american seed trade association

COMMON PRACTICES OF PLANT BREEDERS

INTRODUCTION

The plant world contains hundreds of thousands of species with an amazing array of physical and chemical diversity. For over a billion years, nature has created new genes, altered existing genes, combined them in different ways, and then selected for those that survive and reproduce best. The creation and selection of certain genes and gene combinations led to plants with diverse physical structures for self-defense, support, seed dispersal and water conservation. Evolution also allowed some plant species to become 'biochemical specialists' in producing unique and characteristic phytochemicals, resulting in a broad range of compounds produced in fruits, vegetables, beans and many other plants. For example, peppers produce capsaicin, tomatoes are a good source of lycopene, and grapes produce resveratrol.

Over the last several thousand years, humans have joined Mother Nature in directing the evolution of plants by selectively saving and planting seed from wild, gathered plants with attributes humans valued: better flavors, larger fruits, fewer thorns, more nutrients and seeds that don't scatter. As soon as humans began to save some seeds for planting while discarding others, they altered the genetic makeup of the wild plants they had been gathering. The initial result of human-directed selection was the conversion of some wild plants into domesticated food crops that were easier to harvest and use and provided a reliable and convenient source of nutritious food. By 5000 BC, virtually all major food crops humans rely on today had been genetically changed from wild plants to domesticated crops. As the human population expanded into new areas, their crops moved with them to new environments, and humans continued to shape their genetic makeup so that the crops could adapt and serve human needs.

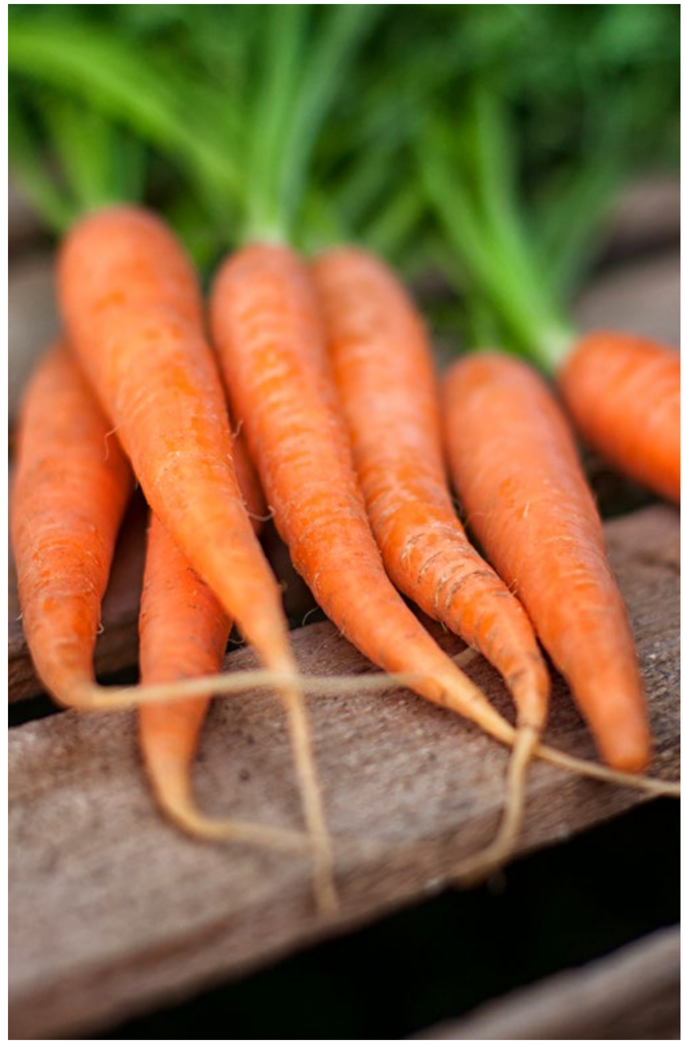
This process of constant genetic improvement continues today, though in a more formalized way, using the science and art of plant breeding. Plant breeding programs can be thought of as small-scale, human directed evolution projects that are increasingly informed by scientific understanding and facilitated by technological advances. As is true of evolution by natural selection, genetic variation is the essential resource upon which plant breeding programs are built. However, unlike biological evolution, plant breeding is goal-oriented, and the overarching goal is an array of crop varieties, with improved characteristics and traits, adapted to diverse environments. Plant breeders use cross-breeding, selection and other methods to both create new genetic variants and shape existing genetic diversity. The breeding and selection process takes multiple generations and results in plant hybrids and varieties that look, smell, taste, and yield in a more reliable and predictable way. Although each crop breeding effort may have unique practices, they all follow common selection practices with goals of improving plant productivity, quality or quantity.

During the thousands of years that humans have genetically changed plants, the value of these plants and plant products was assessed by how they looked, smelled, tasted and met human needs. From these interactions, humans learned which plant species are good sources of building materials, medicine and, most importantly, food. We learned which plants are nutritious, have useful phytochemicals, and are safe to eat, and which plants to avoid. Generally, those plants that tasted good and provided sustenance were considered safe to eat. Others were found, through trial and error, to be safe to eat only after processing or heating to inactivate factors that could negatively affect health or nutrition. Over time, as science progressed, biochemists were able to verify the chemical basis of what plant breeders had originally learned through trial and error. That same science continues to inform the ultimate evaluation of the safety and nutritional value of a given plant species as a food source.

This document provides a generalized description of the typical timelines, steps and procedures used in plant breeding programs today, focusing on hybrid breeding for both vegetables and field crops.

OVERVIEW OF HYBRID BREEDING

Commercial hybrids are a cross between two inbred parents, each derived from divergent genetic pools. In the hybrid breeding process, a key strategy is to identify genes in these divergent genetic pools so that the two inbreds being crossed as hybrid parents are as genetically unique as possible. Because hybrids have a full set of genes from each unique parent, they have much more genetic diversity than either inbred parent alone. Hybrids also have a combination of traits from each parent that enables the hybrids to have an overall performance that exceeds the performance of either of its inbred parents. The combination of the genetic diversity between the two inbred parents is the source of what is called "hybrid vigor" or heterosis. The value of hybrid vigor in plants can be measured in characteristics that deliver increased value to the grower, such as higher yield, larger fruit/vegetable size, better disease resistance, and broader environmental adaptability.



A hybrid breeding program normally consists of two phases: inbred development and hybrid testing. In the inbred development phase, breeders screen populations of plants that are genetically and phenotypically diverse, selecting individual plants with the desired characteristics. These individually selected plants are then self-pollinated in a repetitive fashion, called "selfing." During the selfing process, a plant acts as both mother and father to subsequent generations of seeds. The selection and selfing cycles are repeated until inbred lines that no longer show significant phenotypic variation between individuals are generated. These lines are often called "true breeding" inbred lines. Plant lines are considered inbred when the offspring (progeny) from a self-fertilized plant look identical to the parent, as well as identical to one another. In the second phase of a hybrid breeding program, the breeder crosses different inbreds to make hybrids and then conducts extensive field trials and testing of different hybrid combinations looking for the best field performance for yield, agronomic and product quality traits.

It is important to note that plant species vary in their generation time (time from seed to offspring seed). For example, some widely-grown vegetable species have a two year generation time, i.e., they are biennial. For other species it is possible to get 2 or 3 generations in a single year through special techniques such as embryo rescue. Utilization of tropical environments with year-long growing conditions can sometimes provide 5-6 generations/year for some species.

PLANT SPECIES		ONE GENERATION
Fruit Trees	➡	5 - 6 Years
Carrot, Onion	➡	2 Years
Tomato, Pepper	➡	0.4 Years
Melon, Spinach	➡	0.3 Years
Corn	➡	0.4 - 0.2 Years
Rice	➡	0.5 Years

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Some of the most common traits of interest in modern vegetable breeding programs include features such as reliable field performance across multiple environments, high yield, high quality, disease resistance, flavor, color, size and shape. Similarly, in field crops, the traits of interest range from disease resistance, tolerance to abiotic stress, improved processing and nutritional characteristics. Even today some of the primary tools for selecting the best plants rely upon a breeder's sense of smell and taste along with visual observations, just as has been done for thousands of years.

INBRED DEVELOPMENT

- Introduce genetic variation from (a) related plants, (b) wild relatives, or (c) random induced mutation
- Field trials, selecting and selfing
- 6-9 selfed generations to make inbred.

HYBRID TESTING

- Cross inbreds to make many experimental hybrid combinations
- Field testing at multiple locations
- Test for 3-5 growing cycles

COMMERICAL HYBRID

- Seed production of hybrids selected for commercial sale
- Commercial sale to growers

DEVELOPING INBRED LINES

In the Inbred Development Phase, the breeder begins by crossing two plants with different traits, such as yield, size, shape, color, taste, or disease resistance. The objective is to combine valuable traits from each parent to generate and select offspring containing the best characteristics of both parents. For example breeders are often trying to improve certain weaknesses that exist in one parent with complementary strengths in the other parent. The breeder's goal is to ultimately develop inbred lines with performance that results in an improvement over both of the original parents. The breeder accomplishes this thru repeated cycles of selection of plants with the best combination of characteristics and selfing.

The breeder selects the best performing inbred lines by using a breeder-directed process that includes assessing phenotypes through a field trial process. Through multiple generations (cycles) of plant selection, the breeder typically selects a few dozen elite, "true breeding," inbred plant lines from a population of thousands of individual plants. This process can last, on average, about six plant generations for typical breeding targets and through field trialing over multiple years and environments. For more complicated traits, such as multiple genes controlling a particular trait or, for example, introducing new disease resistance genes from a genetically distant wild relative, more than six plant generations may be required.

When the objective is to enhance an existing inbred line by adding a new characteristic from a donor inbred line, a technique called backcrossing is often deployed. Backcrossing minimizes changes to the inbred line, referred to as the recurrent parent. During backcrossing, the progeny of the original cross is repeatedly crossed to the recurrent parent while selecting for the target trait at each generation. When the donor of a new trait is otherwise wild or inferior to the recurrent parent, this approach enables the transfer of a trait while minimizing the dilution of other valuable traits. The contribution from the donor genome is reduced by half with each backcross. This process is illustrated below.

50%
F1 Seed
Elite Alleles

75%
BC1 Seed
Elite Alleles

87.5%
BC2 Seed
Elite Alleles

For all selection procedures during this period, measurements undertaken by plant breeders to include or eliminate plants from subsequent generations in a breeding program depend upon the requirements of the farmer, the handler, the processor or the consumer. Such measurements may involve simple procedures such as measuring plant height or attractiveness. Others may be complex, such as measuring the response of individual plants to artificial inoculation with a plant pathogen or the chemical analysis of oil.

DEVELOPING HYBRIDS

To make hybrids, breeders cross selected members of the elite, true-breeding inbreds they have developed. The offspring seed from these crossed inbred lines are the hybrids the breeder further evaluates for field performance and quality traits to assess which hybrid combinations have the potential to be commercial hybrid variety. Each new hybrid generated goes through extensive field trialing and testing during this evaluation.

Before any new hybrid is released to the market it undergoes several years of development and testing through a stringent, multi-year process designed to identify those hybrids that have the best combination of field performance and product quality. Hybrids are grown under standard or typical production conditions side-by-side with industry leading hybrids. These leading hybrids are called "checks". Yield data is recorded from both hybrids and the checks. In addition, those hybrids with the most commercial promise are further subjected to other analytical and observational tests, as appropriate for the crop. Hundreds or even thousands of new hybrid combinations are evaluated each year. Hybrids are commercially released only if their combination of field performance and product quality will make them more competitive in some aspect compared to existing commercial hybrids. As a result, only a very small percentage--typically less than 1%-- of the hybrids originally made by the breeder are ultimately commercially released. At every point during this testing process, hybrids that are not competitive will be eliminated from the pool of candidates.

THE TESTING PROCESS

Plant breeding is often said to be a process not of selection, but of elimination. Any off-types, unstable lines, or lines showing characteristics such as significant differences in nutrient content, detrimental responses to environmental stresses, diseases, or the presence of other undesirable traits are discarded as soon as they are identified. An off-type is a seedling or plant that differs in one or more characteristic, such as flower color or height. This winnowing takes place over several years, so the remaining lines identified for prospective commercial release will perform as expected. The environment in which a crop is grown often plays a significant role in affecting plant characteristics, such as the levels of certain anti-nutrients, overall yield and flowering. The trialing process occurs over multiple geographies and multiple years in order to observe that potential variability, keeping only the varieties that will meet consumer and grower expectations.

Genetically stable, potentially commercial hybrids and varieties are normally evaluated for:

- Geographic and production system adaptation
- Performance characteristics, relative to existing commercial hybrids/varieties
- Processing characteristics appropriate for that crop, such as milling for wheat, sugar yield for sugar beets, oil quality for canola and sunflower or storage characteristics for fruits and vegetables
- End-user characteristics (as appropriate for that crop), such as protein content for soybeans, bread-making characteristics for wheat, cooking quality for rice and flavor characteristics for fruits

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While this paper focuses on the process for developing new hybrid varieties, the same testing procedures occur for the development of non-hybrid varieties as well.

The majority of substances in food from plants are not toxic or harmful to humans -- less than 1/10th of one percent of all substances in all foods are toxic or otherwise harmful. Genetic changes, such as a mutation in a DNA nucleotide sequence and DNA rearrangements, were once thought to potentially produce novel, unknown toxins. In fact, however, there is not a single documented example whereby these changes have led to the production of previously unknown toxins. It is now recognized that these kinds of small genetic changes occur routinely and spontaneously in crop plants and during the breeding process. Thus, the range of natural variability that exists within a particular plant species is much broader than scientists could have previously measured and appreciated.

The advent of genomics, the ability to precisely sequence the DNA of crops, and ultimately utilize molecular knowledge about favorable traits, has led to improved efficiencies in plant breeding and plant improvement capabilities. As an example, it was discovered that corn inbred lines can vary by up to 15% in the genes that are present in specific inbred. In other words, inbred lines of corn, historic or recently developed, differ on average by 15% in the genes they contain and many genes will be absent from ones and present in others. This presence-absence variation is a natural phenomenon in corn that was only discovered by using modern genomics tools.

The discovery of this residual genomic variation may lead to new approaches to improving uniformity in products or further maximizing genetic potential. Genomics in breeding has not in any way changed the safety of the products; plants developed through breeding continue to have a record of safety while providing increased value (e.g. improved taste or disease resistance) for consumers and farmers. All reported cases of crop toxicity have been associated with the elevation of known toxins, such that testing for their presence has become a routine part of the breeding process to prevent inadvertent increases in toxin levels. This provides a very strong scientific basis to use breeding, with its genetically discernible, yet phenotypically indistinguishable variations, as a baseline for safety evaluations.

Various physical or chemical tests are used to measure product quality for new hybrids/varieties. The particular tests vary with the crop species and intended use of the final product. For example, processing tomatoes may be tested in the laboratory for fruit pH, sugar and acid levels, color, juice viscosity or even how easily they can be peeled. Fresh market tomatoes may be tested for firmness and shelf life. Hot pepper varieties may be tested for capsaicin levels, to quantitate how 'hot' they are compared to industry standards. Melons may be measured for their ability to be shipped, fruit sweetness, fruit color and for certain compounds that affect aroma and flavor. Rice is tested for stickiness or fluffiness after cooking to identify rice types for different types of dishes.

For some crops, product quality is related to the lack of certain phytochemicals. For example potatoes are selected to have very low levels of glycoalkaloids, squash is selected for low levels of cucurbitacins, soybean for low levels of trypsin inhibitors, and sweet peppers for the lack of capsaicin, canola for low glucosinolates. Some of these attributes can be measured by simple taste-testing. Detailed quantitative analysis, if appropriate, generally requires laboratory evaluations for accurate measurement. Again, these tests are performed on both candidate inbred lines and potential commercial hybrids, to be grown under a variety of environmental conditions, locations, and over multiple years to accurately measure the product quality consumers expect to see in the grocery store.

Phenotypic characteristics provide important information related to the suitability of new hybrids and varieties for commercial distribution. In the case of corn, breeders evaluate stand count and seedling vigor in the early stages of growth. As the plant matures, disease data is evaluated, such as gray leaf spot, anthracnose, fusarium and head smut infestations. As the plant continues to develop, root lodging, stalk lodging, brittle snap, time to silk, and time to shed are evaluated. The mature plant is measured for plant height, ear height, dropped ears, and husk cover. The harvested grain is measured for yield, moisture, and test weight.

CROP		TARGET MARKET		MAJOR CONSUMER QUALITY TRAITS
Sweet Corn	➡	Fresh Market	➡	Ear Size, Shape, Kernel Color, Flavor, Tenderness
Sweet Corn	➡	Processing	➡	Yield, Kernel Size, Color, Flavor, Tenderness
Vegetables	➡	Fresh Market	➡	Appearance, Flavor, Shelf Life
Vegetables	➡	Processing	➡	Yield, Appearance, 'Processibility', Flavor

Plant breeders developing new varieties of soybeans evaluate many parameters at different stages in the developmental process. In the early stages, breeders evaluate flower color, plant vigor, stand count, relative maturity, plant habit, pubescence color, hila color, pod wall color, plant morphology, days to flowering, emergence, and general disease resistance. The latter disease screening depends on the maturity and area in which the seeds are being grown. Later on, as a variety gets closer to commercialization, breeders measure yield at larger sites at increasing numbers of sites. Plants are also screened for resistance to various diseases. In some cases, breeding was directed towards specific increases in certain components, and the plant breeder would be expected to analyze for such components. For a crop, such as wheat, end-use characteristics, including milling and baking qualities, and grain quality are often identified in the initial stages of performance evaluation. Performance evaluations are carried out and include exposure to winter injury, saline soil, high soil temperatures and natural disease epidemics or insect infestations.

CONCLUSION

As our knowledge of plant genetics and biology have developed over time, new tools, technologies and strategies have been adopted by plant breeders. Thousands of years ago plant breeding was based entirely on breeders selecting plants based on their appearance, smell, taste and ease of production. As more sophisticated methods emerged to better measure plant characteristics, breeders adopted these tools. Today breeders use an array of tools such as whole genome sequencing data, computers, and digital imaging to measure plant performance and characteristics. Breeding is a living and dynamic science that is enabled by a multitude of other scientific disciplines, including but not limited to genetics, statistics, plant physiology, agronomy, entomology, plant pathology, molecular biology, computer science, soil science, ecology, and even human and animal nutrition. The effective incorporation of this vast array of scientific disciplines will continue to improve the effectiveness of plant breeding to deliver plants with even greater improvement in offering value to the global human population. Regardless of the tools used, the goal is still the same: To first create genetic diversity in a population of plants and through multiple years of field trials and testing develop new plant varieties that reliably produce safe, nutritious, good tasting food.

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