

# Lecture 9. Creation of Plant Hybrids: Hybridization Methods, Induction of Polyploidy, and Applications in Plant Breeding

## Introduction

Plant hybridization is one of the fundamental approaches used in plant breeding to create new genetic combinations and develop cultivars with improved characteristics. A hybrid is produced by combining genetic material from genetically different parental plants through sexual reproduction or, in some cases, through biotechnological methods. The main objective of hybridization is to bring desirable characteristics from different parents into a single genotype. These characteristics may include high yield, improved product quality, resistance to diseases and pests, tolerance to drought, salinity, heat or cold, early maturation, improved nutritional composition, and adaptation to particular environmental conditions. Hybridization has been used in agriculture for centuries, but modern genetics, cytogenetics, molecular biology, tissue culture, genomics, and genome editing have greatly increased the precision with which parental plants and their offspring can be analyzed. Another important approach closely connected with hybrid breeding is **polyploidy**, the presence of more than two complete sets of chromosomes. Polyploidy occurs naturally in many plant species and can also be induced for breeding purposes. Together, hybridization and polyploidy provide powerful sources of genetic diversity for the development of improved agricultural plants.

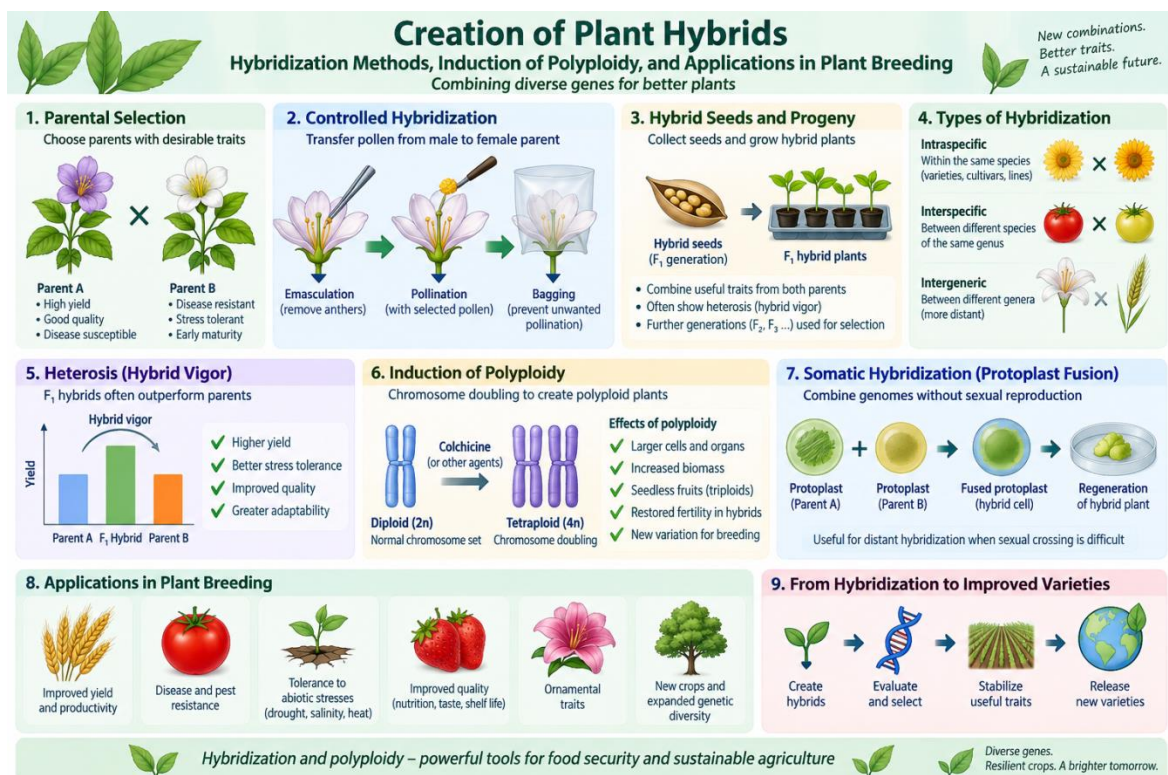


Figure 1. Graphical Abstract of Plant Hybridization: Hybridization Methods, Induction of Polyploidy, and Applications in Plant Breeding

## Principles of Plant Hybridization

Sexual hybridization is based on the fusion of male and female gametes originating from genetically different plants. During meiosis, parental chromosomes undergo segregation and recombination, producing genetically diverse gametes. Fertilization combines genetic information from the two parents and produces a hybrid zygote. Consequently, hybrid offspring contain genetic material inherited from both parental lines. Plant breeders select parents according to the characteristics they want to combine. One parent may possess high productivity but be susceptible to a particular disease, while another may show strong disease resistance but have lower yield or poorer product quality. Crossing these plants creates a population containing new combinations of alleles, and breeders can subsequently identify individuals in which useful characteristics from both parents have been combined. The success of hybridization depends on the genetic relationship between the parents, flowering time, reproductive compatibility, fertility, chromosome behavior, environmental conditions, and the heritability of the target characteristics.

Hybridization can be classified according to the genetic relationship between parental plants. Intraspecific hybridization occurs between different varieties, cultivars, lines, or populations belonging to the same species and is one of the most common approaches in conventional crop breeding. Because the parents belong to the same species, reproductive compatibility is generally relatively high. Interspecific hybridization involves plants belonging to different species of the same genus and can introduce useful traits that may not be available within the cultivated species. Intergeneric hybridization involves plants belonging to different genera and represents a more distant form of hybridization. Distant crosses can provide valuable genetic diversity, but reproductive barriers become increasingly important as the genetic distance between parental plants increases.

### Controlled Hybridization in Plant Breeding

Controlled hybridization allows breeders to determine which plants will serve as the maternal and paternal parents of a cross. The parental lines are first selected according to breeding objectives and evaluated for important agronomic characteristics. Flowering must be synchronized so that compatible reproductive structures are available at the appropriate developmental stage. In species capable of self-pollination, breeders must prevent unwanted self-fertilization if they wish to produce a controlled hybrid. Pollination is then carried out using pollen from the selected paternal parent, and the resulting seeds are collected and evaluated as hybrid progeny.

The first generation produced by crossing genetically distinct parental lines is generally called the  $F_1$  generation.  $F_1$  plants may show a relatively uniform phenotype when the parental lines are genetically stable. When  $F_1$  plants are self-fertilized or crossed with each other, the resulting  $F_2$  generation usually displays greater genetic variation because alleles segregate and recombine. Breeders can use this variation to identify individuals with desirable combinations of traits. Additional generations of selection may then be required to stabilize the desired genotype and phenotype. Modern molecular markers can assist this process by allowing breeders to identify particular alleles without relying exclusively on visible characteristics.

### Heterosis and Hybrid Vigor

One of the most important phenomena associated with hybrid breeding is heterosis, also known as hybrid vigor. Heterosis occurs when hybrid offspring show superior performance for one or more characteristics compared with the average performance of their parents and, in some contexts, compared with a particular parental line. It can influence biomass, growth rate, fertility, yield, stress tolerance, or other quantitative characteristics. Heterosis is especially important in crops such as maize, sunflower, rice, sorghum, and many vegetable species.

Several genetic explanations have been proposed for heterosis. The dominance hypothesis suggests that favorable dominant alleles from one parent can compensate for less favorable recessive alleles from the other. The overdominance hypothesis proposes that heterozygous combinations at particular loci may provide advantages compared with either homozygous condition. Epistatic interactions between different genes may also contribute. Modern genomic research indicates that heterosis is a complex phenomenon involving interactions among many genetic and regulatory factors. Hybrid breeding attempts to identify parental combinations that produce consistently valuable  $F_1$  progeny.

### Distant Hybridization and Reproductive Barriers

Crossing genetically distant plants can provide access to useful characteristics such as disease resistance, environmental adaptation, or quality traits that are absent from cultivated varieties. However, distant hybridization is often limited by reproductive barriers. Prezygotic barriers operate before fertilization and may include differences in flowering time, incompatibility between pollen and stigma, or failure of pollen tubes to reach the ovule. Postzygotic barriers operate after fertilization and may cause embryo abortion, abnormal development, weakness, or sterility of hybrid plants. Chromosome incompatibility is particularly important because chromosomes from different species may fail to pair correctly during meiosis.

Plant biotechnology provides several approaches for overcoming some reproductive barriers. Embryo rescue involves the *in vitro* cultivation of immature hybrid embryos that would otherwise fail to develop normally within the seed. Tissue-culture techniques can therefore make some crosses possible that would be unsuccessful under ordinary reproductive conditions. Molecular markers and genomic analysis can subsequently confirm hybrid identity and determine which regions of parental genomes have been inherited.

### Somatic Hybridization

Hybridization does not always require sexual reproduction. Somatic hybridization is a biotechnology-based approach in which protoplasts originating from different plants are fused. A protoplast is a plant cell from which the cell wall has been removed. Without the cell wall, cells can be brought into direct contact and their membranes can fuse, producing a hybrid cell containing genetic material from both parental cells. Under appropriate tissue-culture conditions, some fused cells can divide and regenerate into complete plants.

Somatic hybridization can be particularly useful when conventional sexual crossing is limited by reproductive incompatibility. It can also combine nuclear and cytoplasmic genetic material in ways that differ from ordinary sexual reproduction. However, regeneration from protoplasts is technically difficult in many plant species, and somatic hybrids may show

chromosome instability or reduced fertility. Therefore, somatic hybridization is a specialized breeding and research tool rather than a universal replacement for conventional crossing.

### Polyploidy in Plants

Polyploidy is the condition in which an organism contains more than two complete sets of chromosomes. Diploid organisms normally possess two chromosome sets, represented as  $2n$ , whereas polyploids may contain three sets ( $3n$ ), four sets ( $4n$ ), six sets ( $6n$ ), or higher numbers. Polyploidy is particularly common in plants and has played a major role in plant evolution and the origin of many agricultural species. Important crops have complex histories involving chromosome duplication, hybridization, or both.

Polyploids can be classified into two major categories. Autopolyploids contain multiple chromosome sets originating from the same species or closely related genetic background. Allopolyploids contain chromosome sets originating from different species, usually following hybridization and chromosome duplication. Allopolyploidy is particularly important in plant evolution because chromosome doubling can sometimes restore fertility to otherwise sterile interspecific hybrids. When each chromosome obtains a sufficiently similar pairing partner, meiotic chromosome behavior can become more regular.

Polyloid plants may differ from their diploid relatives in cell size, organ size, morphology, physiology, fertility, gene expression, and environmental adaptation. Larger cells can sometimes contribute to larger leaves, flowers, fruits, or other plant organs, although polyploidy does not automatically produce superior plants. Its effects depend on the species, genotype, chromosome number, and breeding objective.

### Induction of Polyploidy

Polyploidy can arise naturally through errors in cell division or the formation of unreduced gametes. Plant breeding can also induce chromosome duplication experimentally. The general biological principle involves interference with normal chromosome segregation during cell division so that duplicated chromosomes remain within the same nucleus. The resulting cells may contain an increased number of complete chromosome sets.

After chromosome duplication, plants must be carefully evaluated because not every treated cell or regenerated plant will have the desired chromosome number. Cytogenetic analysis, chromosome counting, flow cytometry, morphological observations, and molecular methods can be used to characterize candidate polyploid plants. Stable polyploids must also be evaluated for fertility, growth, yield, quality, and other agronomic characteristics. Thus, chromosome duplication itself is only the beginning of a breeding process; extensive selection and characterization are necessary to determine whether the resulting polyploid has useful properties.

### Polyploidy and Hybrid Fertility

One of the most important applications of polyploidy is restoration of fertility in certain distant hybrids. When two diploid species with different chromosome complements are crossed, the hybrid may inherit one chromosome set from each parent. During meiosis, chromosomes may fail to find sufficiently homologous partners, resulting in irregular segregation and sterility. If the entire chromosome complement is duplicated, each

chromosome can potentially obtain a corresponding partner. This may allow more regular meiosis and improve fertility.

This relationship between hybridization and chromosome duplication has played an important role in the evolution of new plant species. It is also useful in breeding because it provides a mechanism for combining genomes from different species. Many cultivated plants have evolutionary histories involving repeated hybridization and polyploidization events. Wheat is a well-known example of a crop whose genome reflects complex historical combinations of ancestral genomes.

### Seedless Plants and Polyploid Breeding

Polyploidy can also be used in the development of seedless or low-seed fruits. Triploid plants, containing three chromosome sets, often show reduced fertility because an odd number of chromosome sets makes regular meiotic segregation difficult. This biological characteristic can be useful in certain fruit crops where consumers prefer seedless products. Seedlessness can therefore be achieved through breeding systems that generate triploid plants.

Polyploid breeding is also important in ornamental horticulture. Changes in chromosome number can influence flower size, leaf morphology, plant architecture, pigment intensity, and other characteristics valued in ornamental plants. However, responses are highly species-specific, and chromosome multiplication may also reduce growth or fertility. Candidate polyploid plants must therefore be evaluated rather than assumed to possess improved characteristics.

### Molecular Technologies in Hybrid Breeding

Modern hybrid breeding increasingly uses molecular markers and genomic technologies to characterize parental plants and hybrid populations. DNA markers can identify genetic differences between parents, confirm hybrid origin, and track inheritance of important genomic regions. Marker-assisted selection allows breeders to select plants carrying desired alleles even when the associated phenotype is difficult or expensive to evaluate directly. This can be particularly useful for disease resistance genes and other characteristics influenced by environmental conditions.

Genome-wide markers and sequencing technologies provide even more detailed information about genetic diversity and inheritance. Genomic selection uses information from large numbers of markers across the genome to estimate the breeding value of individuals for complex quantitative traits. These approaches do not eliminate the need for field testing, but they can improve the efficiency with which breeding populations are managed and selected. Hybrid breeding is therefore increasingly becoming an integration of conventional crossing, quantitative genetics, molecular markers, genomics, bioinformatics, and phenotyping.

### Hybridization and Genome Editing

Genome-editing technologies such as CRISPR/Cas provide additional possibilities for plant improvement but do not eliminate the importance of hybridization. Hybridization can combine thousands of genetic variants from different parents simultaneously, whereas genome editing can introduce targeted changes at selected genomic locations. The two

approaches can therefore complement each other. A breeder may use hybridization to create a genetically diverse population and then use molecular selection to identify superior individuals. Genome editing may subsequently modify a specific gene responsible for disease susceptibility, quality, flowering time, or another characteristic.

Alternatively, an edited trait can be introduced into a breeding program and combined with additional characteristics through crossing. Modern plant improvement therefore increasingly integrates hybridization, molecular markers, genomic selection, genome editing, tissue culture, and advanced phenotyping. This integrated approach provides greater flexibility than relying on any single technology.

### Applications in Plant Breeding and Agriculture

Hybridization has contributed to the improvement of many major agricultural crops. Hybrid breeding can increase productivity, improve resistance to pathogens and pests, modify maturity, enhance product quality, and improve adaptation to different environmental conditions. Interspecific crosses can introduce useful genes from wild relatives into cultivated crops, expanding the genetic resources available to breeders. Wild species can be valuable sources of resistance to diseases, tolerance to environmental stress, and other characteristics that may have been lost during domestication and intensive selection.

Polyploid breeding has applications in cereals, fruits, vegetables, ornamentals, forage crops, and medicinal plants. Depending on the biological system, changes in chromosome number may affect biomass, organ size, fertility, secondary metabolism, or stress responses. Allopolyploid breeding can combine genetic material from different species, while triploid breeding can contribute to seedless fruit production. The agricultural value of any hybrid or polyploid must ultimately be established through careful phenotypic and field evaluation.

### Advantages and Limitations

The major advantage of hybridization is its ability to create new genetic combinations and bring useful traits from different parental plants into a common genetic background. Hybrid breeding can exploit heterosis, while distant hybridization can introduce characteristics from related species. Polyploidization provides additional genetic variation and can modify fertility, morphology, and physiology. These approaches use natural biological processes and have a long history in plant improvement.

However, hybridization also has limitations. Desired traits may be genetically linked to undesirable characteristics, requiring several generations of selection and backcrossing. Distant crosses may fail because of reproductive barriers, and hybrids may be sterile. Polyploids may show irregular chromosome segregation, reduced fertility, slow growth, or undesirable morphology. Complex traits such as yield and drought tolerance are influenced by many genes and environmental factors, making their improvement challenging. Modern molecular and genomic technologies can improve breeding efficiency, but they cannot completely replace careful evaluation under realistic agricultural conditions.

### Future Perspectives

The future of plant hybrid breeding will involve increasingly close integration between classical genetics and modern biotechnology. Genomic information can help

breeders select genetically complementary parents, while high-throughput phenotyping can measure plant performance across different environments. Artificial intelligence and computational methods may assist in analyzing large genomic and phenotypic datasets and identifying promising parental combinations. Molecular markers can accelerate selection, and genome editing can introduce precise changes into breeding material.

Polyploid research will also benefit from advanced sequencing technologies because complex polyploid genomes can now be studied in much greater detail. Researchers can investigate how duplicated genes interact, how gene expression changes following chromosome multiplication, and how different parental genomes function within allopolyploid plants. Understanding these mechanisms can improve the rational use of polyploidy in crop breeding.

### Conclusion

Plant hybridization and polyploidy are fundamental mechanisms for generating genetic diversity and developing improved crop varieties. Intraspecific, interspecific, and intergeneric hybridization allow breeders to combine genetic material from different parental plants, while heterosis can provide valuable improvements in growth, productivity, and other characteristics. Biotechnological approaches such as embryo rescue and somatic hybridization can help overcome some reproductive barriers associated with distant crosses. Polyploidy, whether naturally occurring or induced, can modify plant morphology, physiology, fertility, and genetic diversity and can sometimes restore fertility in interspecific hybrids or contribute to the development of seedless crops.

Modern plant breeding combines these traditional genetic principles with molecular markers, genomics, tissue culture, bioinformatics, genome editing, and advanced phenotyping. Hybridization therefore remains a central component of crop improvement even in the era of precision biotechnology. By combining useful genetic diversity with increasingly precise methods of selection and molecular analysis, plant breeders can develop crops with improved productivity, quality, resistance, and adaptation to changing environmental conditions.