Introduction

- **Marker :** Marker is signpost used as reference to find out variation among the individuals . Markers are use to discriminate b/w the individuals.

- There are three types of markers

- Morphological marker
- Biochemical marker/ Protein marker
- Molecular marker / DNA marker

Types of Marker

- **Morphological - Presence or absence of awn, leaf sheath coloration,** height, grain colour, aroma of rice etc.

- **Biochemical- A gene that encodes a protein that can be extracted and observed;** for example, isozymes and storage proteins.

- **Genetic or molecular - A unique (DNA sequence), occurring in** proximity to the gene or locus of interest, can be identified by a range of molecular techniques.

Molecular marker / DNA Marker

- DNA marker firstly developed in 1980.
- Considered to be essential tools in plant breeding .
- Most widely used types of marker
- Arises from mutation in the DNA
- Molecular marker are large or abundant in number
- DNA marker represent genetic differences between individual

Application

- Molecular markers applied in.
- Testing in parentage.
- Map based cloning of the gene.
- Measurement of the genetic diversity.
- Phylogenetic analysis.
- Fingerprinting of the varieties or cultivars.

[1] RAPD (Random amplified polymorphic DNA)

- RAPD is a PCR based marker which employed single primer of arbitrary nucleotide sequence with ten nucleotide to amplified anonymous PCR fragments from genomic template DNA

- RAPD marker need to be converted to stable PCR markers.
- It is used a template and re – PCRed
- The polymorphic RAPD marker band is isolated from the gel.

Advantages

- The main advantages of RAPD is that they are quick and easy assay
- Low quantities of template DNA are required, usually 5-50 ng per reaction.
- Since random primers are commercially available, no sequence data for primer construction are needed.
- Moreover, RAPD have a very high genomic abundance and are randomly distributed throughout the genome.

Disadvantages:

- ✓ RAPD analyses generally required purified, high molecular weight DNA, and precaution are needed to avoid contamination of DNA samples because short random primers are used that are able to amplify DNA fragments in a variety of organism.
- ✓ Dominant markers
- ✓ Reproducibility problems

Application

- Distinct pattern of amplification is seen in different samples. This is why RAPD can be used for studying polymorphism.
- RAPD is applicable for the mapping of genome, analyzing linkage, and individual specific genotyping.
- RAPD markers are dominant in nature so it has restrictions for mapping purpose.
- RAPD is strictly laboratory dependent so it requires sensitivity.

[2]Restriction Fragment Length Polymorphism (RFLP):

- It was one of the first methods used for the analysis of DNA in various fields such as forensic science.
- It is a hybridization based technique.
- It was invented by **Alec Jeffreys,** an English scientist in 1984 during his research in genetic diseases.
- RFLP uses particular restriction endonuclease enzymes that cuts at its specific site yielding fragments of various lengths along with the fragment of interest.
- The length of the distinct fragments is determined by using blotting, now replaced with sequencing.
- RFLP markers are largely locus-specific and are co-dominant in nature due to the nature of restriction endonuclease used.

Applications

- RFLP was one of the first techniques applied for genetic fingerprinting/profiling.
- It is used for identification of inherited diseases, carrier of that diseases, genetic mapping, and heterozygous detection.
- The molecular basis of the RFLP is that any point mutations as such deletions, substitutions and insertions or alterations like duplications, inversions within the genome can eliminate or form new restriction sites. These alterations in genome can be detected by analyzing fragments of variable length, digested with restriction endonuclease enzyme

RFLP techniques

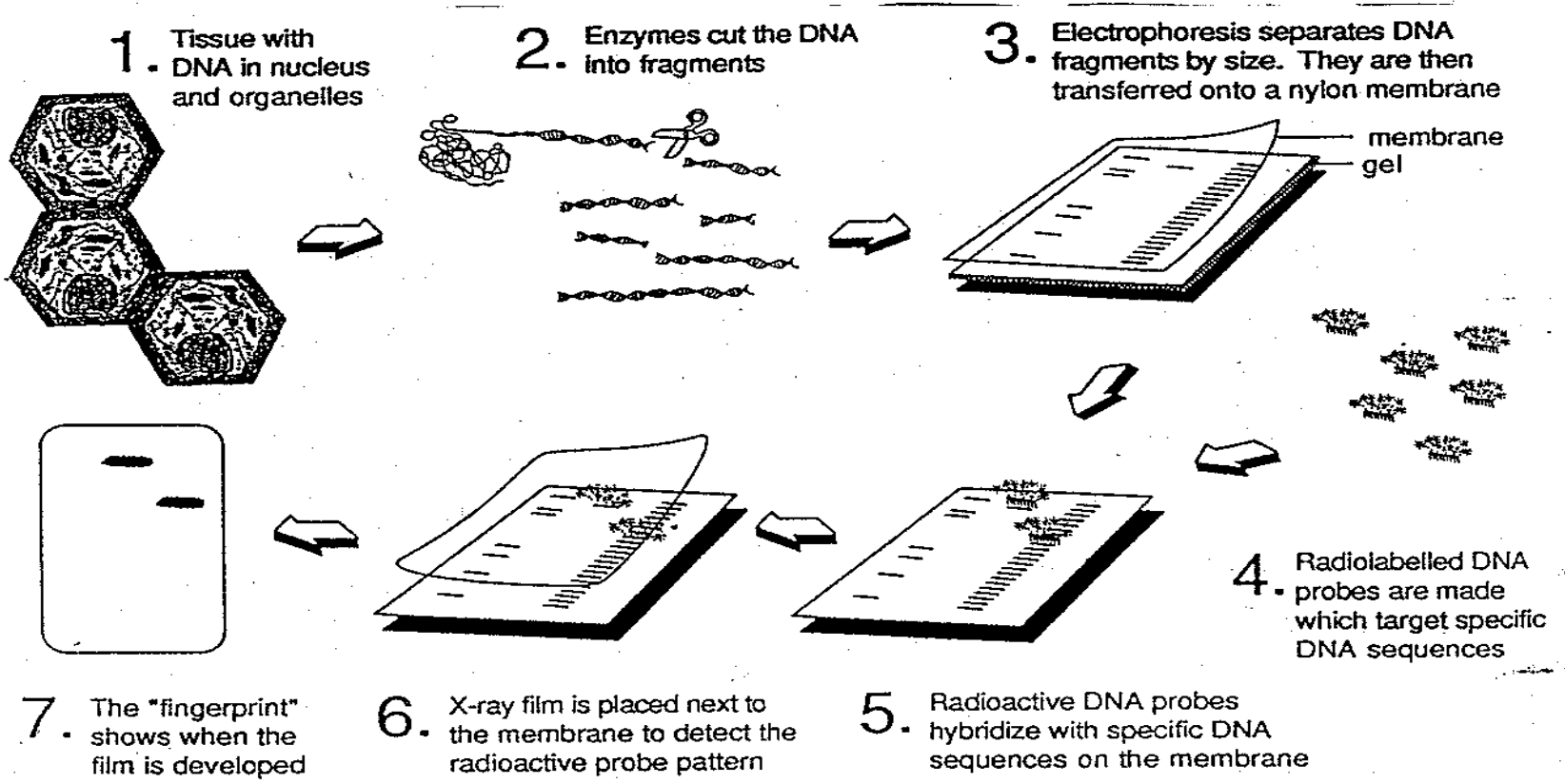


Figure 1. Schematic representation of DNA isolation, restriction nuclease digestion, electrophoresis, and Southern hybridization.

DNA fingerprinting- The powerful new tool for the Genetic Detective. 1990. Peter M. Gresshoff *et al.* Tennessee Farm and Home Science.

Demerits

- requires relatively large DNA sample
- laborious and tedious process
- sensitivity and more precautions for contamination required

[3]Amplified Fragment Length Polymorphism (AFLP):

- Zabeau and Vos invented the AFLP technique in 1993.
- AFLP was originally developed by the KeyGene in 1990.
- It is a PCR based technique for fingerprinting. It includes both PCR and RFLP.
- The basis of AFLP is the amplification of selected fragments followed by restriction digestion of whole genomic DNA of specific organism.
- The steps for the AFLP are as follows:
- DNA extraction and its restriction digestion followed by ligation with the short adaptor sequences.
- Amplification of restricted fragments by PCR
- Analysis of results in gel electrophoresis or PAGE followed by autoradiography.

Applications

- AFLP has its ability for rapid generation of marker fragments for any organism without prior sequencing of DNA is required.
- Also, it needs only small fragments of starting template DNA relative to RAPD and ISSR (inter-simple sequence repeats) and has much higher reproducibility.
- AFLP is largely used for crop improvement programs, parentage and genomic interpretation of various crop species.

Demerits

- AFLP require large DNA samples and require purification

[4]Microsatellites or simple sequence length polymorphisms (SSLPs)

- Microsatellite was termed by Jeffery et al. in 1985.
- Microsatellites or simple sequence repeated (SSR) loci are PCR based markers which needs previous knowledge of gene sequence.
- In literature it is referred to as **variable number of tandem repeats (VNTRs)** or **simple sequence length polymorphisms (SSLPs**) or **sequence tagged microsatellites (STMS).**
- They are dispersed throughout the nuclear genomes in eukaryotes and to a few extent in prokaryotes.
- Microsatellite primers are short tandem repeats (STRs), or simple sequence repeats (SSRs), having 1-6 base pair long sequences repeated several times.
- Usually microsatellites are repeated less than 100 times.
- Microsatellites can be recognized by constructing a small-insert genomic library followed by screening of library and sequencing of positive clones.

Applications

- Microsatellite consists of co-dominance of alleles and requires low quantities of DNA templates.
- It has high reproducibility and is economical in nature.
- The screening of microsatellite variation can be automated.

Demerits:

- Assay is costly if sufficient primer sequences for the species of interest are not available.
- Errors in genotype scoring occurs if alterations are seen in primer annealing sites.
- chances of homoplasy (some characters are present in more than one species but not present in their common ancestor because of convergence evolution)

Comparision of properties of RFLP& RAPD&SSR

S.No	Characterstrics	RFLP	RAPD	SSR
1	Principle	Restriction digestion	DNA amplification	DNA amplification
2	Detection	Southern blotting	DNA staining	DNA staining
3	DNA quality	High	Low	Low
4	Primer requirement	None	Yes	Yes
5	Probe requirement	Set of specific probe	None	None
6	Use of radio isotopes	Yes	No	No
7	Part of genome survey	Generally low copy coding region	Whole genome	Whole
8	Dominant/ Co dominant	Co dominant	Dominant	Co dominant
9	Polymorphism	Medium	Medium	High
10	Reliability	High	Intermediate	High

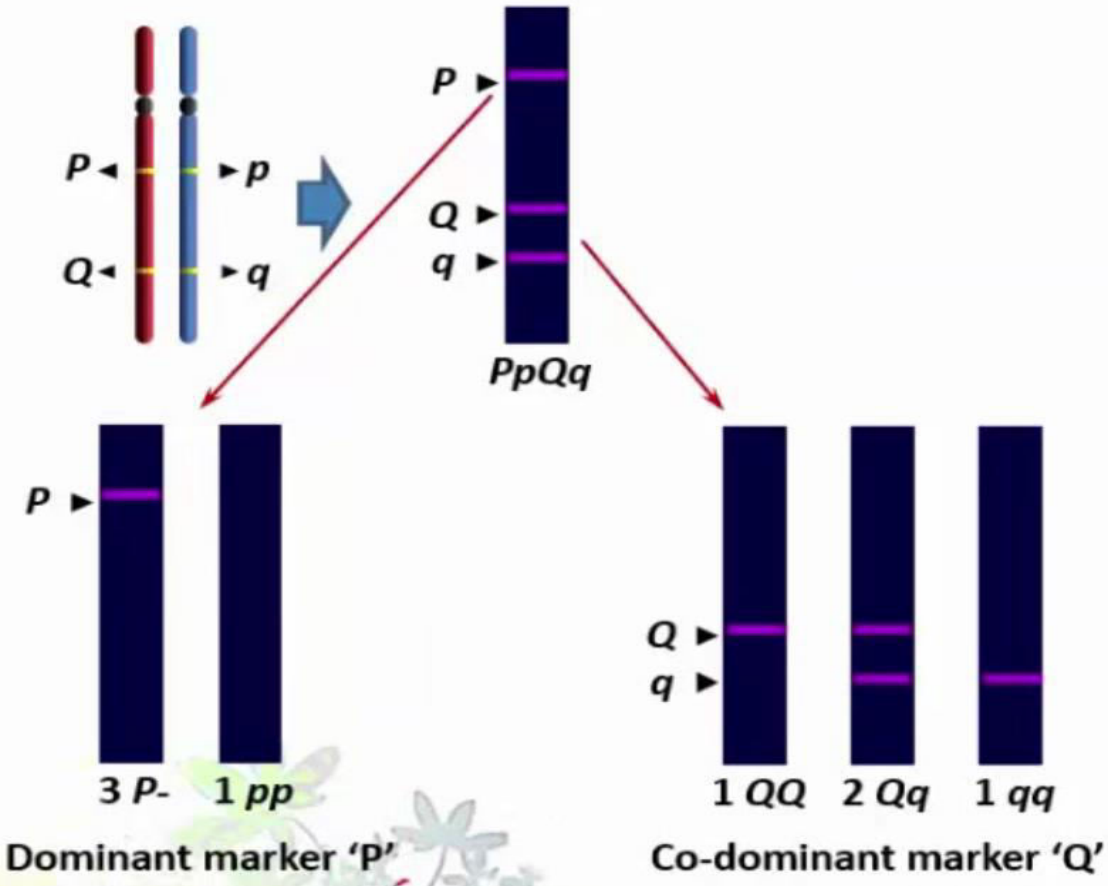
Application of DNA marker

- Testing the parentage
- Genetic mapping and tagging of the loci governing economic traits
- Measurement of the genetic diversity
- Phylogenetic analysis
- Fingerprinting of the varieties of cultivars
- Map based cloning of the gene

IMPORTANT PROPERTIES OF IDEAL MARKERS FOR MAS

- Abundant in number
- Highly polymorphic
- Codominent in nature
- Evenly distributed throughout the genome
- Easy recognition of all possible phenotypes (homo- and heterozygotes) from all different alleles.
- Low or null interaction among the markers allowing the use of many at the same time in a segregating population

Types of marker inheritance



PROCEDURE OF MAS

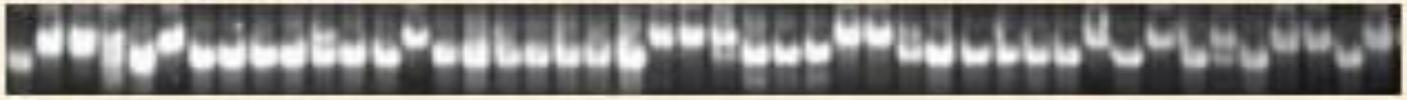
(1) LEAF TISSUE SAMPLING

(2) DNA EXTRACTION

(3) PCR

(4) GEL ELECTROPHORESIS

(5) MARKER ANALYSIS



QTL mapping and MAS

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
verifation of QTLs in independent population and
testing in different genetic backgrounds
fine mapping

Marker Validation
testing of marker in important breeding material
identification of polymorphic marker

Marker - Assisted selection

APPLICATION OF MAS IN PLANT BREEDING

- Marker Assisted Evaluation of Breeding Material
- Marker-Assisted Backcrossing
- Marker-Assisted Pyramiding
- Early generation Marker-Assisted selection
- Combined Marker-Assisted selection

Marker Assisted Evaluation of breeding Material

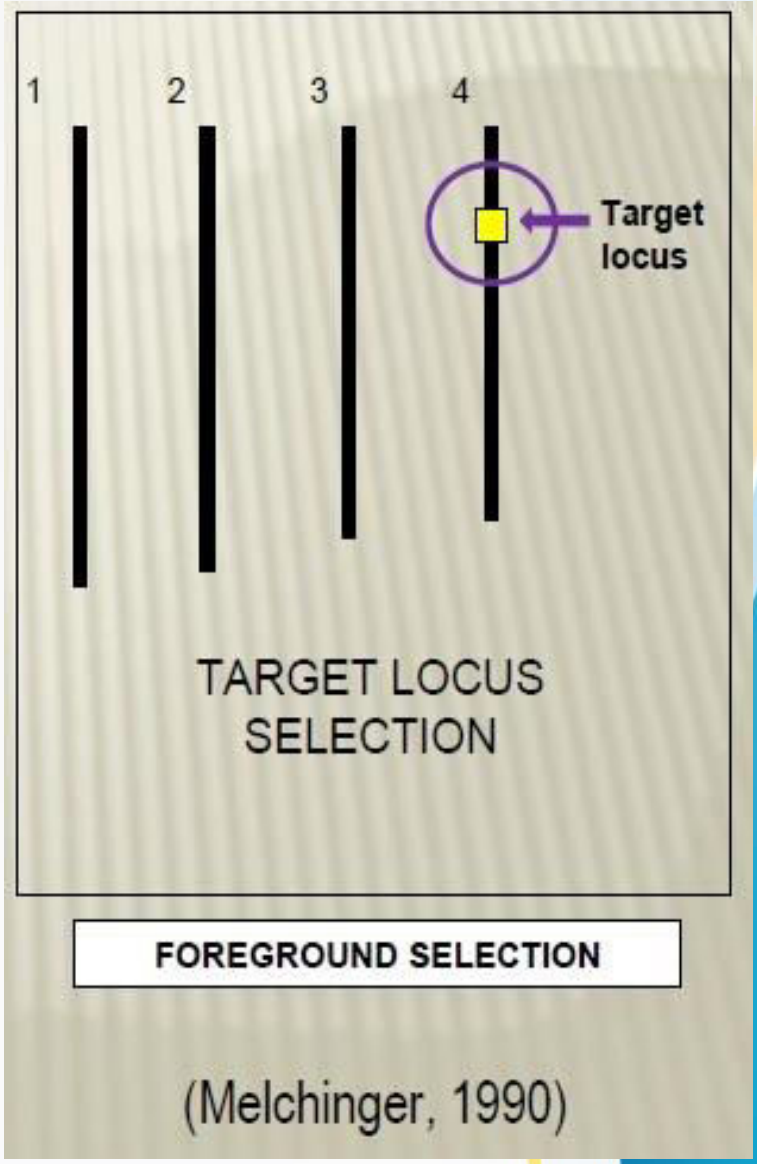
- cultivar identity/assesment of 'purity'
- Assement of genetic diversity and parental selection
- Study of heterosis
- Identification of genomic regions under selection

Marker Assisted Backcrossing

- MAB has several advantages over conventional backcrossing
- Effective selection of target loci
- minimize linkage drag
- Accelerated recovery of recurrent parents

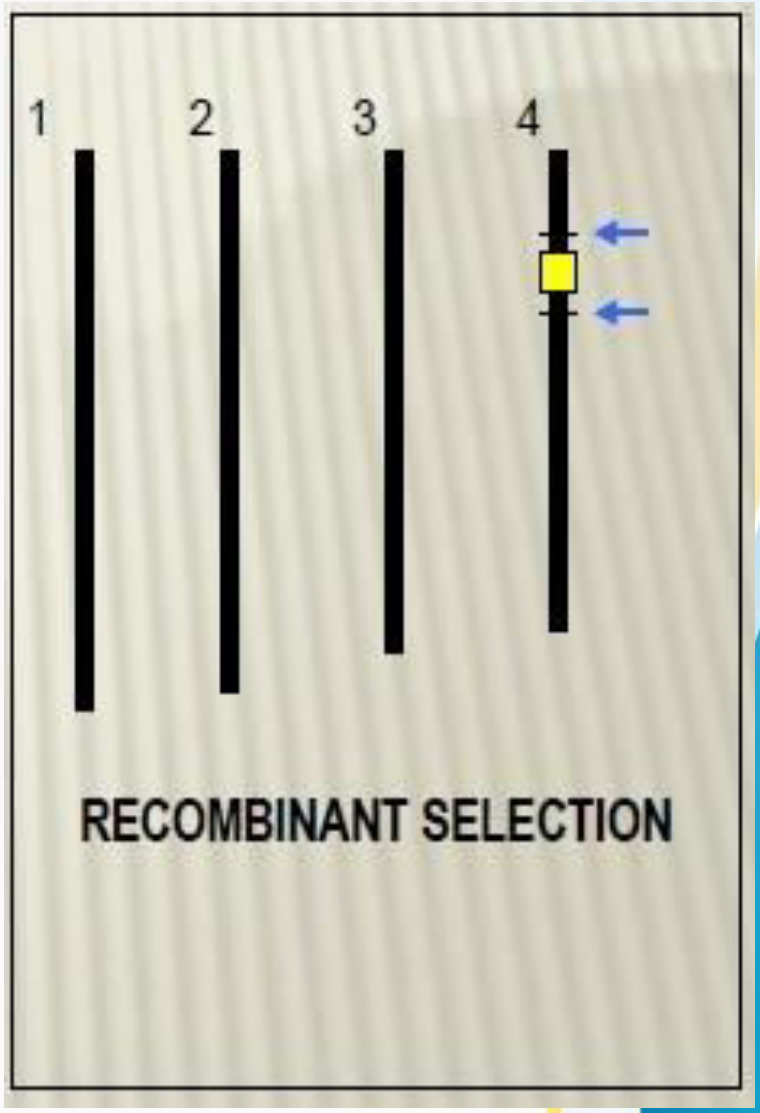
MAB:1st LEVEL OF SELECTION FOREGROUND SELECTION-(TANKSELY,1983)

- Marker that are flanking the target gene in the donar genotype are used for the selection for target gene
- Useful for traits that are difficult to evaluate
- Useful for recessive genes
- It can help in selecting plant at seedling stage
- Identification of best plants for backcrossing with the use of markers



MAB: 2nd LEVEL OF SELECTION RECOMBINENT SELECTION

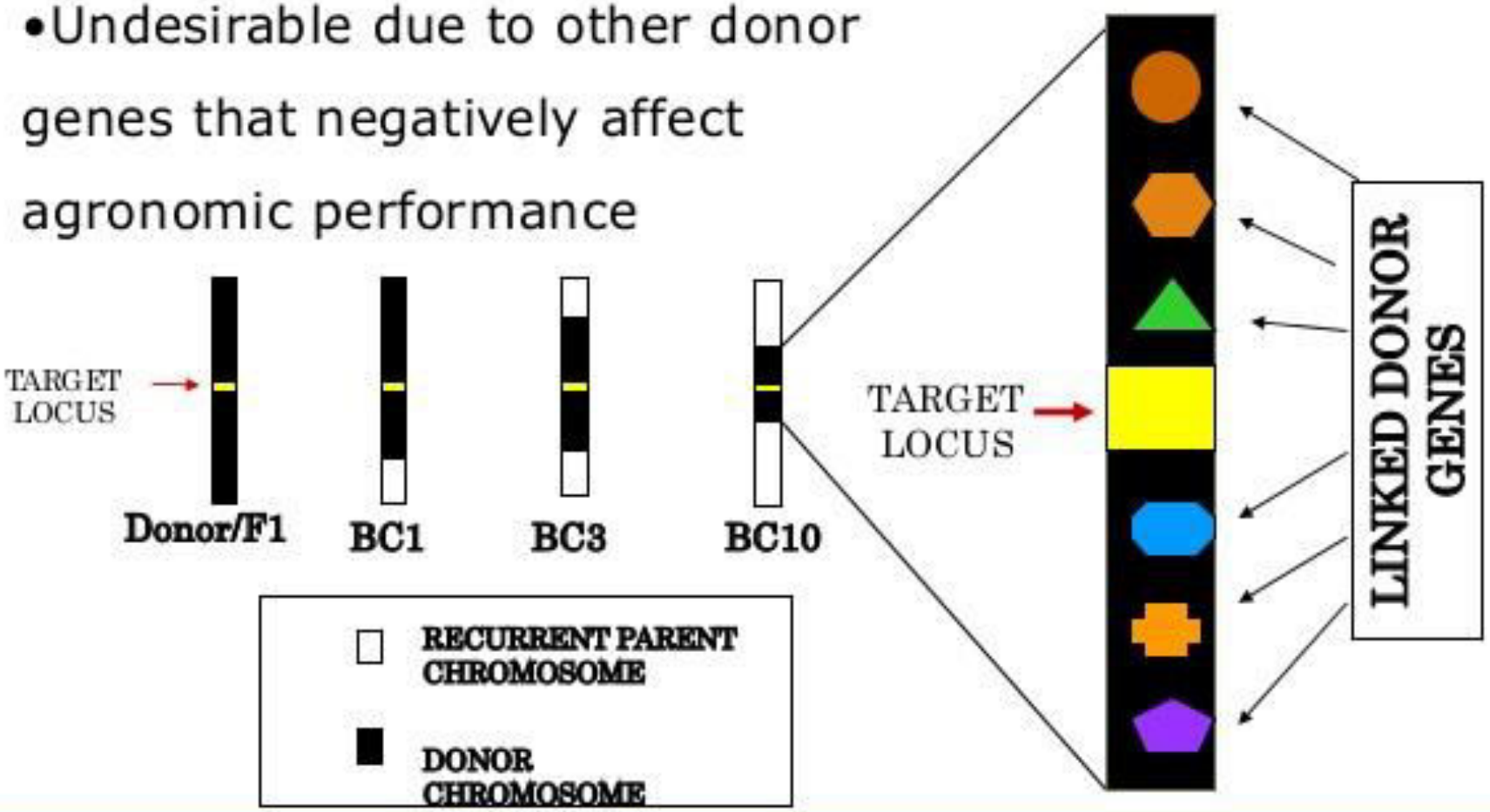
- Purpose: To reduce the size of the donar chromosome segment that is linked with the gene of interest
- Linkage drag is minimize by using flanking marker (less than 5cM on either side) of target gene
- Require large population sizes
- Depends on distance of flanking markers from target locus)



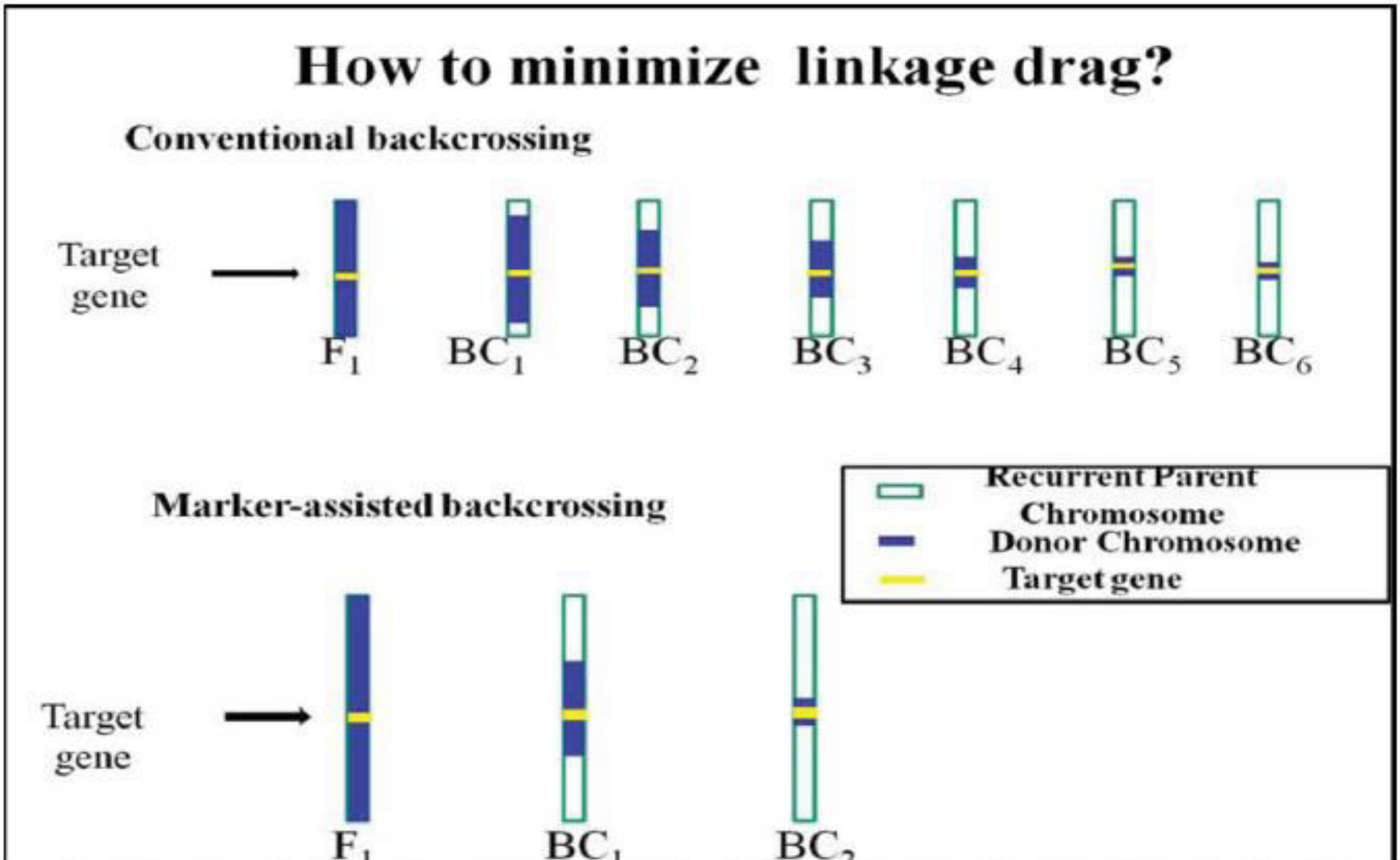
Concept of 'linkage drag'

- Large amounts of donor chromosome remain even after many backcrosses

- Undesirable due to other donor genes that negatively affect agronomic performance

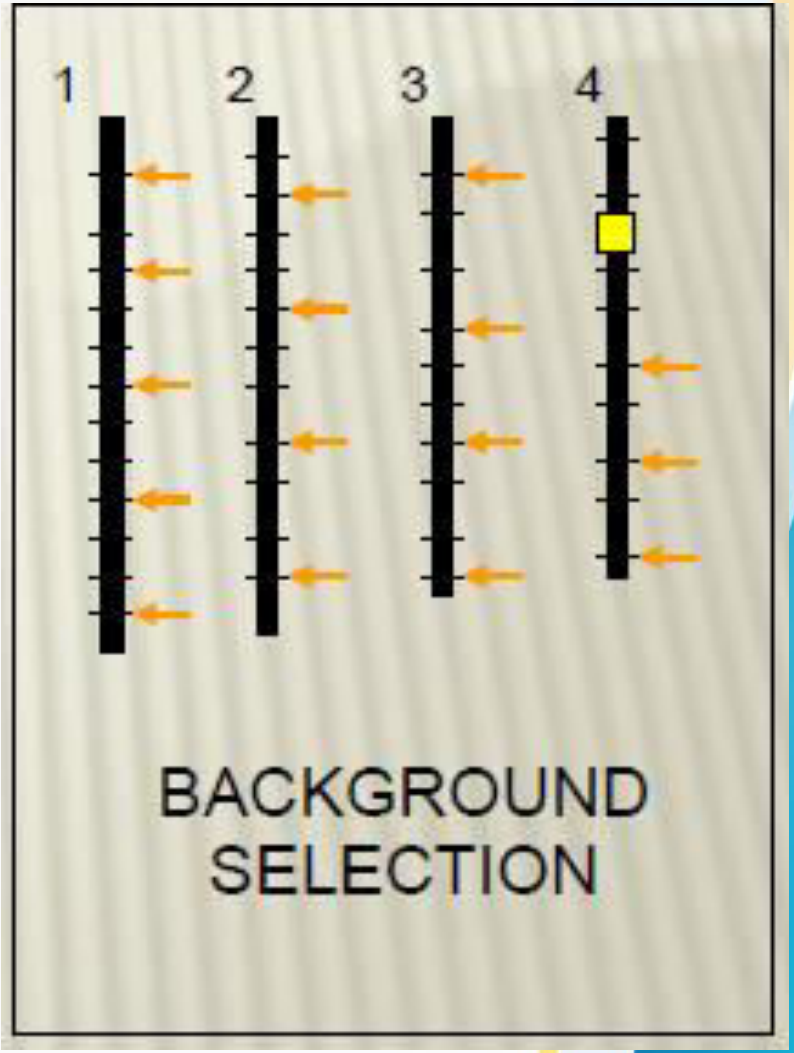


MARKERS CAN BE USED TO GREATLY MINIMIZE THE AMOUNT OF DONOR CHROMOSOME

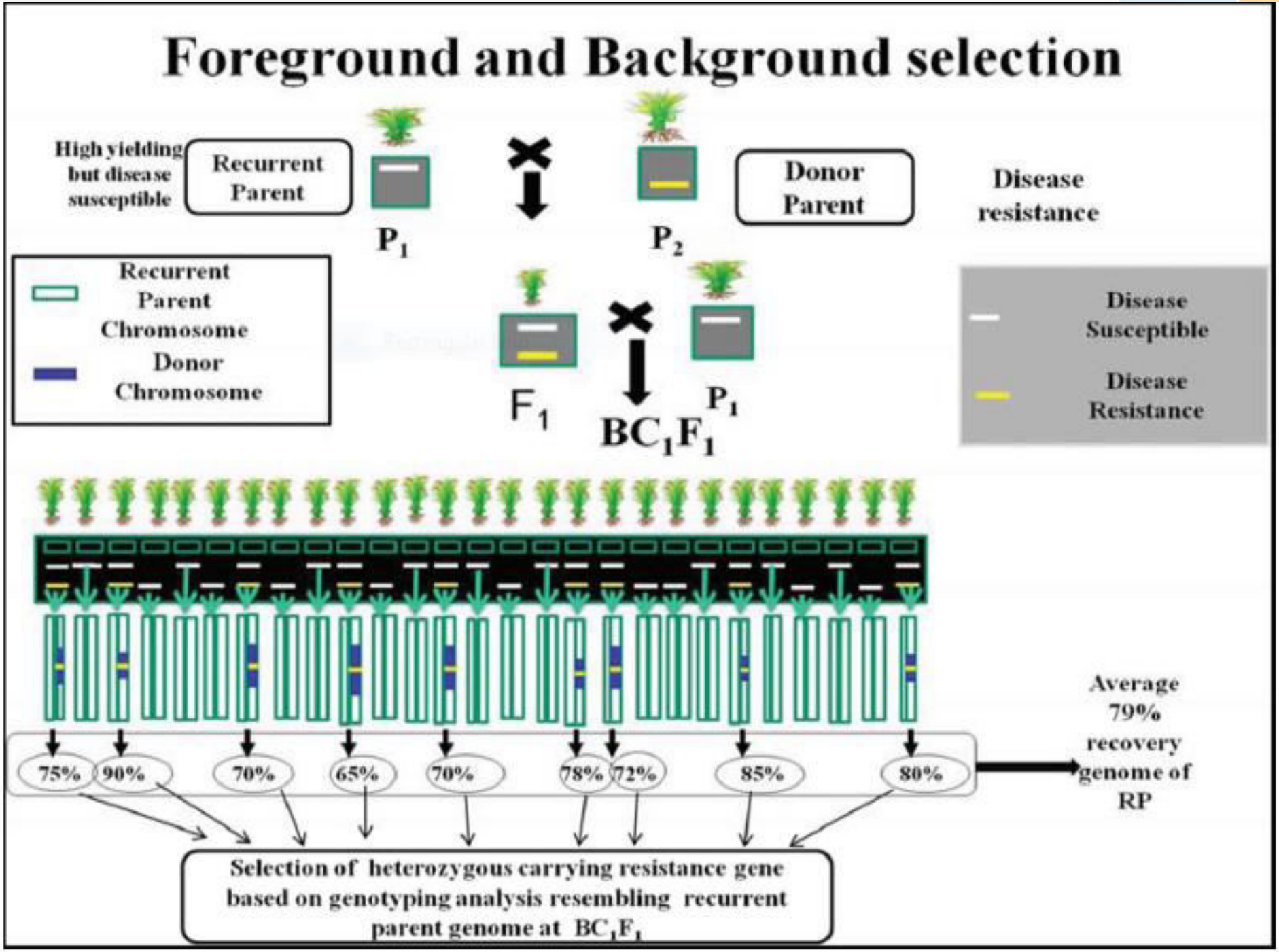


MAB: 3rd LEVEL OF SELECTION BACKGROUND SELECTION

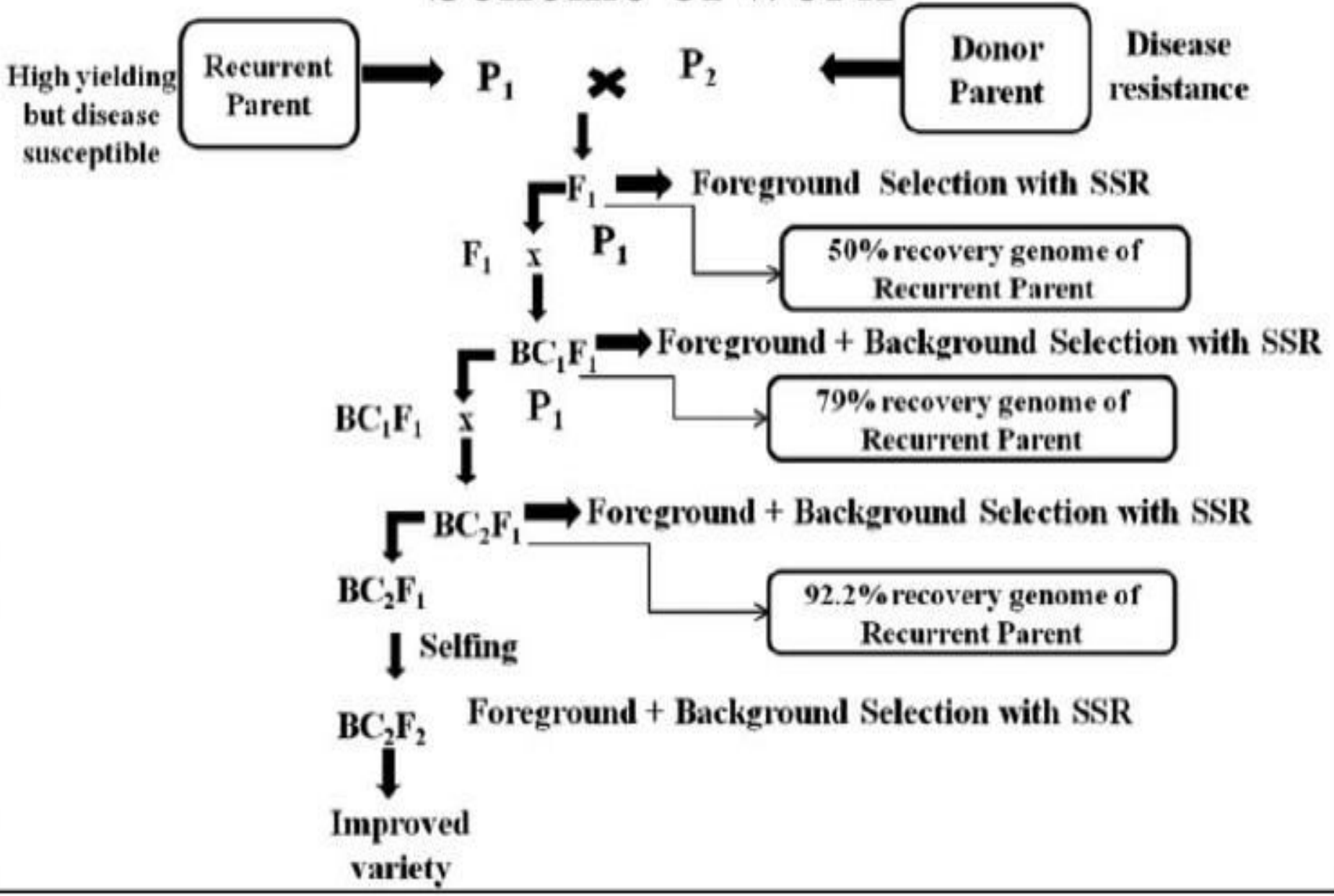
- Used unlinked marker to select against donor genome
- Accelerates the recovery of the recurrent parent genome
- Saving of 2,3 or even 4 backcross generations may be possible
- It is the selection of backcross progeny with greatest proportion of recurrent parents



Foreground and Background selection



Scheme of work



MARKER ASSISTED PYRAMIDING

- Marker assisted pyramiding involve breeding several different varieties in order to develop a genetically distinct 'pedigree'
- Marker can be developed for specific traits on each of the varieties being inbred and then applied to determine the grain of the trait or its subsequent loss over several cycle of breeding.

EARLY GENERATION MAS

- Early generation MAS facilitates the elimination of F_1 hybrid which do not carry the desirable traits as reflected by their DNA profile.
- Plants with desirable genes/QTLs are selected and alleles can be 'fixed' in the homozygous state

  plants with undesirable gene combinations can be discarded
- Advantage for later stages of breeding program because resources can be used to focus on fewer lines

COMBINED MAS

- In some cases, a combination of phenotypic screening *and* MAS approach may be useful
  - To maximize genetic gain (when some QTLs have been unidentified from QTL mapping).
  - Level of recombination between marker and QTL (in other words marker is not 100% accurate).
  - To reduce population sizes for traits where marker genotyping is cheaper or easier than phenotypic screening.

Marker Assisted Selection Possibilities

- The ability to screen in the juvenile stage for traits that are expressed late in the life of the plant
- The ability to screen for trait that are extremely difficut,expensive or time consuming to score phenotypically
- The ability to distinguish the homozygous from the heterozygous without the need for progeny testing
- The ability to perform simultaneous MAS for several characters at one time

Advantages of Marker Assisted selection

- Precise targeted selection
- Expression of traits are not affected by envirnment
- selection can be done even when trait is absent
- Selection is rapid and hence considerable time is saved
- Breeding can be accelerated
- combining several genes are possible

Achievements of MAS
(details not being discussed)

- MAS Programs World-Wide: USA,Canada, Australia, CIMMYT, IRRI - Many Cultivars Released
- MAS in India: Cultivars Released-1. Rice(Improved PB-1; Improved Sambha Mahsuri; 2. Pearl-millet (HHB-67-2); 3. Maize (Vivek-QPM9)
- Submergence tolerance: Sub1A

Select example of marker assisted selection in rice through back cross programme

S. NO	**Characters**	**gene/QTL**	**MAS**
1. .	Salinity tolerance	*saltol*	*SSR*
2.	Bacterial leaf blight resistant	*xa5,xa13,xa21*	SSR
3.	Blast resistant	*pi1*	SSR
4.	Submergence	*Sub- 1*	SSR
5.	Drought tolerance	*Drab – 1*	SSR
6.	Gall Midge	*GM 4(T) and GM 6(T),*	---
7.	Yield	*Yld 1.1 and yld 11.1*	----

Some possible reason to explain the low impact of MAS in crop improvement

- Resource (equipment) not available
- Markers may not be cost-effective
- Accuracy of QTL mapping studies
- QTL effect may depend on genetic background or be influenced by envirnmental conditions
- Lack of marker polymorphism in breeding material
- Poor integration of molecular genetics and conventional breeding

MAS IN SALT TOLERANCE

- Very recently in Vietnam, the Saltol QTL obtained from the highly salt tolerant rice variety FL478 has been transferred into the high-yielding and widely grown cultivars,ASS996 by following the MABC strategy.
- In this study, in each backcross generations, AP3206, RM3412 and RM10793 were used for screening heterozygous plants and 63 polymorphic markers were used to be distributed on 12 chromosomes.
- Two plants P284 and P307 had the highest recipient alleles up to 89.06% and 86.36% were used to develop BC_2F_1 populations. The recombinant
- selection was done with RM10694, RM562 RM7075 along the Saltol region on chromosome 1.
- Plant P284-112-209 was the best BC_3F_1 individual with all the recipient alleles screened based on a total of 63 markers.
- The four plants P307-305-21, P284-112- 195, P284-112-198 and P284-112-213 were the second ranking with only one loci heterozygous.
- All those five plants were chosen as the breeding lines for result of Saltol-AS996introgression.

Conti....

- Saltol QTL derived from FL478 was introgressed in genetic background of Bacthom 7 cultivar. The background analysis in the introgression line revealed the recovery up to 96.8%_x0001_100% of RP alleles based on the screened markers after three generations.
- In this study, 8 markers were used to identify Saltol locus and 81 markers were used in other loci between the parents. Then, 88 markers were applied to analyse genotyping of each backcross generation with the three steps of selection (foreground, recombinant and background).
- The results revealed that the best plant of BC_3F_1 generation bear the highest recovery of recipient genome i.e. 96.8%_x0001_100%.
- This study revealed that the introgression lines can be directly developed into the salinity tolerance variety, which is suitable for cultivating in coastal areas of theVietnamese Deltas using MABC.

MAS FOR BLAST RESISTANCE

- Pusa1602 (PRR78CPiz5)andPusa1603(PRR78CPiz54) lines have been developed through incorporation of blast resistance genes Piz-5 and Pi54 derived from donor lines C101A51 and Tetep into the background of PRR78 (highly blast susceptible) through MABC breeding strategy.
- Foreground selection for the genes Piz-5 and Pi54 were effected using tightly linked molecular markers, AP5930 and RM206, respectively in two independent backcross series. Background analysis revealed the RP genome recovery up to 89.01% and 87.88% in Pusa1602 and Pusa1603 lines, respectively.
- The hybrids produced by crossing Pusa6A with improved lines of PRR78, were on par with original Pusa RH10 in terms of yield, grain and cooking quality traits with an added advantage of blast resistance.

SUBMERGENCE TOLERANCE IN RICE

Theor Appl Genet (2007) 115:767–776
DOI 10.1007/s00122-007-0607-0

ORIGINAL PAPER

A marker-assisted backcross approach for developing submergence-tolerant rice cultivars

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Mega varieties

BR11	**Bangladesh**
CR1009	India
IR64	**All Asia**
KDML105	Thailand
Mahsuri	India
MTU1010	India
RD6	Thailand
Samba Mahsuri	**India**
Swarna	**India, Bangladesh**

- Flash floods or short-term submergence regularly affect around 15 million hectares of rice (Oryza sativa L.) growing areas in South and Southeast Asia
- An economic loss of up to one billion US dollars annually has been estimated
- Submergence tolerant varieties have been developed but have not been widely adopted
- Poor agronomic and quality characteristics
- Many popular and widely-grown rice varieties - “Mega varieties”
- A major QTL (Sub1) for submergence tolerance identified and fine mapped on chromosome 9 in the submergence tolerant cultivar FR13A (Xu and Mackill, 1996)

Conclusion

MAS is a methodology that has already proved its value.

It is likely to become more valuable as a larger number of genes are identified and their function and interactions elucidated.

Reduced costs and optimized strategies for integrating MAS with phenotypic selection and needed before the technology can reach its potential.

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