

Molecular Breeding and Marker-Assisted Selection



The process of developing new crop varieties can take almost 25 years. Now, however, biotechnology has considerably shortened the time that new crop varieties are brought to the market. One of the tools which can make it easier and faster for scientists to select plant traits is *marker-assisted selection* (MAS).

Molecular shortcut

The differences that distinguish one plant from another are encoded in the plant's genetic material, the DNA. DNA is packaged in *chromosomes* (strands of genetic material), one coming from each parent. The genes, which control a plant's characteristics, are located on specific segments of each chromosome. Together, all of a plant's genes make up its *genome*.

Some traits, like flower color, may be controlled by only one gene. Other more complex characteristics, however, 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 done in the laboratory, as well as in the field. This is done by using a process called *marker-assisted selection* (MAS). To help identify specific genes, scientists use what are called *molecular* or *genetic markers*. The markers are a string or sequence of nucleic acid which makes up a DNA sequence. The markers are located near the DNA sequence of the desired gene and are transmitted by the standard laws of inheritance from one generation to the next (Figure 1). Since the markers and the genes are close together on



the same chromosome, they tend to be inherited together as each generation of plants is produced. This is called *linkage*. This linkage can be used to predict whether a plant will have a desired gene. If researchers can find the marker for the gene, it means the desired gene is present.

As scientists learn where markers occur on a chromosome, and how close they are to specific genes, they can create a *genetic linkage map*. Such a map would show the location of markers and genes, and their distance from other known genes. Scientists can produce detailed maps in only one generation of plant breeding.

Using very detailed genetic maps and better knowledge of the molecular structure of a plant's DNA, researchers can analyze only a tiny bit of plant tissue, even from a newly germinated seedling. Once the tissue is analyzed, scientists know whether that seedling contains the appropriate gene. If it doesn't, they can quickly move on and concentrate on analysis of another seedling, eventually working only with the plants which contain a specific trait.

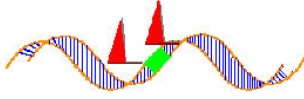


Figure 1. Molecular/genetic markers.

"The black section indicates the presence of a desirable gene in an organism's genetic code that is associated with two genetic markers (flags)."

Source: <http://usda-ars-beaumont.tamu.edu/dh/helix.pdf>

It should be noted, however, that molecular breeding through MAS is somewhat limited in scope compared to genetic engineering or modification because: 1) it works only for traits already present in a crop; 2) it cannot be used effectively to breed crops which have long generation times (e.g. citrus); and 3) it cannot be used effectively with crops which are clonally propagated because they are sterile or do not breed true (this includes many staples such as yams, bananas, plantain, sweet potato, and cassava).

Sources:

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<http://www.ostp.gov/html/plantgenome/abstract.html>

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Molecular Markers

Several marker systems have been developed and are applied to a range of crop species. These are the Restriction Fragment Length Polymorphisms (RFLPs), Random Amplification of Polymorphic DNAs (RAPDs), Sequence Tagged Sites (STS), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) or microsatellites, and Single Nucleotide Polymorphism (SNPs). The advantages and disadvantages of these marker systems are provided in Table 1.

Table 1. Comparison of most commonly used marker systems (Adapted from Korzun, 2003).

Feature	RFLPs	RAPDs	AFLPs	SSRs	SNPs
DNA required (µg)	10	0.02	0.5-1.0	0.05	0.05
DNA quality	High	High	Moderate	Moderate	High
PCR-based	No	Yes	Yes	Yes	Yes
Number of polymorph loci analyzed	1.0-3.0	1.5-50	20-100	1.0-3.0	1.0
Ease of use	Not Easy	Easy	Easy	Easy	Easy
Amenable to automation	Low	Moderate	Moderate	High	High
Reproducibility	High	Unreliable	High	High	High
Development cost	Low	Low	Moderate	High	High
Cost per analysis	High	Low	Moderate	Low	Low

These molecular techniques have been widely used to monitor differences in DNA sequence in and among species. They also allow the creation of new sources of genetic variation by introducing new and desirable traits from wild varieties into elite lines. While RFLP markers have been the basis for most genetic work in crop plants, AFLPs and SSRs are currently the most popular techniques used due to ease in detection and automation. The adoption of the new marker system, SNPs, is now highly preferred, with the increasing amount of sequence information, and the determination of gene function due to genomic research.

Applications of molecular markers for crop genetic studies

The main uses of these molecular markers in crop genetic studies are as follows:

- Assessment of genetic variability and characterization of germplasm
- Identification and fingerprinting of genotypes
- Estimation of genetic distances between population, inbreds, and breeding materials
- Detection of monogenic and quantitative trait loci (QTL)
- Marker-assisted selection
- Identification of sequences of useful candidate genes



MAS for Pathogen Resistance in Tomato

One of the major constraints in tomato cultivation and production are severe harvest losses caused by a number of pathogens, including viruses, bacteria, fungi, and nematodes. Farmers have adopted control measures, such as applications of agrochemicals and use of resistant lines. Although conventional breeding has had a significant impact on improving resistance of tomato, the time-consuming process of making crosses and backcrosses, and the selection of the desired resistant progeny make it difficult to react adequately to the evolution of new virulent pathogens.



Molecular markers are now being widely used for breeding tomato. More than 40 genes that confer resistance to major classes of tomato pathogens have been mapped, cloned, and/or sequenced (Grube, et. al., 2000). These maps have allowed for "pyramiding" resistance genes in tomato through MAS, where several resistance genes can be engineered into one genotype. Currently, tomato breeding through MAS has resulted in varieties with resistance or tolerance to one or more specific pathogens.

Glossary

AFLP: Amplified Fragment Length Polymorphism. A highly sensitive method for detecting DNA polymorphism. Following restriction enzyme digestion of DNA, a subset of the DNA fragments is selected for PCR amplification and visualization.

Genetic Map: A map of the relative positions of genetic loci on a chromosome, determined on the basis of how often the loci are inherited together.

Linkage Map: A map of relative positions of genes on a chromosome. Genes inherited together are close to each other on the chromosome, and said to be linked.

Microsatellites: Very short DNA motifs (1-10 base pairs) which occur as tandem repeats at numerous loci throughout the genome. Also known as simple sequence repeats (SSR), simple tandem repeats or simple repetitive sequences.

Monogenic trait (Mendelian trait): a trait determined by the action of a single genetic locus
Nucleic acid: molecule found in all living cells, in which the hereditary information is stored and from which it can be transferred. The two chief types are DNA (deoxyribonucleic acid), found mainly in cell nuclei, and RNA (ribonucleic acid), found mostly in cytoplasm.

PCR: Polymerase Chain Reaction. A method for amplifying a DNA sequence in large amounts using a heat-stable polymerase and suitable primers to direct the amplification of the desired region of DNA.

Polymorphism: A detectable difference at a particular gene or marker occurring among individuals.

RAPD: Random Amplification of Polymorphic DNA. A widely-used technique for amplifying anonymous stretches of DNA using PCR with arbitrary primers.

RFLP: Restriction Fragment Length Polymorphism. Variations which occur in the length of DNA fragments produced when DNA is broken down by restriction enzymes (enzymes which recognize specific sequences of DNA, usually 4-6 base pairs long, and cleave the DNA at these points, known as restriction sites).

SNP: Single Nucleotide Polymorphism. A common, but minute, variation that occurs in DNA sequences of a genome. These variations can be used to track inheritance in families or species.

QTL: Quantitative Trait Locus. Location of a specific gene that affects a measurable or quantifiable trait. These traits are typically affected by more than one gene, and also by the environment. Examples of quantitative traits are plant height (measured on a ruler) and body weight (measured on a balance)

Quantitative (continuous) traits - phenotypes that exhibit a range of measurable outcomes.