

PLANT BREEDING AND GENETICS

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Summary

Despite advances in technologies, plant breeding is still based on Mendel's original concept of inheritance which is extended to encompass the inheritance of quantitative and other complex traits. Plant breeding involves the systematic production of crop populations exhibiting genetic segregation and selection within that population to establish lines with favorable allele combinations. For cross-pollinating plants this is accomplished by recurrent selection of heterozygous plants, whereas for self-pollinating crops, pure-line breeding is preferred. Breeding of vegetatively-propagating species presents special challenges and relies heavily on culture techniques and induction of somatic mutations.

The majority of traits pursued by breeders are quantitative in nature and, thus, environmental factors must be given heavy consideration during selection. Experimental design and replication are critical for selection to be instructive.

Breeding techniques are largely focused around expediting the production of superior and stable lines; in the case of self-pollinating crops this is largely a focus on establishing more rapid homozygosity to produce an isogenic population for variety release. Wide hybridizations, tissue culture and mutagenesis are employed by breeders to generate new alleles and broaden available genetic resources. Molecular markers are used to assist breeders through marker-assisted selection and to identify quantitative trait loci (QTL) for traits of interest. Transgenic technologies are being increasingly used as a rapid, targeted means of introducing new genes, or introducing desirable novel alleles.

1. Introduction to Plant Breeding

Plant breeding is the use of natural and artificial selection to produce heritable variations and novel combinations of alleles in plants and to identify plants with novel and useful properties. The goals of plant breeding are to produce crop varieties that boast unique and superior traits for a variety of agricultural applications. The most frequently addressed traits are those related to biotic and abiotic stress tolerance, grain or biomass yield, end-use quality characteristics such as taste or the concentrations of specific biological molecules (proteins, sugars, lipids, vitamins, fibers) and ease of processing (harvesting, milling, baking, malting, blending, etc.).

The specific goal of a plant breeding project is highly dependent upon the market for which the product is intended. In wheat, for example, varieties bred to produce high levels of gluten protein are used to produce noodles and breads, whereas varieties specifically selected for low protein grains are used in the production of pastry flours. The varieties of grapes developed for various applications such as juices, red and white wines, and jams, vary considerably in chemical composition.

Plant breeders employ a variety of techniques to improve the genetic composition of the crop and a successful strategy is dependent on the physical, physiological and hereditary characteristics of the plant. Different breeding approaches are required for self-pollinating, cross-pollinating and clonally-propagated plants. The methods used by plant breeders have developed along with the advancement of human civilization and have expanded to incorporate humanity's increased knowledge of genetics.

2. History of Plant Breeding

The most rudimentary forms of plant breeding likely originated during the transition of early human societies from hunter-gatherer groups to agrarian communities. However it has also been argued that forms of plant breeding occurred even before the human diet was based on cultivated crops, because many plants were used in medicinal or religious capacities, even during the nomadic periods of human pre-history. It is not then difficult to imagine that these plants were selected from local regions based on phenotypes deemed desirable for ceremonial and other purposes. It is possible that these selected varieties were even cultivated on a small scale in village gardens.

As agriculture began to take hold and human populations increased, the most basic forms of plant breeding were employed as the most efficient calorie-conferring plant varieties were selected from wild stands, with seeds saved and replanted. The genes that conferred efficient harvest characteristics were the ones that distinguished wild from cultivated species. Thus, some form of rudimentary selection within these wild populations must have been performed. As today, the crops that provided humans with the greatest caloric intake were self-pollinating annual species. These plants are among the most successful crop species because as annuals they invest all their energy into a single reproductive event and thus produce large seeds rich with photosynthate.

Furthermore, as self-pollinators these species have more reliable reproduction and are not contingent upon weather patterns or other species to ensure pollination. For this reason, both early and modern plant breeding have been focused more intently on self-pollinating species. Maize is an exception as it is mostly out-crossing and thus harder to manipulate; it is suggested that the lack of self-pollinating grain plants on the American continent necessitated the use of maize. However, many non-grain crops that are out-crossing or vegetatively propagated are of significant agricultural importance and, thus, methods for their breeding must also be addressed.

2.1. Pre-Mendelian Plant Breeding

Plant breeding has been practiced for over ten millennia, and arose independently in several centers, including South and Central America, Asia, the Fertile Crescent and New Guinea. While early farmers recognized that, at least for inbred crops, “like beget like,” the genetic aspects of plant breeding were enigmatic until the relatively recent work of Mendel. As such, early plant breeders practiced a form of mass selection, in which plants of superior phenotype were selected and seed of these plants was replanted during the next season.

Prior to the 18th century there is little evidence of the intentional, controlled crosses between plant varieties which typify modern plant breeding. Thus, the lines developed by early plant breeders are what we would now term landraces, locally adapted lines shaped largely through either natural selection or farmer-driven selection in a particular region; these are colloquially known as folk varieties.

The first implementation of controlled crosses is attributed to the late 18th century German botanist Joseph Kolreuter. However, his study used the ornamental plant

Dianthus herbatus and thus was more botanical in nature rather than aimed at the improvement of a crop population. The use of controlled pollination to make new varieties was employed with increasingly greater precision during the 19th century, particularly in maize which received wide attention from early plant breeders due to its many visible phenotypes. However, while the physical principles of plant reproduction were better elucidated by botanists during the late 18th and early 19th century, the underlying mechanisms of inheritance were still enigmatic.

Theories regarding heredity abounded in the 19th century among both zoologists and botanists. In 1856 Louis de Vilmorin published his studies on heredity in beetroot and reported that progeny from two seemingly similar plants can exhibit emerging traits. De Vilmorin was thus among the first to recognize the distinction between genotype and phenotype although he did not yet formally identify these features as such.

The work of these pre-Mendelian plant biologists represented the early synthesis of botany and heredity into the emerging sub-discipline of plant breeding. However, it was not until the work of Mendel that modern techniques of quantitatively manipulating heredity of plant populations became possible.

2.2. Post-Mendelian Plant Breeding

Mendel's 1865 paper outlining and quantifying the principles of inheritance provided the basis for all modern genetics, but was sadly undiscovered until the beginning of the 20th century. As human understanding of inheritance and the gene action rapidly deepened, the application of these principles to crop production was pursued with equal alacrity.

A mere three years after the rediscovery of Mendel in 1900 by De Vries, Correns and von Tschermak, the Danish botanist Wilhelm Johannsen developed the pure-line breeding theory that is employed to generate true-breeding (aka. homozygous) lines through repeated self-pollination. Johannsen also defined the role of environment in the inheritance of quantitative traits, a concept that is critical to the proper execution of field experiments in plant breeding. Marcus Rhoades, in 1933, incorporated the use of cytoplasmic male sterility (CMS) genes into maize breeding; this permits the controlled crossing of plants without the need for a labor-intensive removal of male reproductive structures. The establishment of the Haber-Bosch process in 1910 to artificially produce ammonia provided plant-breeders with the new goal of adapting varieties to take advantage of abundant amounts of nitrogen fertilizer.

In the latter half of the 20th century, plant breeders incorporated techniques aimed at increasing diversity and expediting the breeding process. Following the works of Thomas Hunt Morgan and Hermann Muller in the 1910's and 1920's, plant breeders saw the value of physical and chemical mutagenesis for the production of new phenotypes. The application of statistics and population genetics to plant breeding was accomplished through the work of scientists such as Nikolai Vavilov and J.R. Harlan. The elucidation of DNA as the genetic material by Watson, Crick and Wilkins, and the later deduction of the genetic code further empowered the efforts of plant breeders through increased comprehension of gene action.

Transfer of genetic material between disparate species was aided by the use of tissue culture methods and later through transgenic technologies. Initiatives and developments in genomics made possible more rapid identification and targeting of gene sequences conferring desirable qualities. These methods are all discussed in detail in later sections.

Perhaps the most significant advance in plant breeding in the 20th century, and the most defining time for modern plant breeding was the so-called Green Revolution, the dramatic increase in crop yields that occurred between 1945 and 1985, credited in large part to the work of plant breeder Norman Borlaug. While numerous factors, both scientific and political, contributed to the progress of the Green Revolution, the greatest impact came from the development of high-yielding varieties in several of the world's major crops. This was accomplished through alterations to both plant genotypes and farming practices. Yields were dramatically increased in maize, wheat and rice by breeding for both increased nitrogen assimilation and reduced height. These so-called semi-dwarf varieties are deficient in components of the gibberilic acid pathways and thus have limited apical dominance. The net result is the investment of energy into grains without lodging (i.e. falling over before harvest).

The Norin 10 wheat cultivar, produced by plant breeder Orville Vogel, formed the basis for increasing yields in the world's most abundant crop, and the Filipino high-yielding rice cultivar IR8 also had a significant impact on world food production. The increased application of fertilizers, pesticides, and the rise of irrigation and mechanization also contributed to increased yields and improved market efficiency. It is accurate to say that modern plant breeding goals and methods have been set within the context established by the Green Revolution; this includes meeting the challenges and problems which the Green Revolution failed to solve (or in some cases, exacerbated).

3. Genetic Basis of Plant Breeding

Mendel was able to successfully quantify the inheritance of traits primarily because he chose an easily manipulated crop for his experiments: the green garden pea. His investigation used self-pollinating (autogamous) plants and he assessed simply-inherited, qualitative traits in a controlled environment. Plant breeders seldom have such luxury, as most agronomically significant traits are determined by complex gene interactions. Furthermore, many important crop species do not appreciably self-pollinate, instead requiring cross-pollination (allogamous), while others generally undergo vegetative propagation. Wheat, rice, soybean, sorghum and millets are examples of self-pollinating crop plants; corn, sugarcane and sugar beet are cross-pollinating; coconut is cross-pollinating or self-pollinating; potato, sweet potato, cassava, grape, banana and orange have a vegetative mode of reproduction.

Mendel's work helped establish the modern terms "homozygous", indicating an organism with two identical alleles for a given gene, and "heterozygous", indicating an organism with two different alleles for a gene. The term "true-breeding" is often applied to homozygotes, because when self-pollinated (or inbred in the case of cross-pollinating species), these individuals only produce more homozygotes. Self-pollinating heterozygotes, on the other hand, produce 50% heterozygotes and 25% homozygotes of each class.

The plant breeder must also consider the environment in which the plant will be cultivated; for example a variety which boasts high quality under irrigation may well be a poor performer in an arid region. While these factors may make Mendelian inheritance seem inadequate to describe plant breeding efforts, these principles, though in a greatly complicated form, comprise the basis of a crop breeding project. Thus, the fundamental principles of plant breeding can be explained using Mendelian analysis, if we lay aside greater complexities for the moment.

The standard methodology of plant breeding is to create a population of segregating alleles in order to select superior combinations from a diverse population, thus heterozygosity is desired in early generations. However, true-breeding lines, homozygous at all loci, are desired as both the starting materials and end-products of this process, at least when breeding self-pollinating species.

A standard plant breeding project begins with a cross of two parental plant varieties to create an F_1 hybrid population, heterozygous at many loci, which will segregate in subsequent generations. This is generally accomplished through emasculation, removal of the pollen-bearing structures, of one plant to ensure that the two plants cross-fertilize. This is generally done twice, with each parental genotype type serving as the pollen donor in the respective crosses. This is termed a reciprocal cross and is important to measure the effects of cytoplasmic inheritance (mitochondria and chloroplasts are always inherited from the female parent).

In maize, de-tasseling, the removal of the pollen-bearing tassels of the plant, is labor intensive, and the cunning implementation of cytoplasmic male sterility (CMS) genes into the breeding process significantly reduces the effort needed to insure cross pollination. This method is currently used when genetically modified and non-GM crops are grown together, to prevent transfer of transgenes. For self-pollinating species, the parental plants of a cross should be true-breeding, so that an isogenic (genetically identical) F_1 population is produced. If the parental lines are themselves still segregating, the F_1 will not be uniform and some alleles may not be passed on to any F_2 individuals, thus sacrificing diversity. In subsequent generations, the breeder will select segregating lines that demonstrate desired qualities, refining their selection with more stringent criteria in successive rounds of selection.

Genetic recombination, or crossing over, between chromosomes in the early generations of a project allows new combinations of alleles by breaking up linkage blocks, clusters of alleles at closely-linked loci on a chromosome. In general, crosses between genetically distinct lines with differing, desirable characteristics are most successful because segregation produces a population with high variation. Ultimately, for self-pollinated crops, most breeders release true-breeding lines, also called pure lines. Breeders of wheat or rice, for instance, would not release lines for cultivation that retain heterozygosity, as subsequent generations may lose key characteristics through segregation. Fortunately, this task is easy with self-pollinating species, as the heterozygosity of a population is halved in each generation of self-fertilization (Figure 1).

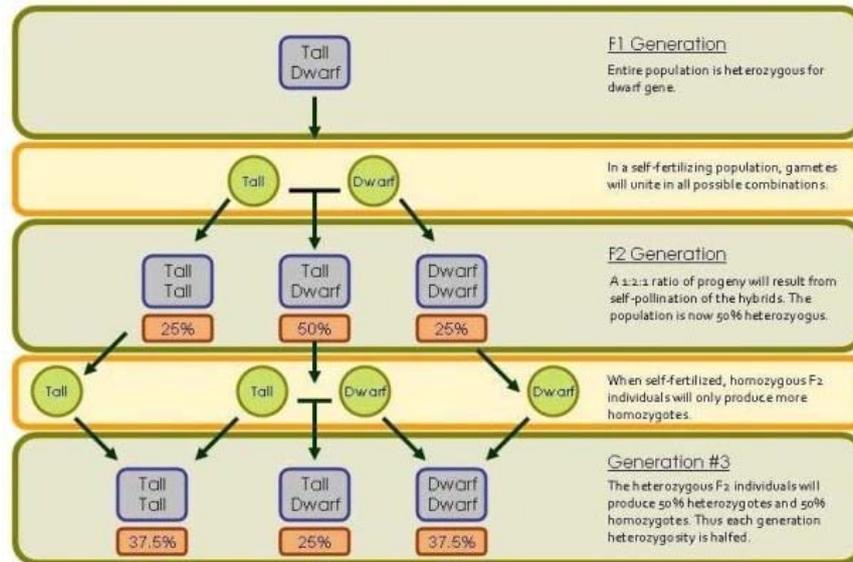


Figure 1. Self-pollination of hybrid plants leads to a halving of heterozygosity in each generation. After six generations, approximately 97% will be homozygous.

Thus in order to establish a true-breeding line, the breeder of a self-pollinated crop merely has to engage in repeated cycles of self-fertilization and selection. Consider the simple example related in Figure 2 of selecting a line homozygous for two genes.

This loss of heterozygosity has prompted the designation of self-pollinating lines in the F₅ or later generations as recombinant inbred lines, because they are the product of recombination in a segregating population, but are likely homozygous at many loci due to inbreeding.

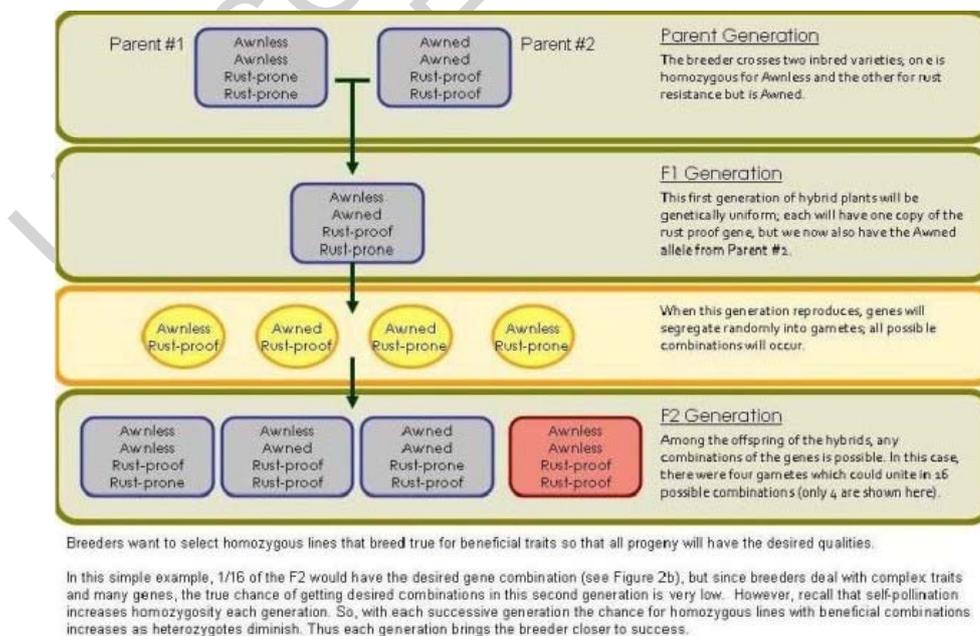


Figure 2. Simplified example of a plant breeding plan in a self-pollinated species.

In this example, a breeder is attempting to make an awnless wheat variety that is resistant to stripe rust disease.

4. Techniques for Breeding Self-Pollinating Plants

In the most basic terms, the general strategy of breeders of self-pollinated crops is to cross two lines of varying genotypes to each other, produce hybrid progeny that are segregating at multiple loci, identify lines with superior allele combinations through successive cycles of selection and produce stable, true-breeding varieties from these lines. While relatively straightforward conceptually, a quick summary fails to convey the physical and temporal scale required for these procedures.

While the initial stages such as establishing crosses and generating F₁ hybrids, can be performed in a greenhouse with a relatively small number of isogenic plants, latter stages must be performed under field conditions on a large scale. This obviously requires a significant amount of labor and physical space, particular to implement adequate replication and controls. Furthermore, in most environments, field conditions allow for only a single generation to be assessed each year. These factors place considerable constraints on the efficiency of plant breeding projects.

As such, modern plant breeding techniques are largely focused on two goals: 1) increasing the efficiency of the selection process and production of true-breeding lines; and 2) acquisition or *de novo* generation of additional genetic diversity in plant populations. Methods used to achieve the former goal will be considered first.

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Biographical Sketches

Matthew Arterburn is an Assistant Professor of Biology at Washburn University, in Topeka, Kansas. He has a Ph.D. degree in Genetics and Cell Biology from Washington State University and entered into his current faculty position immediately after, in 2006. His research focus is on the genetic mechanisms that cause the switch between annual and perennial life cycles in wheat and its relatives. The primary goal of his research is to contribute to the production of perennial wheat for use in sustainable agriculture systems. He teaches courses in Genetics, Cell Biology and Immunology, and contributes to interdisciplinary courses in bioethics and the socially responsible use of genetic research.

Stephen Jones is the winter wheat breeder at Washington State University. He has a Ph.D. degree in genetics from the University of California at Davis and has been at Washington State University since 1991. He has published numerous scientific articles on wheat breeding and genetics. He has also published papers on the history and value of public wheat breeding and the urgent need to keep these and other Land Grant University programs public. Recently he authored the "Sustainable Agriculture" entry and "Wheat" entry for the World Book Encyclopedia. Stephen teaches graduate level courses in advanced classical genetics and in the history and ethics of genetics. The wheat breeding program that Stephen leads has been in existence since 1894.

Kim Kidwell is Associate Dean of Academic Programs for the College of Agricultural, Human and Natural Resource Sciences. She is a professor in the Department of Crop and Soil Sciences, and is currently serving as the interim spring wheat breeder and geneticist, a position she held from October 1994 to July 2007. Combining the use of biotechnology with traditional plant breeding methods, eleven new varieties have been developed through Dr. Kidwell's research efforts that were designed to provide genetic solutions to spring wheat production problems. She also teaches a high enrollment experiential-based interpersonal communication course for undergraduates.