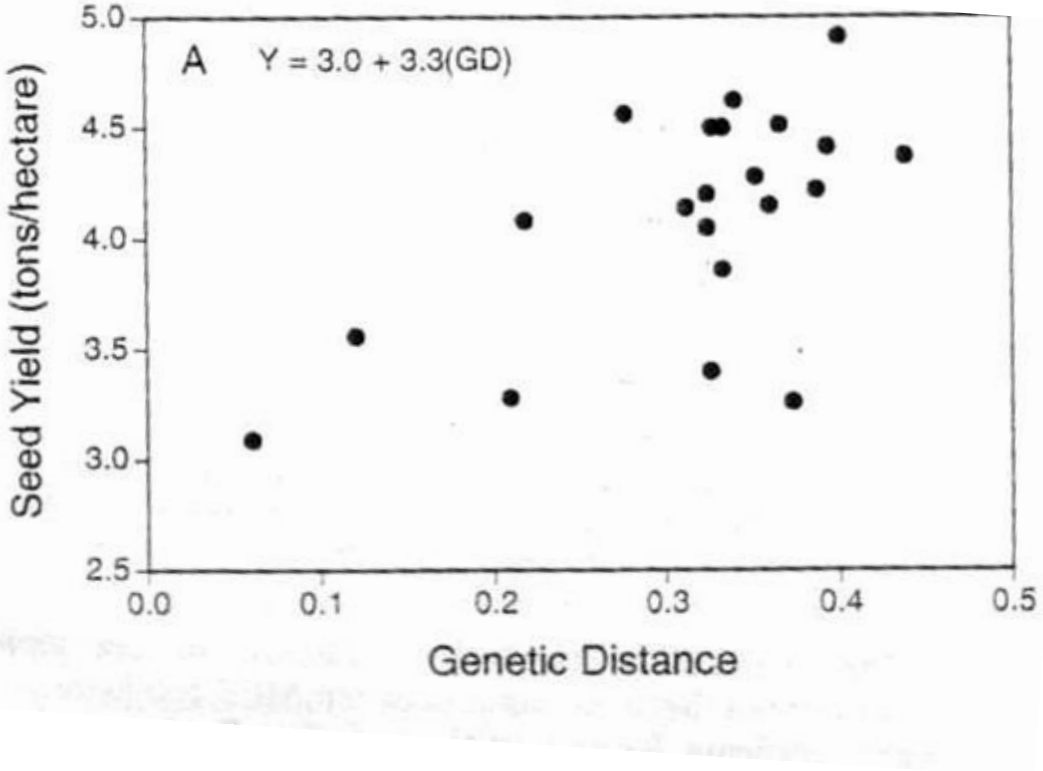
Dendrogram of 8 pigeon pea populations based on iszoymes with Nei's genetic distance

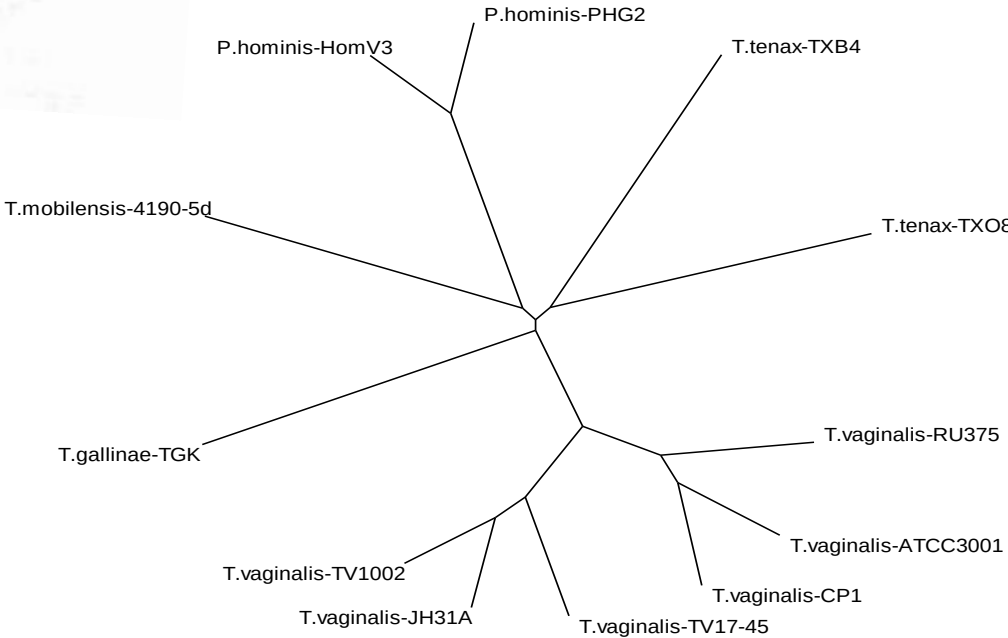


Principal component analysis based on 46 RAPD primers in Fagopyrum cymosum



Divergence and Heterosis study





Phylogenetic (radial) tree of trichomonads based on 731 RAPD traits: Phylogeny and evolution

E. Population structure

Parameters

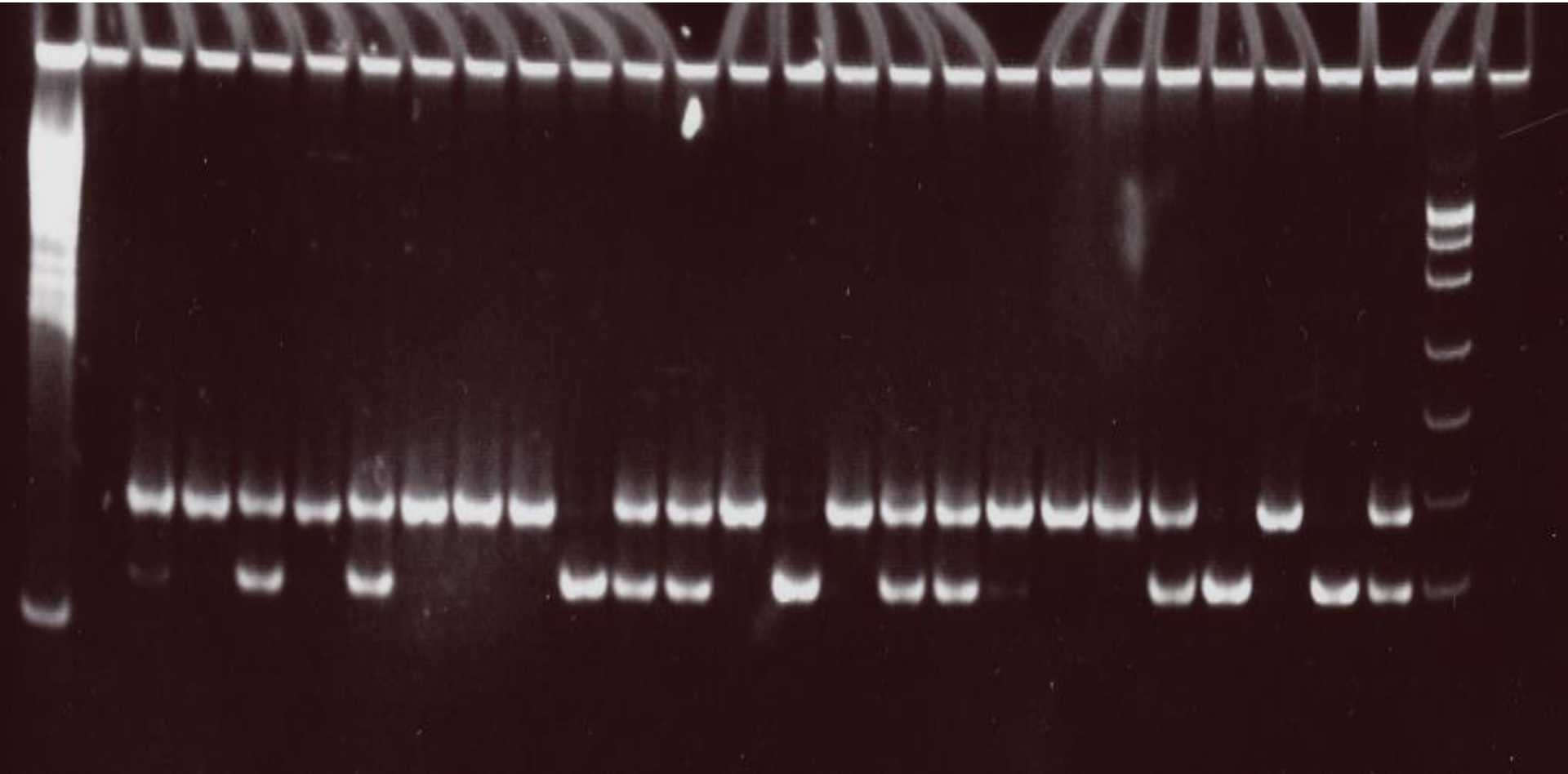
- Wright' F statistics
- Nei's G statistic
- Rho statistics

AMOVA, partitions of variations

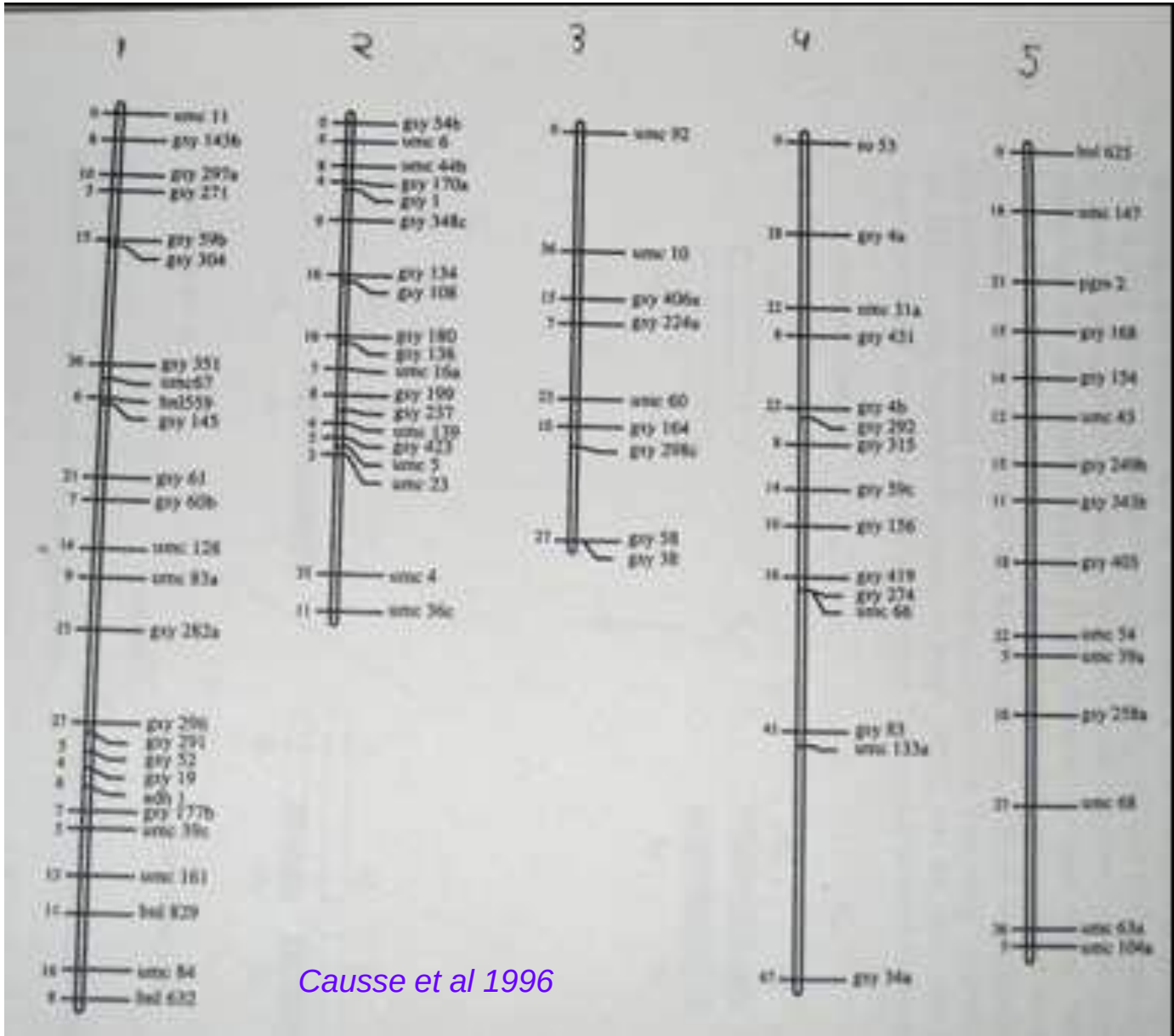
Source of variance	df	SS	EMS
Among groups, species	G-1	SSD (AG)	$n''\sigma^2_a + n'\sigma^2_b + 2\,\sigma^2_c + \sigma^2_d$
Among populations/ within groups	P-G	SSD (AP/WG)	$n\sigma^2_b + 2\,\sigma^2_c + \sigma^2_d$
Among individuals/ within populations	N-P	SSD (AI/WP)	$2\,\sigma^2_c + \sigma^2_d$
Within individuals	N	SSD (WI)	σ^2_d

F. Linkage map construction, gene tagging and QTL mapping

CAPS marker in parents and F2 population of common buckwheat

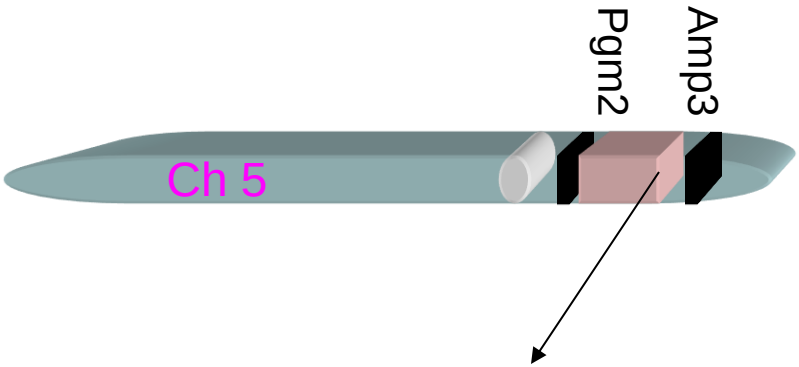


A linkage map of maize using cDNA markers in 5 chromosomes

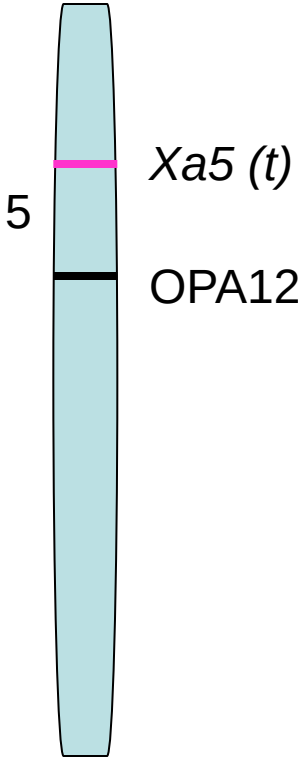


Causse et al 1996

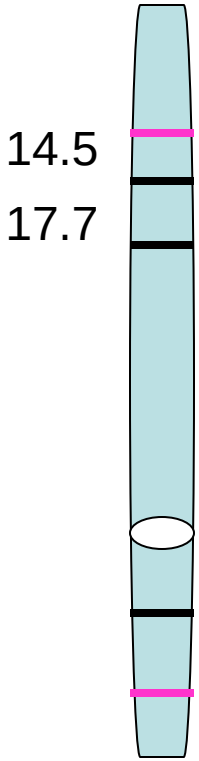
RAPD and SSR markers linked to bacterial blight resistance gene of rice



RAPD maker



SSR markers

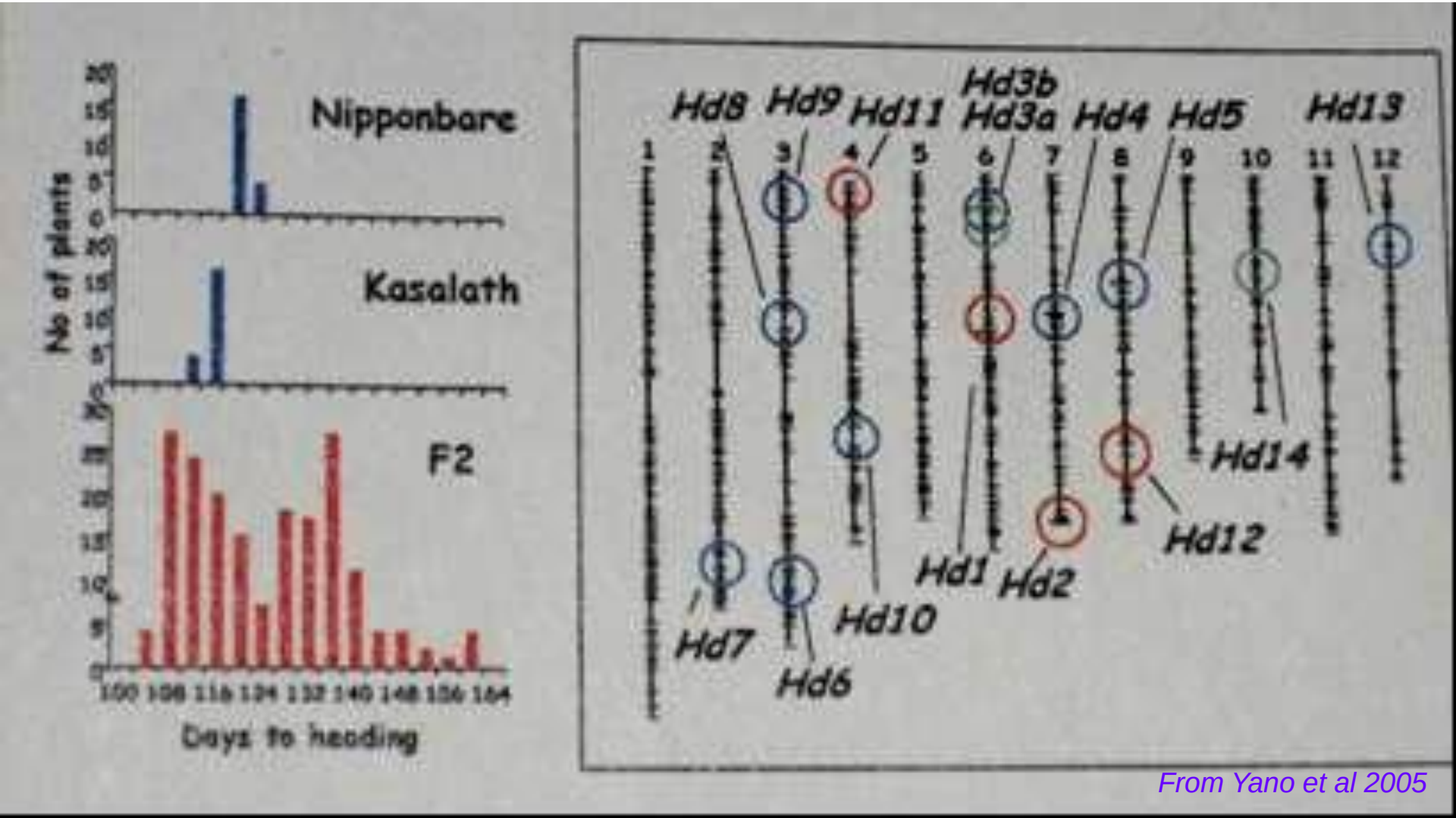


Ch 5

Segment bracketed by isozyme markers: very significant effect on grain yield in maize

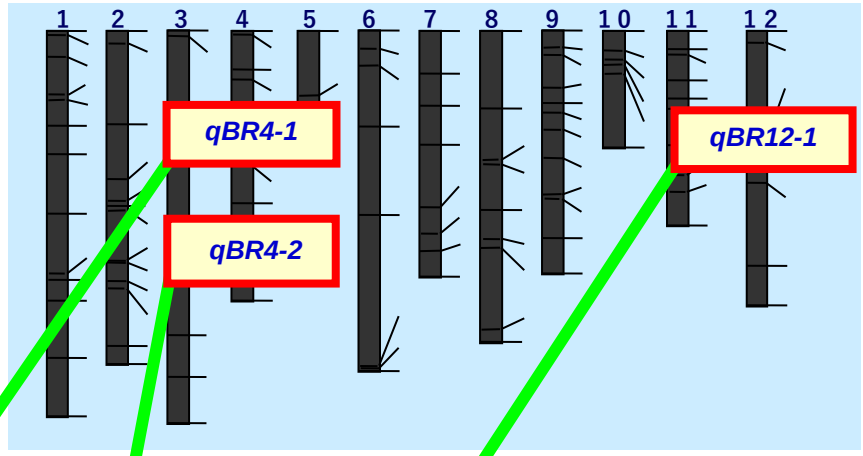
From Rao et al 2003

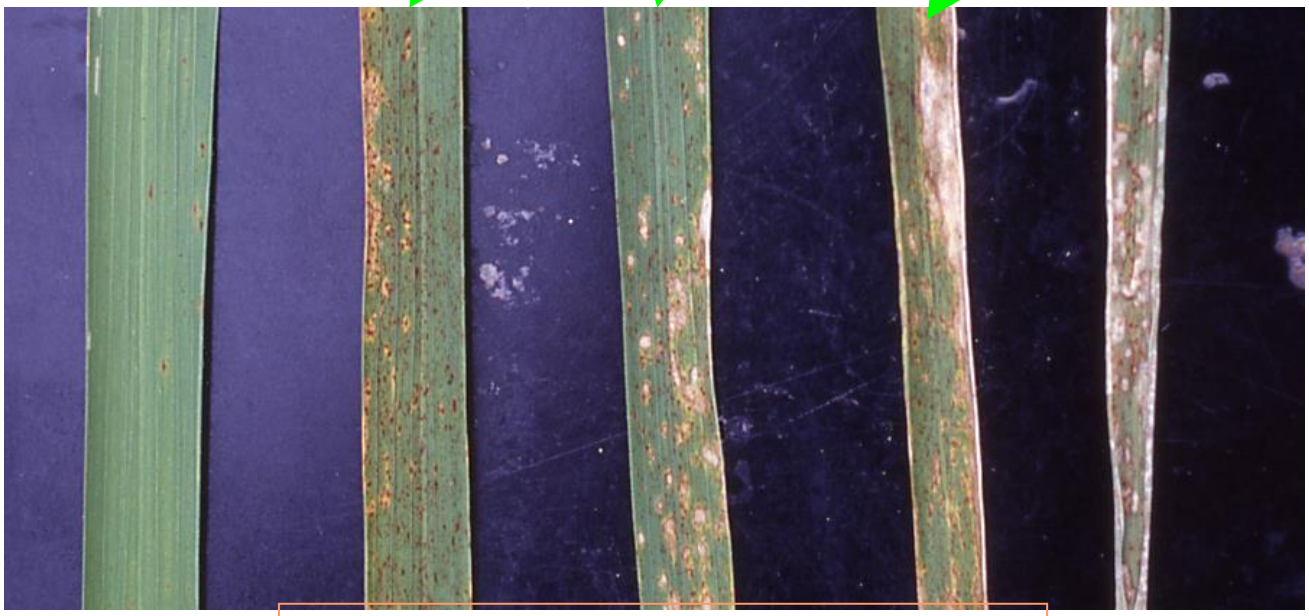
QTL mapping for heading date in rice = ETL



From Yano et al 2005

Effect of each QTL for rice blast





From Fukuoka et al 2006 upland

pi21 (qBR4-1) *qBR4-2* *qBR12-1*

lowland

G. Marker assisted selection (MAS) and marker assisted backcrossing (MAB)

Phenotypic selection

Genotypic selection

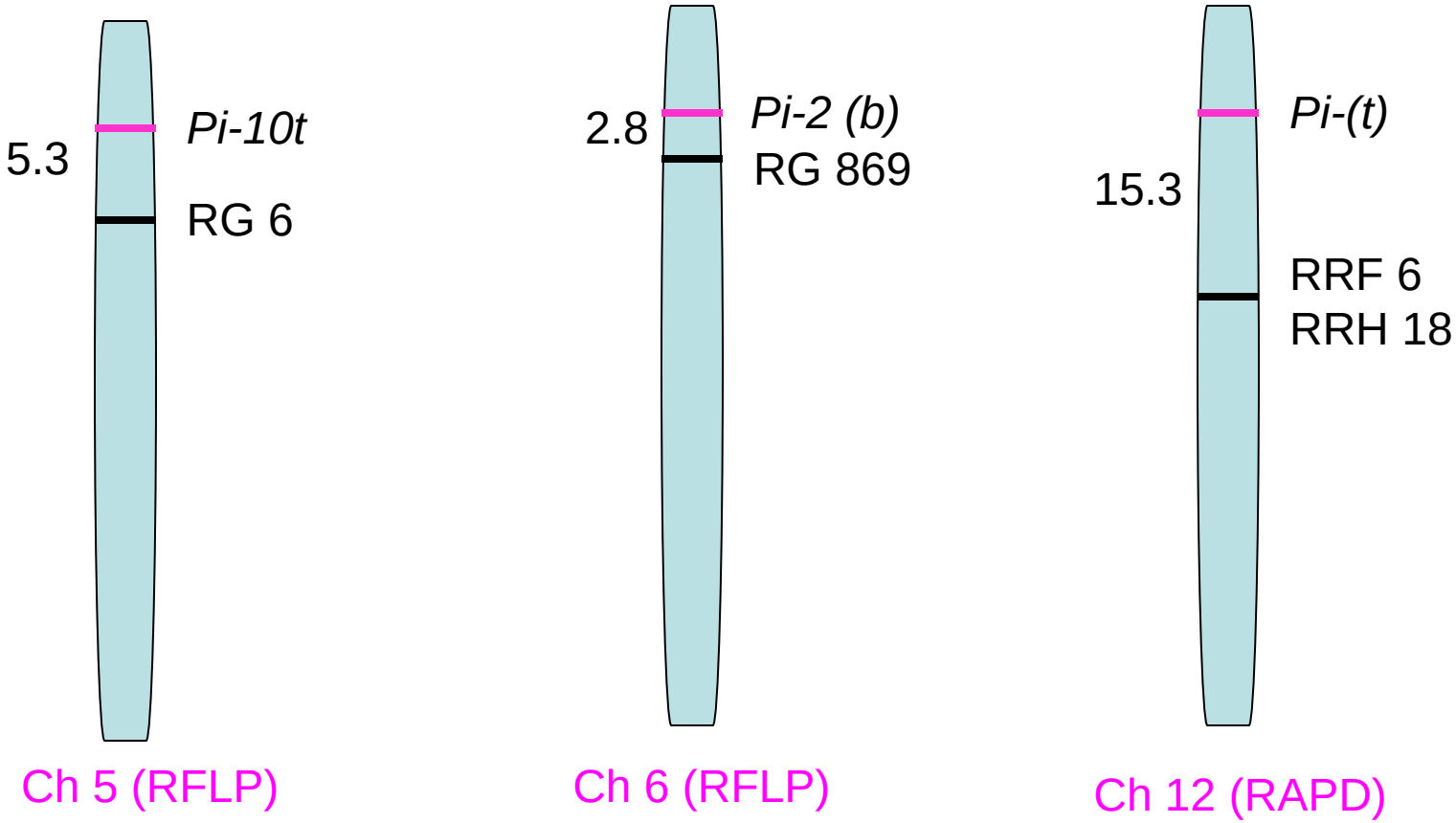
Combined selection

- Parents selection/ tagging desired traits
- Crossing
- Marker assisted selection in segregating population
- Graphical genotyping (Foreground and background selection)

- At any stage of crop development
- Many round of selection
- No need of gene expression
- No need of sample destruction
- Large portions of recurrent parent
- No need of selfing in case of recessiveness of gene

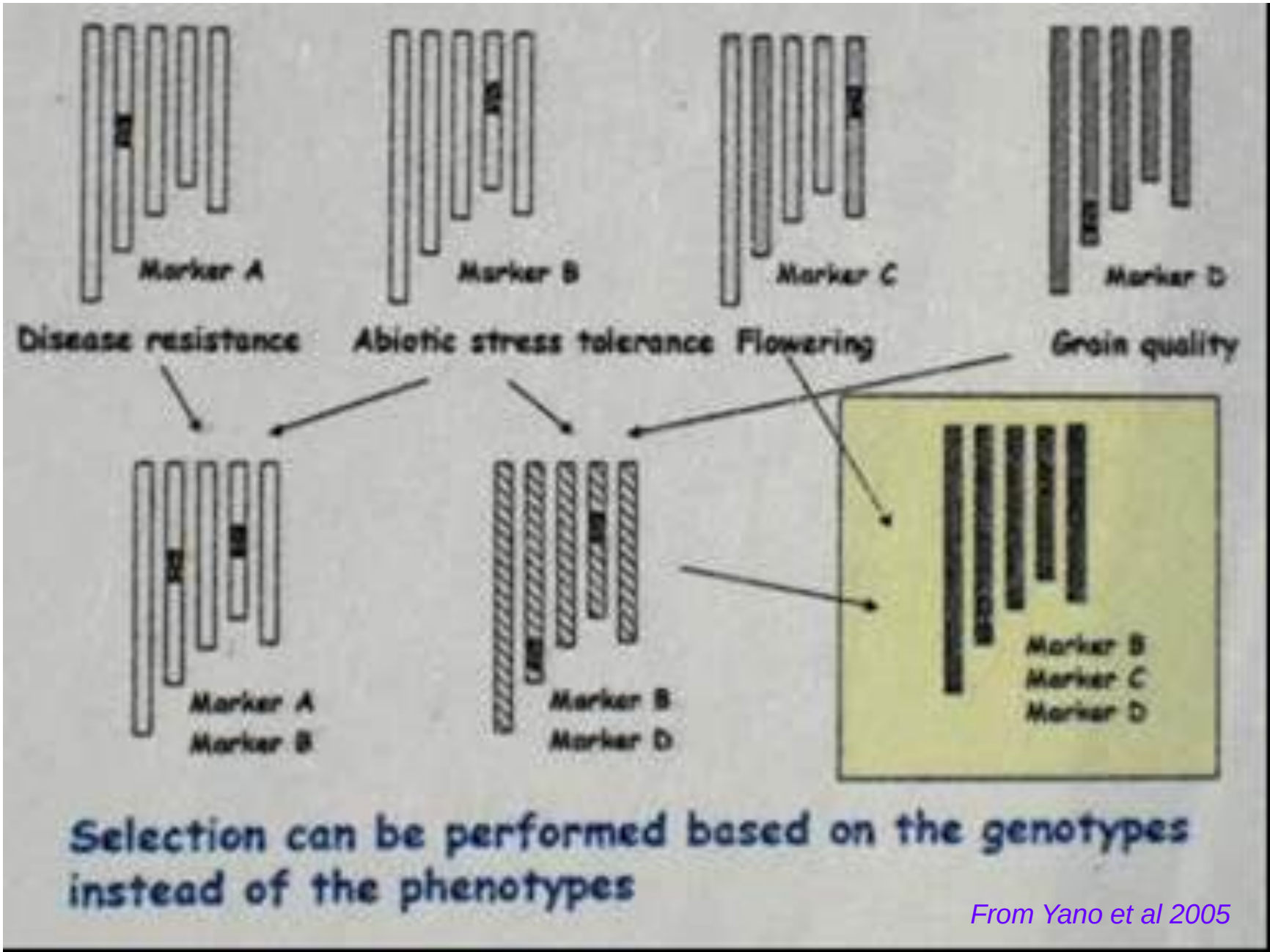
Co segregation of *Pi-g(t)* and SSR marker RM166 in the BC1 population.

Different markers linked to blast resistance gene in rice



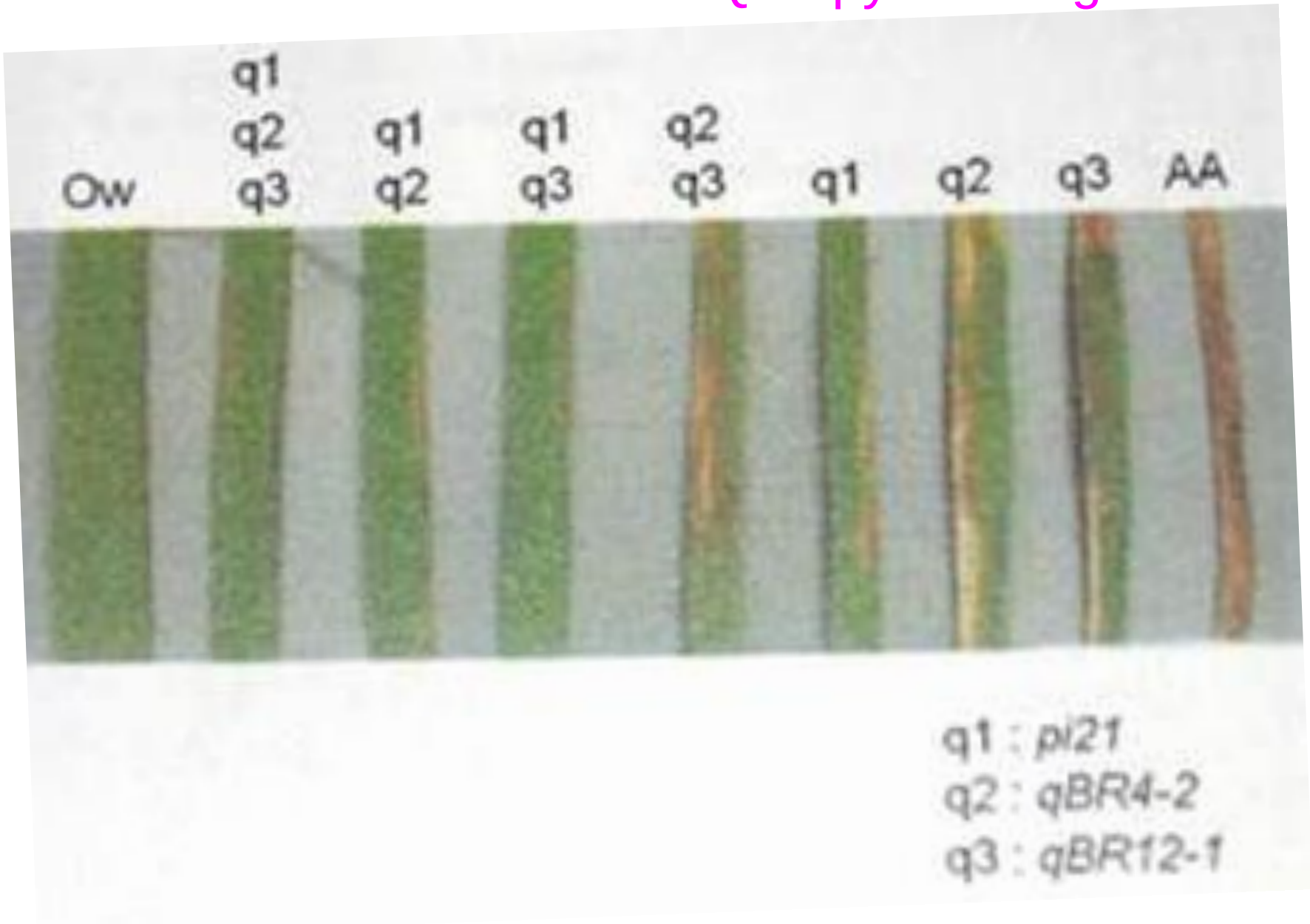
From Mohan et al 1997

Gene pyramiding



From Yano et al 2005

Durable rice blast resistance QTL pyramiding



From Okuno et al 2006

Comparative study

- Vavilov’s law of homologous series in variation
- DNA markers can be utilized among different crop species, not possible in case of phenotypic markers
- Use of complete DNA sequences of Rice, Bacteria, Fungi, Human, Drosophila, Arabidopsis
- Evolutionary study

Scope

- Any areas, if your material content DNA

Issues

Technical

- Biochemical or DNA base
- PCR or non PCR base
- Arbitrary (single primer) or site specific (pair primer)
- Dominance or codominace
- Exon or intron base
- Single or multi locus

Operational

- Cost
- Tiime
- Protocol
- Skill etc

Molecular marker technology

- Should be integrated in our plant breeding program
- Never replace the conventional plant breeding
- Complementary to conventional plant breeding (improve the efficiency and precision)

Plant breeder

- Fingerprinting
- Linkage map construction and QTL mapping
- Graphical genotyping
- Gene tagging
- MAS/ MAB (Parents selection, desired genotypes selection, NIL development, screening)
- Gene pyramiding