MOLECULAR MARKERS AND IT'S APPLICATIONS

Genetic Markers

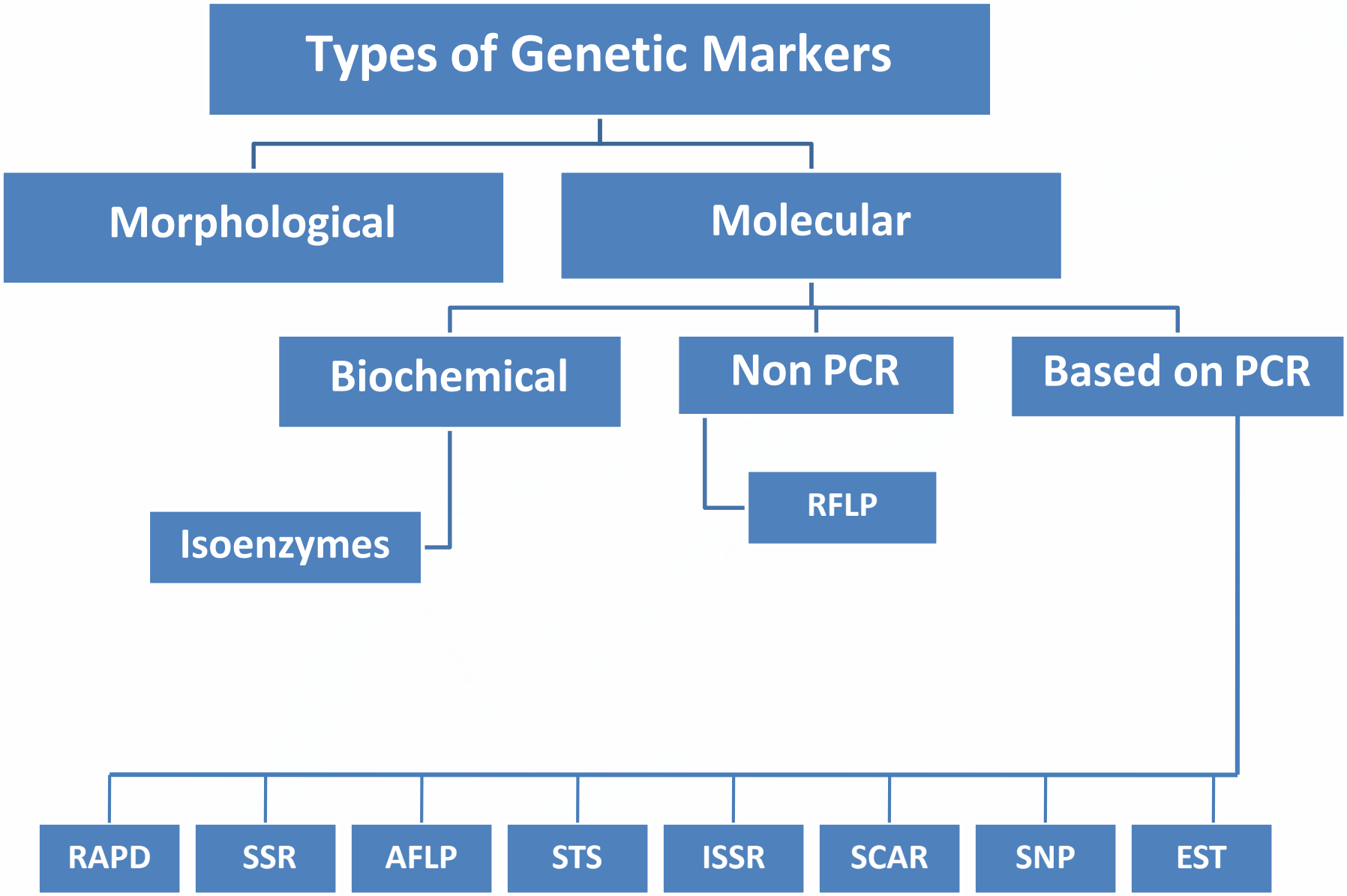
❖A **genetic marker** is a gene or DNA sequence with a known location on a chromosome that can be used to identify individuals or species. It can be described as a variation (which may arise due to mutation or alteration in the genomic loci) that can be observed.

❖ Generally, they do not represent the target genes themselves but act as **'signs' or 'flags'.**

❖ Genetic markers that are located in close proximity to genes (i.e. tightly linked) may be referred to as gene **'tags'.**

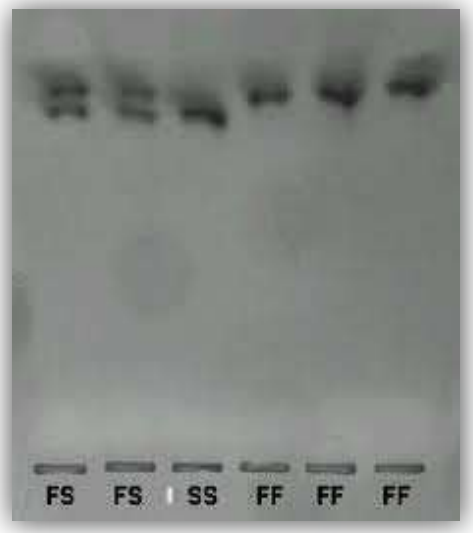
❖Such markers themselves do not affect the phenotype of the trait of interest because they are located only near or 'linked' to genes controlling the trait.

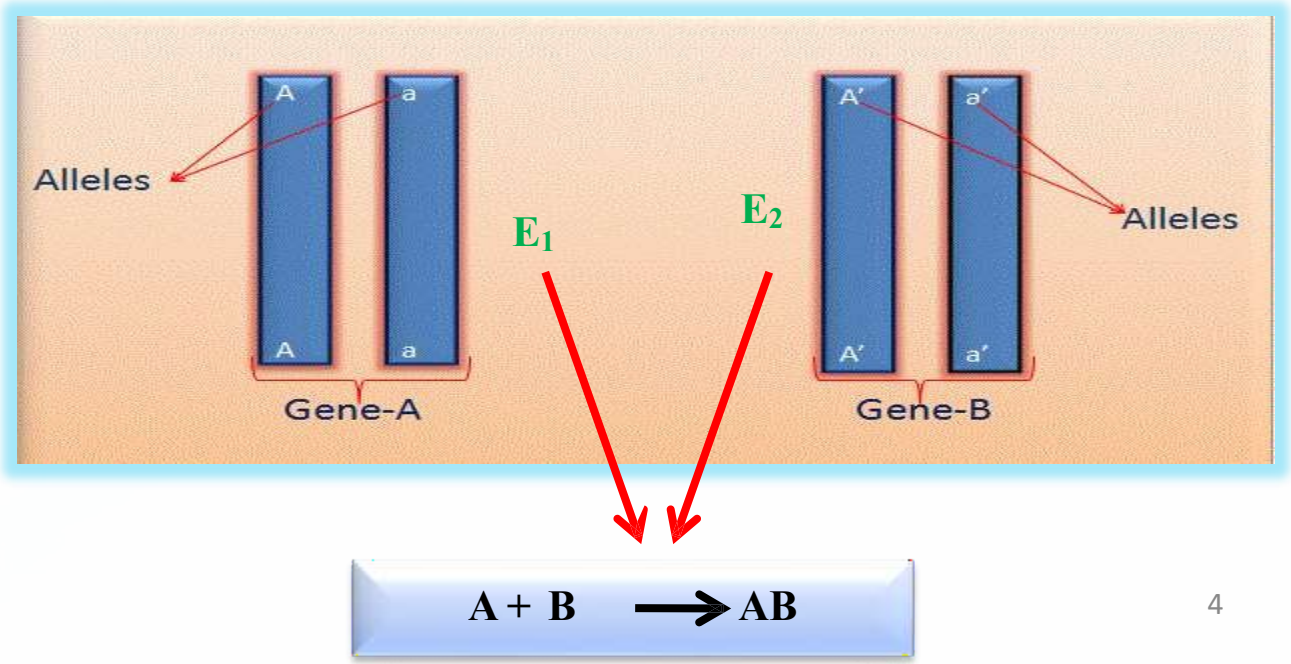
❖All genetic markers occupy specific genomic positions within chromosomes (like genes) called **'loci' (singular 'locus').**


Types of Genetic Markers
Morphological
Molecular
Biochemical
Non PCR
Based on PCR
Isoenzymes
RFLP
RAPD
SSR
AFLP
STS
ISSR
SCAR
SNP
EST

1. Morphological Marker

2. Isozyme/Isoenzyme





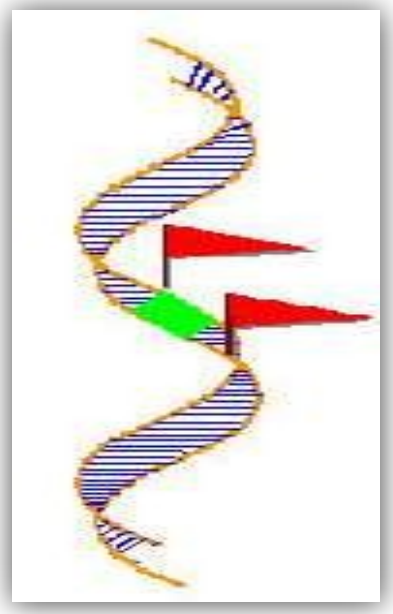
3. Molecular Marker

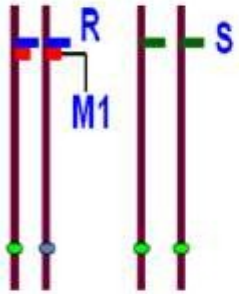
❑**Molecular markers are specific fragments of DNA that can be identified within the whole genome.**

❑ Molecular markers are **found at specific locations of the genome.**

❑**They are used to 'flag' the position of a particular gene or the inheritance of a particular character.**

❑ Molecular markers are phenotypically neutral.

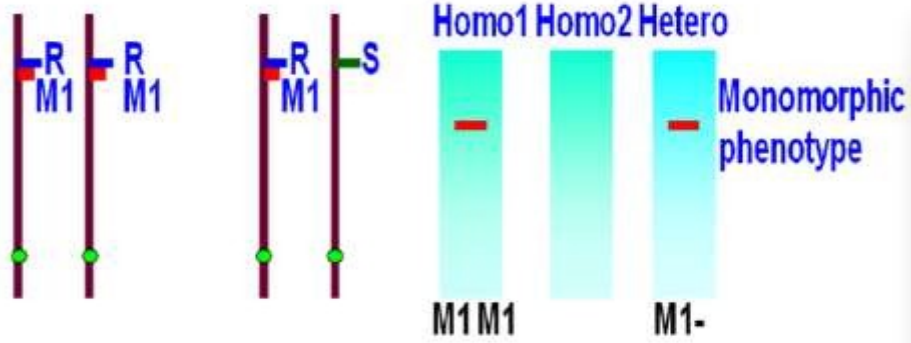


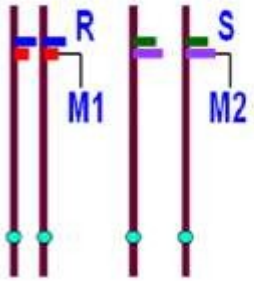


If only one form of the trait (which is targeted to be marked) is associated with the marker whereas the other form of the trait has no marker

Dominant Marker

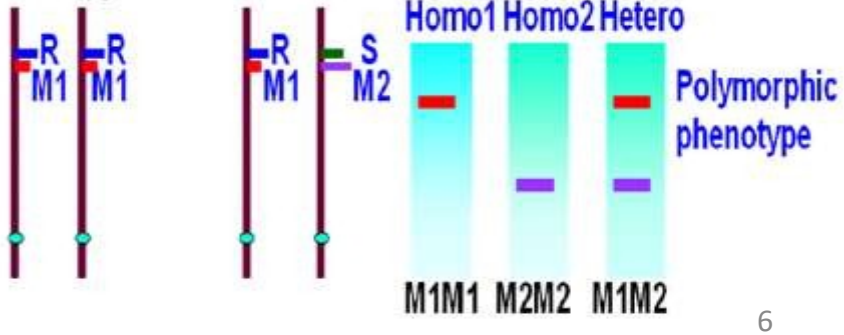
It can not discriminate between heterozygote and homozygote individuals





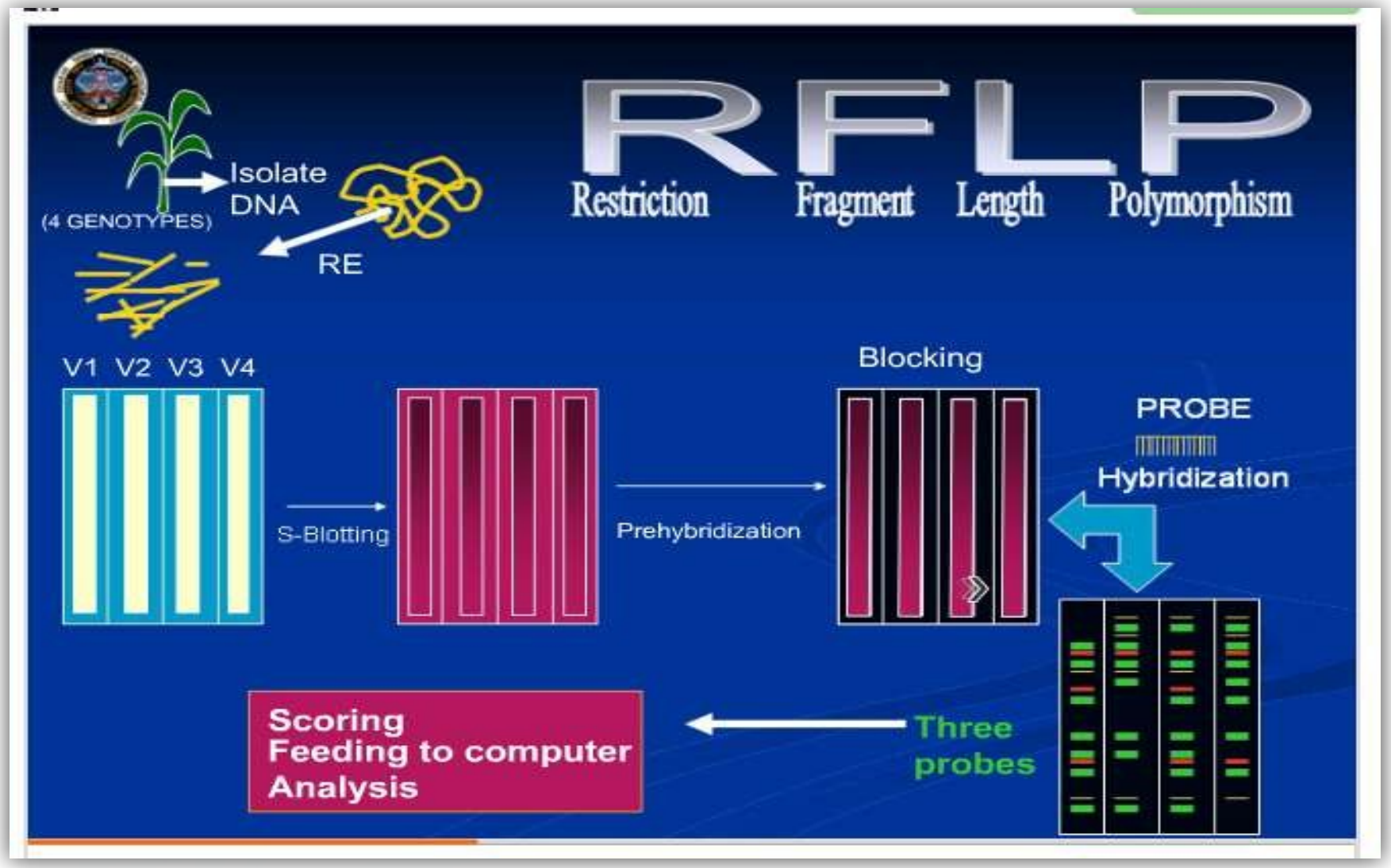
If both forms of the trait (which is targeted to be marked) is associated with the marker

It can discriminate between heterozygote and homozygote individuals



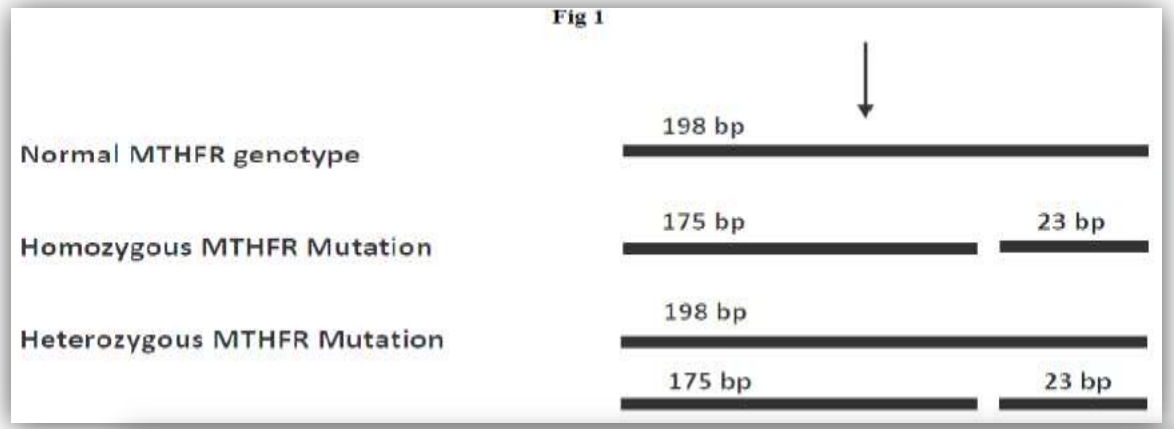
Co-dominant Marker

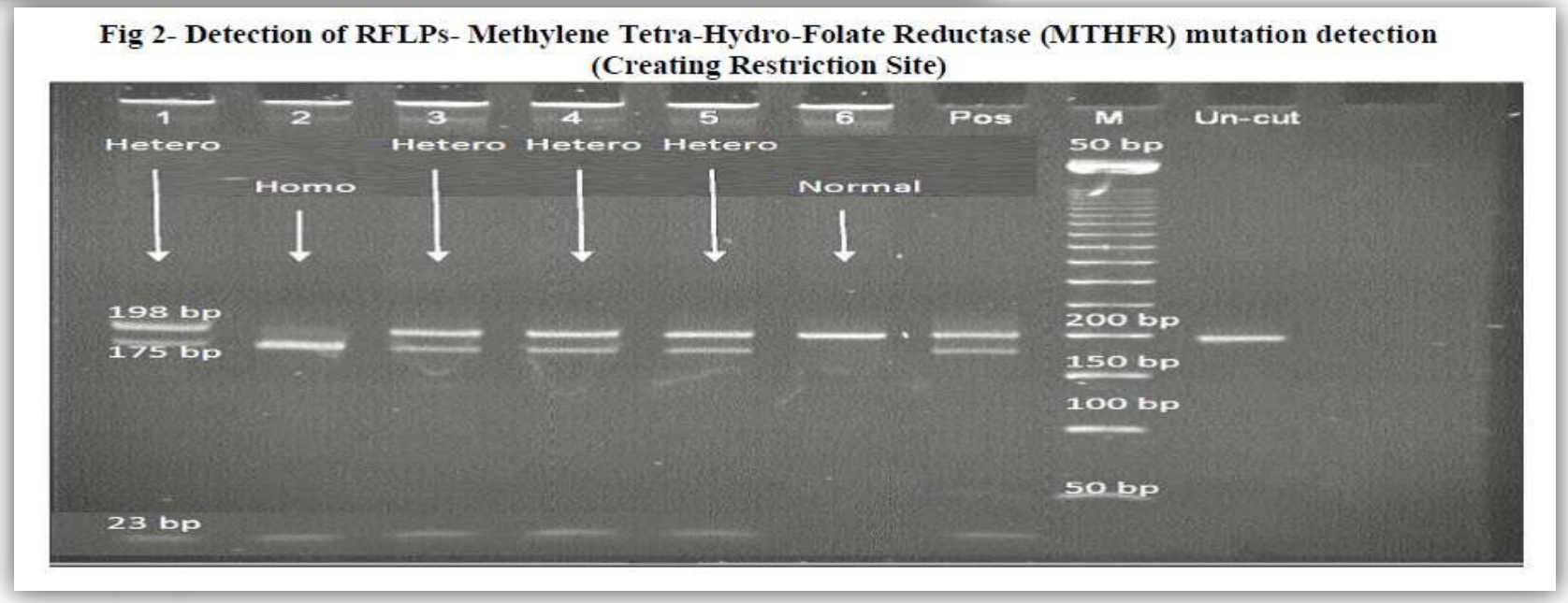
1. Restriction Fragment Length Polymorphism (RFLP)



Examples of RFLPs

Methylene Tetra-Hydro-Folate Reductase (MTHFR) mutation detection (Creating Restriction Site) - (Rate limiting enzyme in methly cycle)





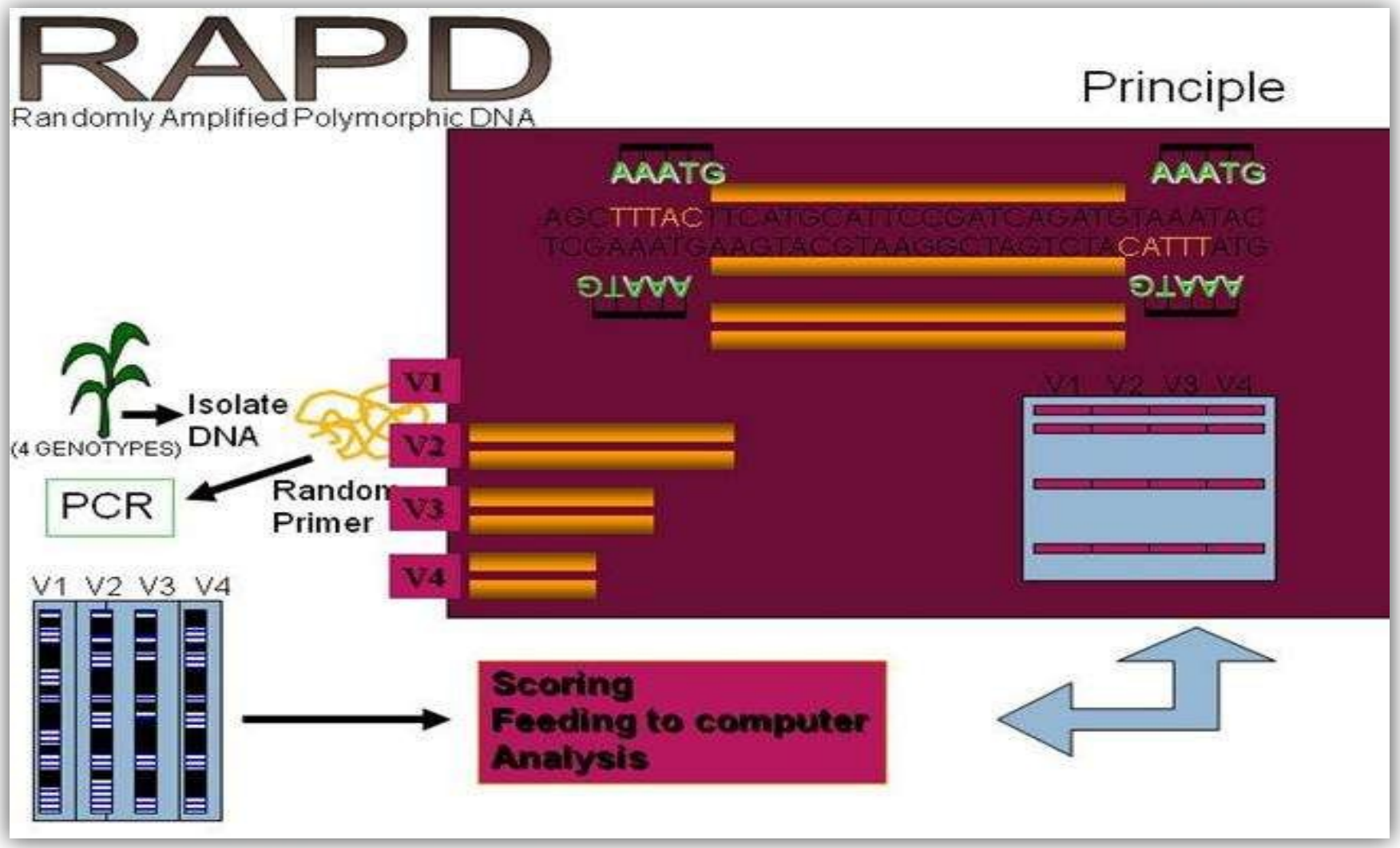
Advantages

- ❑ High reproducibility
- ❑ Show **codominant** alleles
- ❑ Detect coupling phase of DNA
- ❑ Reliable marker in linkage and breeding analysis
- ❑ Easily determine a linked trait present in both homozygous and heterozygous .

Limitations

- ❑ Require **large quantities of high molecular weight DNA (5-10 µg).**
- ❑ Expensive process
- ❑ Time consuming
- ❑ Labor intensive
- ❑ **Radioactive probe**

2. Random Amplified Polymorphic DNA (RAPD)



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IDENTIFICATION OF RAPD MARKER LINKED TO BLAST RESISTANCE GENE IN A SOMACLONE OF RICE CULTIVAR ARAGUAIA*

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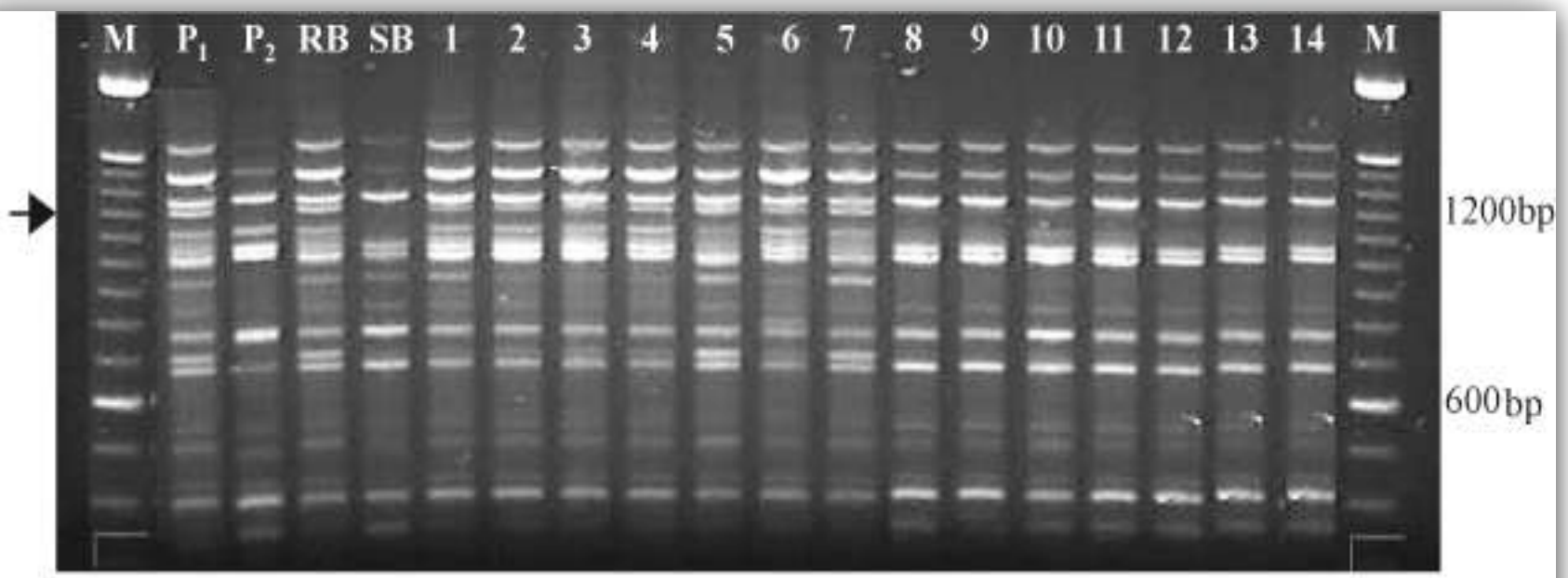


FIG. 2 - RAPD tagging of *Pi-ar* gene by bulked segregant analysis using primer $OPC02_{(1200)}$. Lanes: Resistant somaclone SC09 (P_1), susceptible parental cultivar Araguaia (P_2), resistant bulk (RB), susceptible bulk (SB) followed by 14 F_2 plants of the cross composed of resistant plants (lanes 1 to 7) and susceptible plants (lanes 8 to 14). The arrow indicates the marker linked to the resistance gene *Pi-ar* of somaclone SC09 derived from Araguaia to race IB-45 of *Pyricularia grisea*. M = Marker (100 bp)

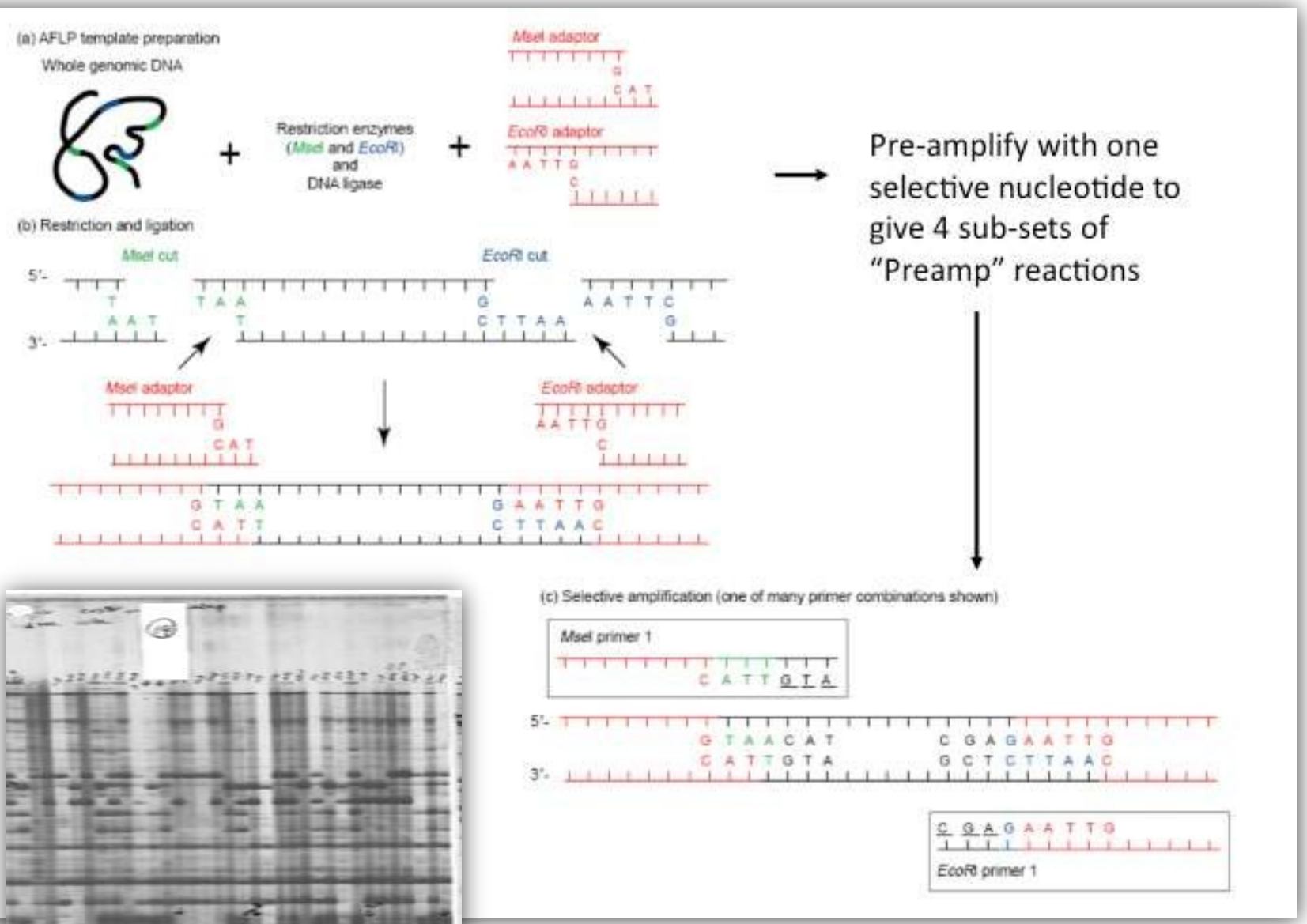
Advantages

- Quick and easy to assay..
- **Low quantities of template DNA required.**
- **Dominant** markers.
- In expensive.
- Do not require any specific knowledge of the target.

Limitations

- It is **not always reproducible.**
- It shows **dominant inheritance.**
- Sometime it reveals homology.

3. Amplified Fragment Length Polymorphism (AFLP)



Advantages

- It is highly **reliable and reproducible.**
- It does not require any DNA sequence information from the organism under study.
- Ability to analyze a large number of polymorphic loci simultaneously with a single primer combination on a single gel as compared to RAPDs.

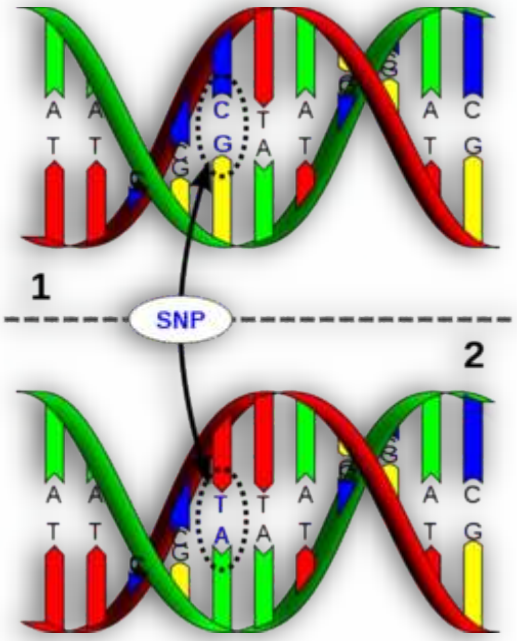
Limitations

- It requires more number of steps to produce the result.
- It involves **additional cost to purchase both restriction and ligation enzymes as well as adapters.**
- Most AFLP loci are **dominant,** which does not differentiate dominant homozygotes fromheterozygotes.

4. Single Nucleotide Polymorphism (SNP)

❑ A single Nucleotide Polymorphism (SNP) describes a **single base difference between two DNA sequences.**

❑For example, a C/T substitution in the DNA of plant 2 compared to the same region of DNA in plant



Female Plant 1 GAATTCCGCAATGCAGGTTAAGAGCTCTGTGAAAGAGGAAAACGAAAAAC

Male Plant 2 GAATTCCGCAATGCAGGTTAAGAGCTTTGTGAAAGAGGAAAACGAAAAAC

SNP

Types of nucleotide substitutions:

Transition [C/T or G/A]

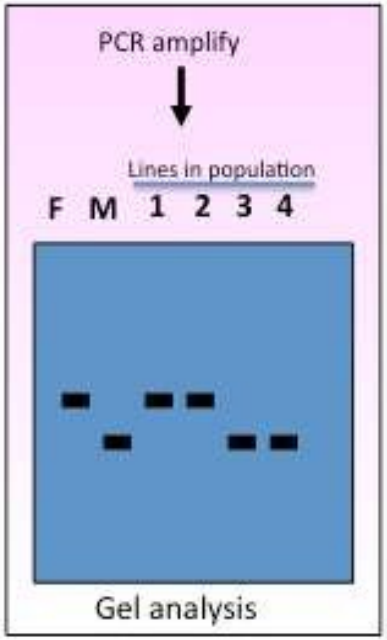
Transversion [C/G, A/T, C/A, T/G]

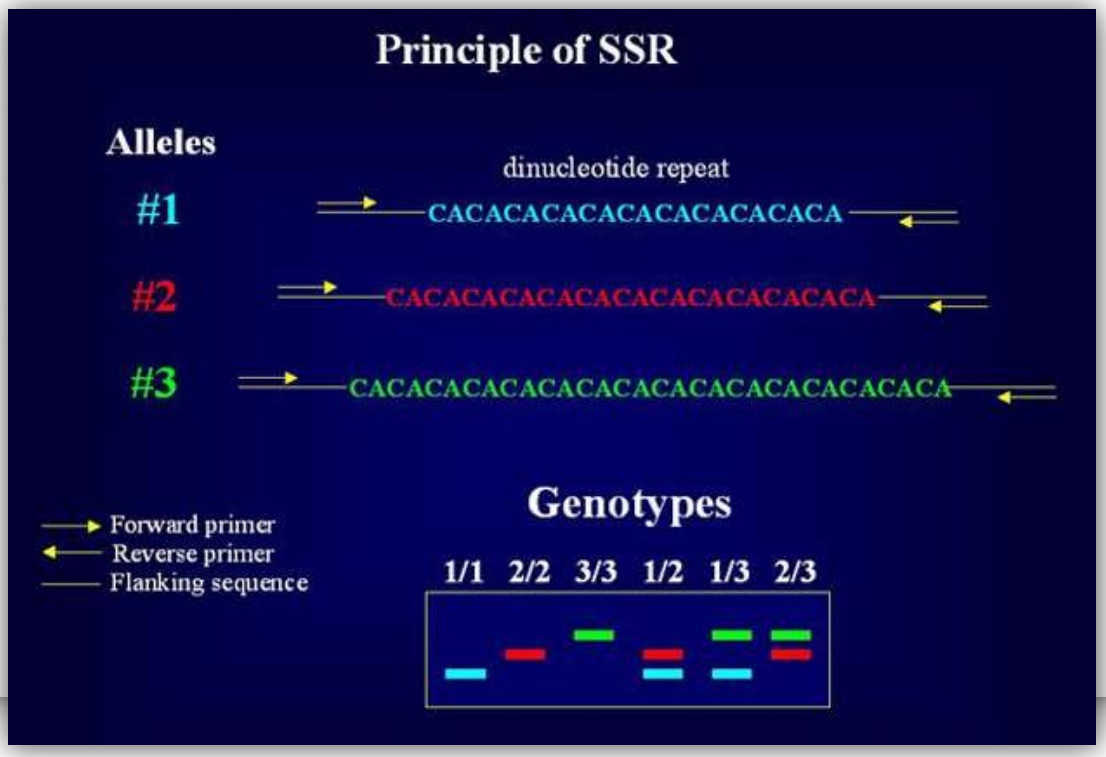
Plant	Sequence
1	GAATTCCGCAATGCAGGTTAAGAGCTCTGTGAAAGAGGAAAACGAAAAAC
2	GAATTCCGCAATGCAGGTTAAGAGCTCTGTGAAAGAGGAAAACGAAAAAC
3	GAATTCCGCAATGCAGGTTAAGAGCTCTGTGAAAGAGGAAAACGAAAAAC
4	GAATTCCGCAATGCAGGTTAAGAGCTTTGTGAAAGAGGAAAACGAAAAAC
5	GAATTCCGCAATGCAGGTTAAGAGCTCTGTGAAAGAGGAAAACGAAAAAC
6	GAATTCCGCAATGCAGGTTAAGAGCTCTGTGAAAGAGGAAAACGAAAAAC
7	GAATTCCGCAATGCAGGTTAAGAGCTTTGTGAAAGAGGAAAACGAAAAAC
8	GAATTCCGCAATGCAGGTTAAGAGCTCTGTGAAAGAGGAAAACGAAAAAC

5. Simple Sequence Repeat (SSR)

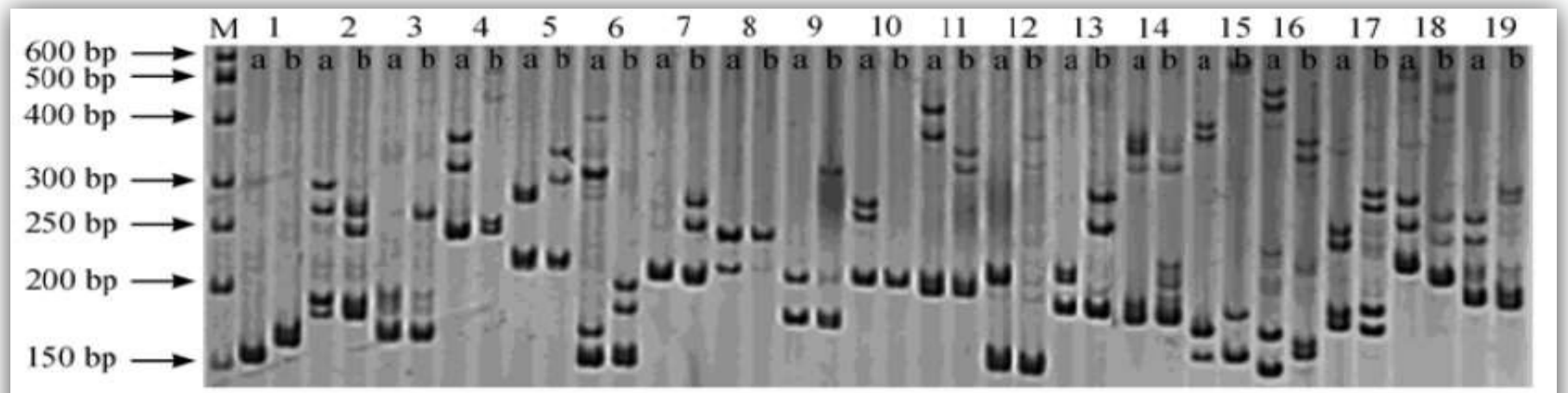
- Short sequences of nucleotides (typically 2 to 5) that are repeated multiple times in tandem
- Often particularly polymorphic

- ❑ Mono: A, T
- ❑ Di: AT, GA
- ❑ Tri: AGG
- ❑ Tetra: AAAC.



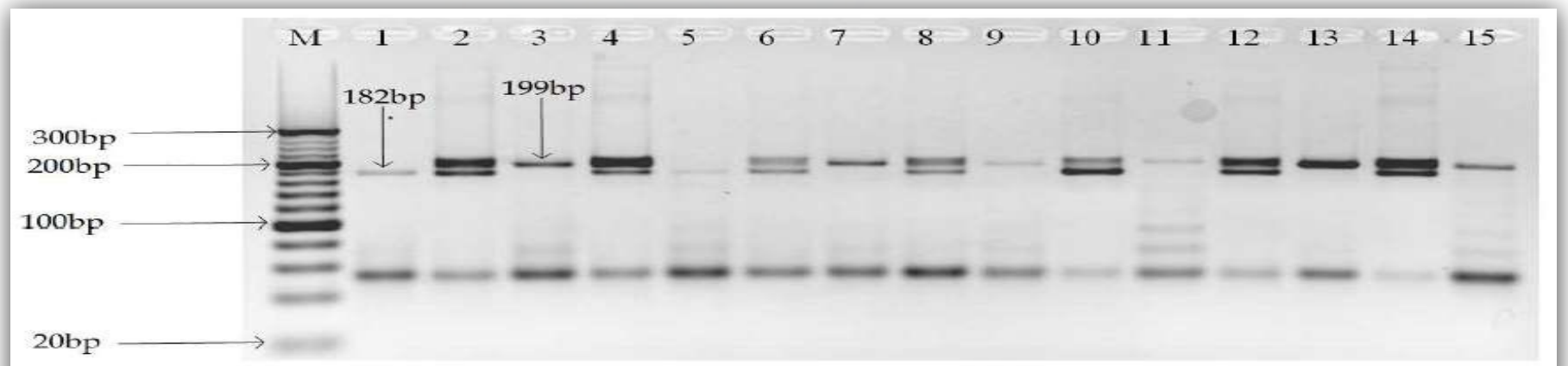


SSR Primer pairs for polymorphisms between two tetraploid cotton



(a, *G. barbadense* and b, *G. hirsutum* M-Molecular weight marker, lane 1-19 different SSR primer amplification product.)

Genetic Purity Testing of hybrids through SSR markers



Homozygous dominant or heterozygous alleles. No fragment is produced from homozygous recessive alleles because amplification is disrupted in both alleles.

Advantages

- **Low quantities of template DNA** (10–100 ng per reaction) are required.
- **Codominant** marker
- **Highly polymorphic**
- **High reproducibility**
- **Population studies**

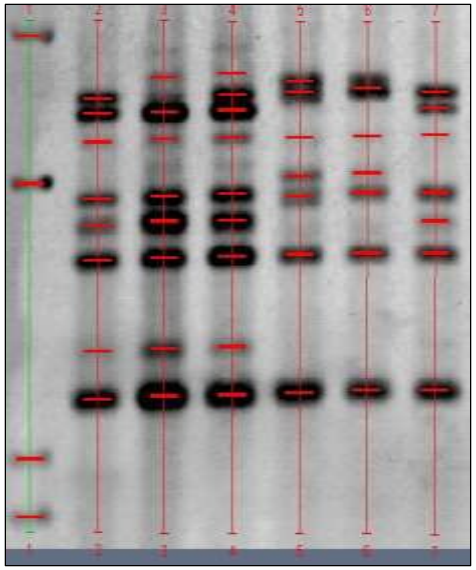
Limitations

- High development costs

Applications of Molecular Markers

- ❑ Phylogenetic studies
- ❑ Trait Identification and Mapping
- ❑ DNA finger printing
- ❑ Genetic diagnostics
- ❑ Expression Profile Analysis
- ❑ Study of genome
- ❑ Gene mapping / Gene tagging
- ❑ Seed testing
- ❑ Identifying location of QTL's
- ❑ Marker Assisted Selection (MAS)

Phylogenetic Relationship



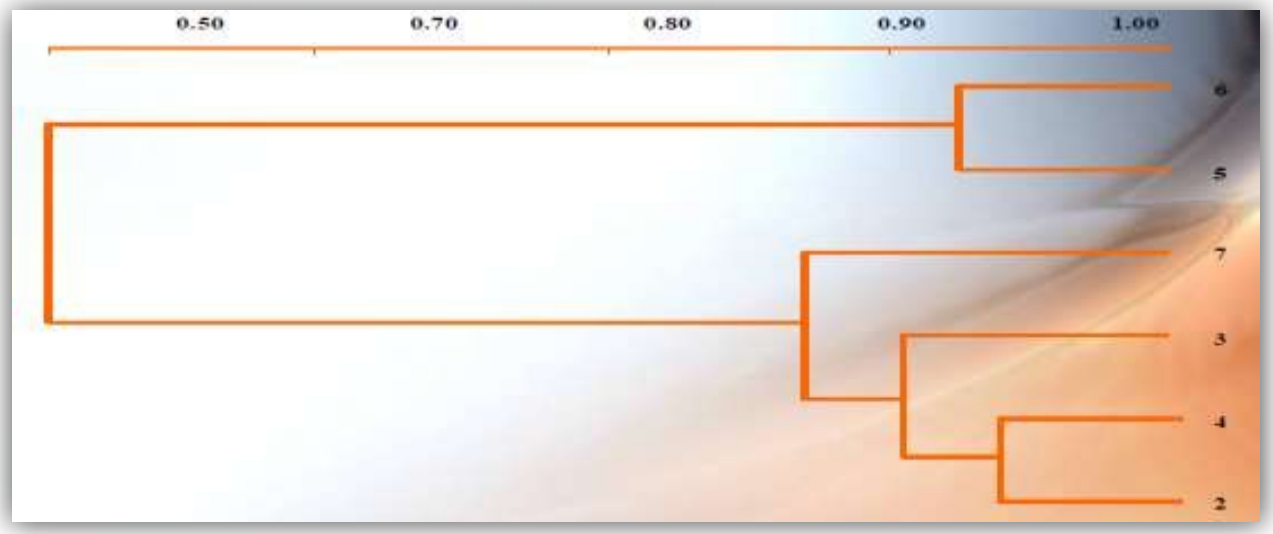
1. PCR product

2	3	4	5	6	7
0	1	1	0	0	0
0	0	0	1	1	0
1	0	1	1	1	1
1	1	1	0	0	1
1	1	1	1	1	1
0	0	0	1	1	0
1	1	1	1	1	1
1	1	1	0	0	1
1	1	1	1	1	1
1	1	1	0	0	0
1	1	1	1	1	1

2. Scoring of bands

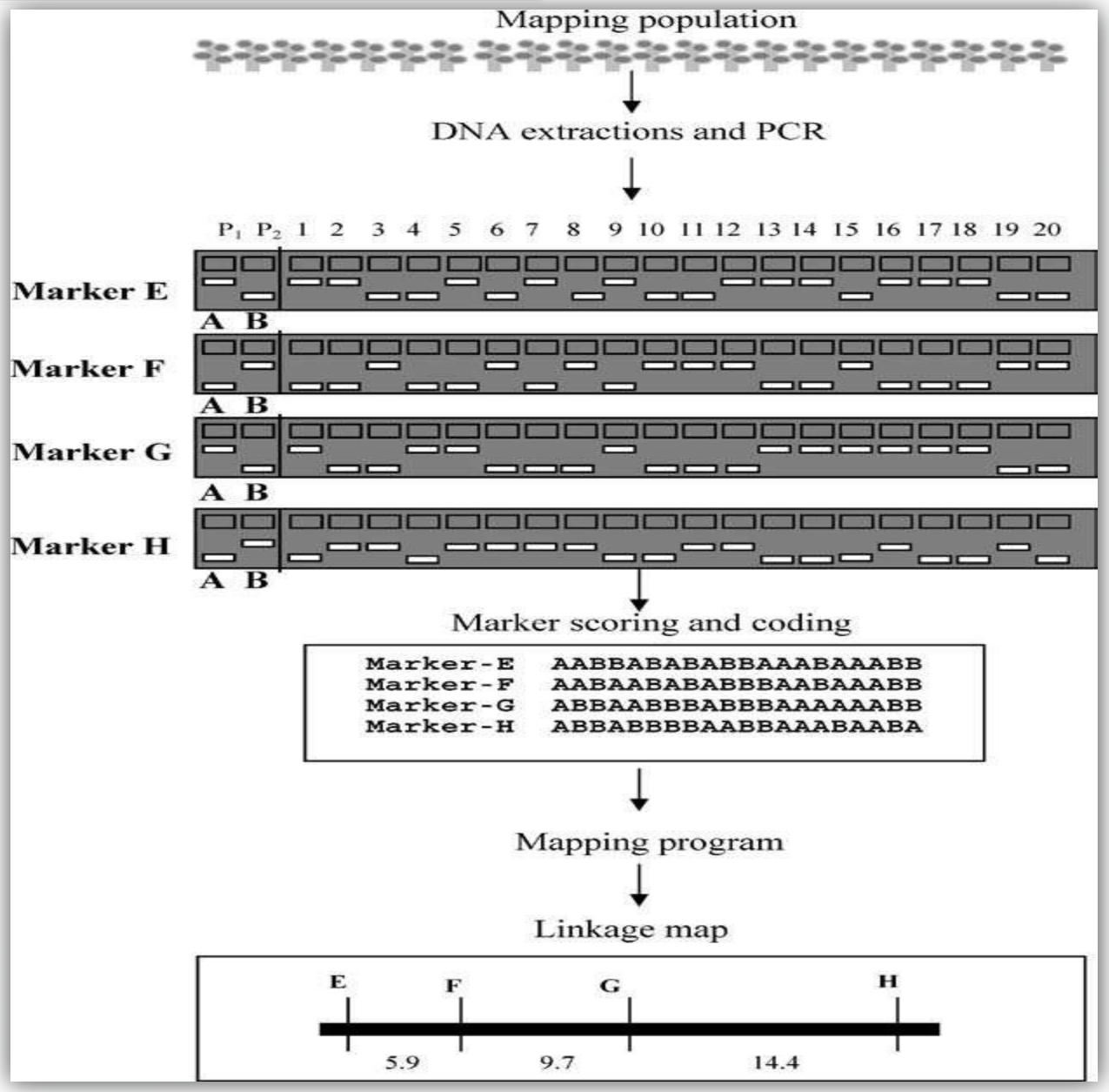
	2	4	7	3	5	6
2	100					
4	94.1	100				
7	93.3	87.5	100			
3	87.5	94.1	80.0	100		
5	66.7	62.5	71.4	53.3	100	
6	57.1	53.3	61.5	57.1	92.3	100

3. Genetic Similarity matrix



4. Dendrogram constructed with UPGMA cluster

Construction of Linkage Map



Marker Assisted Selection

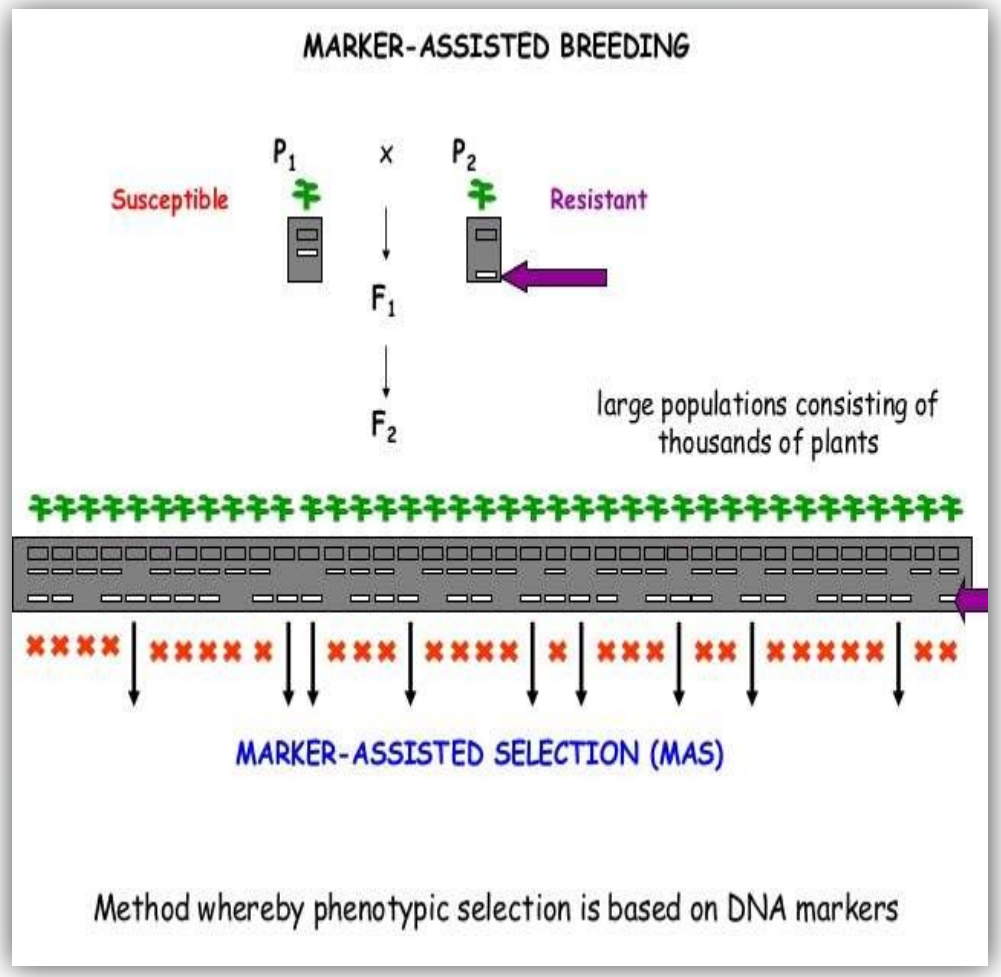
What are the advantages of marker-assisted selection (MAS) ?

The fundamental **advantages of MAS** compared to conventional phenotypic selection are:

1) **Simpler compared to phenotypic screening**
2) **Selection may be carried out at seedling stage**
3) **Single plants may be selected with high reliability.**

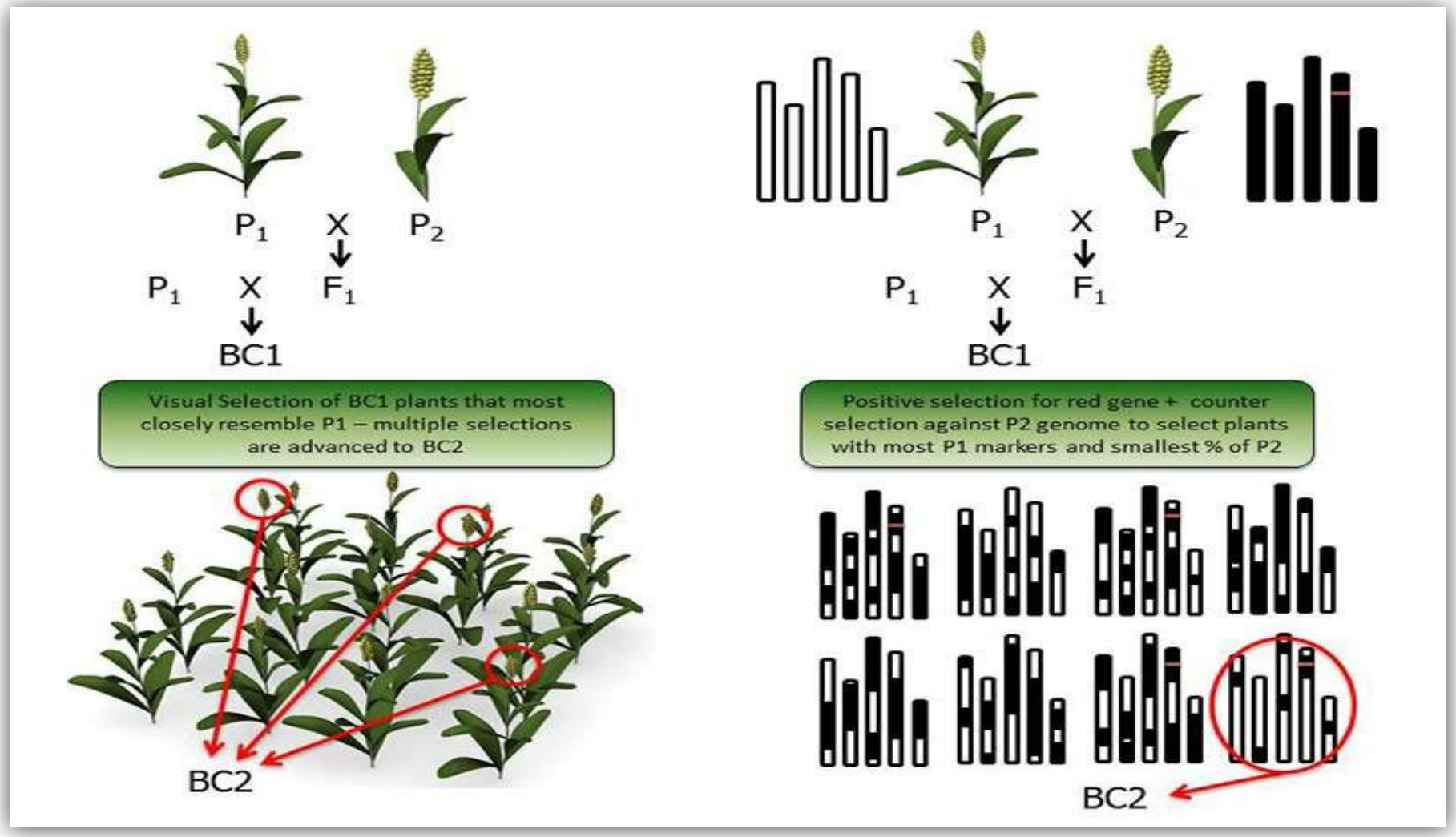
These advantages may translate into

1) **Greater efficiency or**
2) **Accelerated line development in breeding programs.**



Marker Assisted Backcrossing

Classical Breeding Approach **Vs** *Marker Assisted Breeding Approach*



Conclusion

Selection of ideal molecular markers

❑**Highly polymorphic nature:** It must be polymorphic as it is polymorphism that is measured for genetic diversity studies.

❑**Codominant inheritance:** determination of homozygous and heterozygous states of diploid organisms.

❑**Frequent occurrence in genome:** A marker should be evenly and frequently distributed throughout the genome.

❑**Selective neutral behaviours:** The DNA sequences of any organism are neutral to environmental conditions or management practices.

❑ **Easy access (availability): It should be easy, fast and cheap to detect.**

❑ **Easy and fast assay**

❑ **High reproducibility**

References

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- Mishra Kundan K, Fougat R. S., Ballani A., Thakur Vinita., Jha Yachana and Bora Madhumati. (2014): Potential and application of molecular markers techniques for plant genome analysis. *Int. J. Pure App. Biosci.* 2 (1): 169-188.


Thank
you!