



## *"Fingerprinting" Vegetables* DNA-based Marker Assisted Selection

**Faster, Cheaper, More Reliable;** These are some of the goals that vegetable breeders at seed companies and public institutions desire for improving their ability to develop new cultivars with beneficial traits. The quality, efficiency and economy of a breeder's methods for variety selection and advancement of a new cultivar to commercial use determine the number of desirable characteristics that will be included. The screening and testing methods that are used will determine the predictability of maintaining a desired trait, such as pest resistance, during the breeding process.



Cultivars are developed by selecting for desired traits or by removing undesirable traits from existing varieties or breeding lines. Selections are made for measurable or visual traits that are controlled by genes, the "blueprint" for all characteristics in living organisms. Genes are the hereditary units, composed of DNA (deoxyribonucleic acid), that provide all the instructions that determine an organism's characteristics. Some traits are determined by single genes (certain growth habit or pest resistance traits) while others are complex interactions among several to many genes ( yield, quality, fruit size ). Successful selection strategies are dependent on the breeder's ability to distinguish genetic effects from environmental effects on a visible or measurable trait. The timely response of vegetable breeders to the needs of the producers and consumers of vegetables will be continually improved as new selection technologies are developed and implemented.

Advanced breeding technologies that meet these goals have resulted from recent research in plant biotechnology. Applications of this knowledge has created powerful new DNA-based **molecular markers** for plant breeders to more rapidly and efficiently sort through large groups of plants. Molecular markers are 'tags' that can be used to identify specific genes and locate them in relation to other genes. These technologies will help ensure a desired beneficial trait is maintained or an undesirable trait is eliminated. These 'tags' can also be used to identify a corresponding gene in a distant plant species or even in animal or microbial species.

A simple way to appreciate this powerful tool for vegetable selection is to visualize a UPC bar-code, as on products in a supermarket. Each set of bars in the code specifies a product with both a unique set of characteristics and shared characteristics with items of a similar type. The unique product code is deciphered from the series of bars with the proper instrumentation. The code identity gives a traceable "fingerprint" of the product for a diversity of information gathering and retrieval applications in source-tracking, inventory management, marketing, and other applications.

In a similar manner, a modern vegetable breeder can create a bar-code like "fingerprint" that allows the physical identification and tracking of desirable or undesirable traits in a breeding and selection program. The unique ladder-like pattern is the result of differences in the genetic blueprint of the breeding line or hybrid. With specialized techniques, detection can be as sensitive as a single change in the DNA component of a gene.

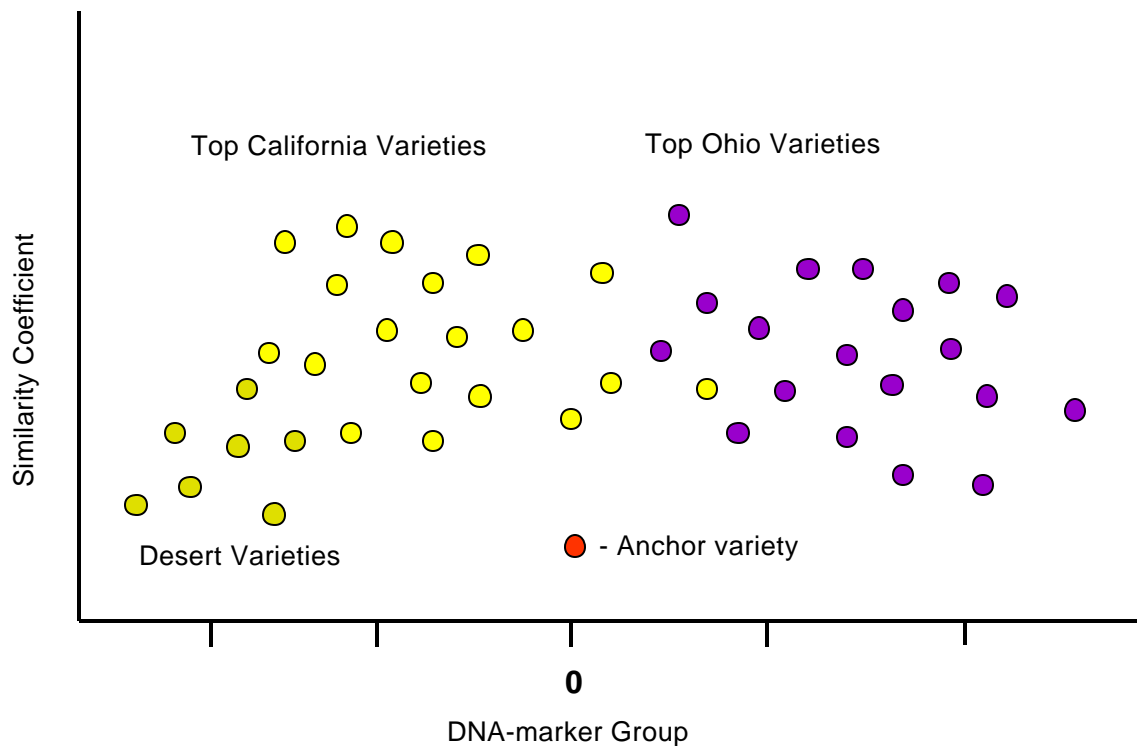
The techniques and process to accomplish this genetic fingerprinting are too detailed to present in this overview ( For those who wish to know more see ABC Series XXX). An example of the application to achieve a desirable variety improvement goal is shown in Fig. X. The techniques have been utilized for some time using a bar-code-like fingerprint of differentiating enzymes and proteins (the products of the genes) for a few important traits to monitor or reveal inheritance in the same way. New, more precise technologies for fingerprinting are based on visualizing uniqueness in the genetic material itself, DNA.

### **Applications to Vegetables**

A variety of DNA-based marker systems have been instrumental in the identification and cloning of disease resistance genes from a number of plants. New highly specific methods, developed at UC Davis, have been used to fingerprint and monitor Downy Mildew resistance genes in lettuce (See ABC-Lettuce). Some additional examples from vegetables are given below.

| <b><i>Crop</i></b> | <b><i>Disease Resistance Trait</i></b>          |
|--------------------|---|
| <b>Common Bean</b> | Antracnose                                      |
| <b>Lettuce</b>     | Downy Mildew                                    |
| <b>Peas</b>        | Powdery Mildew                                  |
| <b>Pepper</b>      | Bacterial Spot                                  |
| <b>Potato</b>      | Cyst Nematode                                   |
| <b>Tomato</b>      | Tobacco Mosaic Virus (transferred from tobacco) |
|                    | Bacterial Speck                                 |

The seed industry is beginning to use DNA-based markers to develop “fingerprint” patterns for grouping or clustering complex beneficial traits. This “cluster analysis” will allow breeders to predict regional performance or environmental adaptation based on shared marker patterns. For example, processing tomatoes adapted for California conditions cluster separately from varieties that perform best in Ohio. The efficiency and outcome of selection strategies can be greatly enhanced by optimizing breeding crosses to a targeted DNA-marker cluster.



Schematic representation of “cluster analysis” applied to processing tomatoes. Regionally adapted varieties fall into distinct groups that guide breeders in designing efficient crosses and broad selections without knowing the specific genes involved.

Another practical application, developed at U.C. Riverside, is being used to identify seed lot purity in asparagus (See ABC Asparagus). Molecular markers were used to show that some seed lots used for crown production contained over 70% poor yielding types. This can be critical information for a perennial crop that is expected to be productive over a 10-12 year span.

## What are the advantages of these DNA-based fingerprinting techniques?

Recent developments have made great progress towards **Faster, Cheaper, and More Reliable**. These specific DNA-based techniques have the following advantages;

- Highly reproducible fingerprint pattern
- Fingerprinting based on DNA can be done at any stage of plant growth  
*In comparison, enzyme or protein-based fingerprinting of traits only found in flowers or fruit may only be done at that stage of plant maturity.*
- Only a small amount of plant tissue is needed.  
*Breeders can make selection determinations at the seedling stage, for example, and save only those plants of interest for a whole plant observation.  
Larger plant populations may be assessed than with conventional techniques ;or space, time, and resources may be more economical applied to variety development.*
- Traits, such as insect and disease resistance, can be selected by the breeder without having to depend on the presence of the pest to confirm that the resistance trait is carried in the varieties.  
*The expensive and regionally unpredictable field resistance evaluations can be postponed, with confidence, until a later stage of variety selection, closer to commercial release. Variety selections are not regionally limited, at the early stages, since issues of plant pest quarantine are removed. No pest introductions for screening are necessary as a simple seedling grow-out is sufficient to monitor the desired trait.*
- DNA-based fingerprinting allows the reliable tracking of beneficial traits during variety selection.  
*These have traditionally been difficult or impractical due to the variable influence of environment, plant tissue dependencies ( seed vs. root vs. leaf vs. fruit etc.), local or regional field conditions, or other factors on enzyme/protein fingerprinting and whole plant visual traits or crop performance.*
- DNA-based fingerprinting is the only practical or available technique for ensuring the presence of multiple beneficial genes in a single variety.  
*Stable and durable pest resistance strategies that depend on "stacking" or "pyramiding" resistance factors, for example, have become much more attainable with the development of these new techniques.*

- DNA-based fingerprinting provides the best currently available technology to establish differentiation for patent protection and Plant Variety Protection Act Certification.
- DNA-based marker systems are more readily automated to increase cost efficiencies.

### **Automated Marker Systems**

Recent advances in “fingerprinting” technology for multiple markers, 10 to 100 times more efficient than prior methods, have been applied to corn, tomatoes, brassicas (the broccoli family), pepper, and lettuce. The technology is readily automated and commercial systems are available. Combined with computer-assisted analysis of the complex pattern, several hundred DNA marker sites can be generated per day. Selection for multigene traits, such as sensory quality, will be greatly aided by these technology systems. Careful economic analysis is required for each crop application, as these approaches are costly. Their use must be justified relative to the effectiveness of other strategies.

Clearly, DNA-fingerprinting techniques are not a replacement for classical breeding and selection techniques. Rather they have become a powerful accelerating tool to improve the rate of development and durability of plant-based pest resistance traits. The specificity of molecular monitoring provides the confidence in trait selection and overall cost-effectiveness of the breeding program for these complex traits. Recent estimates place a cost of using DNA-based marker systems at 100 to 1000 times more expensive than standard phenotype screening. The cost:benefit ratio may not always be in favor of using Marker Assisted Selection technologies but for managing complex traits such as yield and sensory quality, it has a high potential.

Accurate and rapid identification of proprietary vegetable varieties is desirable for both breeders and commercializing companies that have invested in the development of a particular genetic trait or novel combination of traits. Unfortunately, with conventional breeding, the variability among varieties is not always sufficient to create readily detectable “fingerprints “.

New selections from vegetatively propagated crops, such as potato and sweet potato, have improved traits but efforts to apply genetic fingerprinting have yielded no detectable differences. Practical molecular technologies that can uniquely identify one proprietary breeding line or commercial hybrid, for many other crops, from another won't apply in these cases.

Recent research in the Department of Vegetable Crops at U.C. Davis has led to the development of techniques to create and customize a unique “fingerprint” that can be used in variety-specific identification. Inserting a special, movable genetic element found in plants, original identified in maize (field corn), into the existing genetic background of a crop creates a distinct pattern of identifying markers in the parent line of interest. This patented process

was shown to create novel, identical and stable patterns in all the seed, following conventional breeding crosses, by “fixing” the genetic element in a nonmobile form. The fixation step results from the normal plant genetic processes that are part of the well characterized traits of these naturally occurring genes. By repeating this process multiple markers can be developed providing a “fingerprint” that is unambiguous, rapidly determined, inexpensively visualized and insensitive to the effects of multiple parent crosses. In this way, a high level of proprietary insulation can be achieved to protect investments and markets.

Breeders face considerable challenges to breeding for desirable agronomic and quality traits. The table below gives a few examples of the number of genetic loci (regions) that effect individual phenotypes (19 for Phytophthora Late Blight resistance) for a few vegetable crops. The selection for complex traits for cultivar improvement will be more attainable as Marker Assisted Selection technologies improve and costs are reduced.

| <b>Crop</b>        | <b>Trait</b>           | <b>Estimated Interacting Genetic Regions</b> |
|--------------------|------------------------|--|
| <b>Common Bean</b> | Common Blight          | 4  |
| <b>Corn</b>        | Grain Yield            | 6-8  |
|                    | Plant height           | 5  |
| <b>Potato</b>      | Late Blight resistance | 19   |
|                    | Chip Color             | 6  |
| <b>Tomato</b>      | Days to flower         | 7  |
|                    | Number of flower buds  | 10   |
|                    | Fruit Size             | 11   |
|                    | Soluble solids         | 7  |
|                    | Fruit pH               | 9  |

#### Technical Review (Intermediate Complexity)

Staub, Jack, and F.C. Serquen. 1996. Genetic Markers, Map Construction, and Their Application in Plant Breeding. HortScience 31: 729-741

For more information contact:

Vegetable Research and Information Center

University of California

One Shields Ave

Davis, CA 95616

Phone: 530-752-1748

Fax: 530-752-9659

Email: [vrlic@vegmil.ucdavis.edu](mailto:vrlic@vegmil.ucdavis.edu)