

Interspecific Hybridization

The genetic improvement of some cultivated plants has involved incorporation of useful genes from other species or genera. New types of plants have been developed and are being contemplated that represent the merger of the chromosome complements of two existing cultivated species. The unique opportunities and challenges presented by interspecific hybridization can be important for plant breeders interested in cultivar development.

Interspecific hybridization refers to crosses between different species, and intergeneric hybridization represents crosses between different genera. Both types of matings are sometimes referred to as wide crosses. For simplicity, interspecific hybridization will be used in the following discussion to include crosses between different species of the same or different genera.

OBJECTIVES OF INTERSPECIFIC HYBRIDIZATION

Improvement of Cultivars of a Species

The level of difficulty in developing a new cultivar generally is related to the number of undesirable characteristics in the parents. The first choice of parental material for most plant breeders is cultivars and experimental lines with desirable quantitative and qualitative characteristics. If genes for a desired characteristic cannot be found in elite parents, a breeder will attempt to find them in a genotype having a minimum number of undesirable traits. Genotypes within the cultivated species generally are evaluated first. If the characteristic cannot be found within the species, the breeder will consider related species within the primary gene pool, then within the secondary gene pool, and finally within the tertiary gene pool (Chap. 11).

Formation of New Species

The formation of a new cultivated species with desirable characteristics from two or more existing species can be a goal of interspecific hybridization. For example, triticale (*X Triticosecale*) is a cultivated species developed from the hybridization of wheat (*Triticum*) and rye (*Secale*). The hexaploid type ($2n = 42$) has the A and B genomes of wheat and the R genome of rye. An octaploid type ($2n = 56$) also has been formed, but has less potential for commercial use at present. It has the A, B, and D genomes from wheat and the R genome of rye.

TECHNIQUES FOR GENE TRANSFER

The development of improved cultivars of a cultivated species by the transfer of genes from another species involves two factors: obtaining viable seeds or vegetative propagules in the F_1 and later generations and eliminating undesirable characteristics of the donor species. One or both of these factors may be a consideration in gene transfer between species (Hadley and Openshaw, 1980).

Securing Viable Seeds and Plants

The primary gene pool of a cultivated species includes related taxa with which it can be hybridized to obtain viable hybrid seeds and progeny without the use of special techniques. Because noncultivated types commonly have unusual plant or floral characteristics, making crosses between species in the primary gene pool may be more difficult than crossing genotypes within the cultivated species. Noncultivated types also may have chromosome aberrations that reduce seed set of plants in the F_1 or later generations. In soybean, *Glycine soja* is a member of the primary gene pool of the cultivated species *Glycine max*. *G. soja* \times *G. max* crosses generally are more difficult to make than *G. max* \times *G. max* matings, due to the small flower size and prostrate growth habit of *G. soja*. Genotypes of *G. soja* may have a chromosome translocation not found in cultivars of *G. max*, which causes partial sterility in the hybrid offspring. Neither factor, however, necessitates special techniques for obtaining viable hybrid seeds or plants.

Gene transfer from species in the secondary and tertiary gene pools may require special techniques to be successful. Failure to obtain viable seeds or plants in the F_1 or later generations can occur at various phases of the transfer program. Various techniques have been used to overcome these barriers (Hadley and Openshaw, 1980).

Lack of Fertilization. The first phase of the gene transfer program that may be unsuccessful is failure of the male and female gametes to unite to form a zygote. Failure of fertilization may be due to lack of pollen germination, insufficient pollen tube growth, or inability of male gametes that reach the embryo sac to

unite with the egg cell. Lack of success at this phase has been referred to as a prefertilization barrier caused by cross-incompatibility. A number of techniques have been used to successfully obtain zygotes from interspecific crosses that initially demonstrated cross-incompatibility.

Parent Selection. Genetic variability for cross-incompatibility may be present for genotypes within a species. The probability of mating genotypes that are compatible is a function of the number of different genotypes of each species that are used as parents.

Reciprocal Crosses. A guideline that has been used when crossing species that differ in chromosome number is to choose as female the one with the greatest number of chromosomes. Although this guideline has been useful, in some cases use of the species with the lower chromosome number as the female has been more successful. Genetic systems related to cross-incompatibility may permit a successful cross in one direction but not the other, regardless of the chromosome number of the parents. As a result, reciprocal crosses are recommended for interspecific matings that have not been attempted or have not been successful.

Modification of Ploidy Levels. When two species of a cross differ in ploidy level, modifying the ploidy of one species to match that of the other may improve the success of obtaining a zygote. Ploidy level can be modified by inducing chromosome doubling or by crossing plants of the same species with different ploidy levels. An example of this latter possibility would be to obtain tetraploids from crosses between diploid and hexaploid plants of species A to use in crosses to tetraploids of species B.

Pollen Mixtures. Cross-incompatibility is associated with proteins of the pistil that interact unfavorably with proteins of the pollen to prevent normal pollen tube germination and growth (Hadley and Openshaw, 1980). This unfavorable reaction has been avoided in certain interspecific matings by mixing pollen from a compatible species with pollen from an incompatible parent.

Modification of the Pistil. Matings may be hampered by the failure of the pollen tube of a short-styled species to attain a length sufficient to reach the ovule of a long-styled species. Use of the short-styled species as female is one alternative, but the mating may not be successful or the reciprocal cross may be more desirable. It may be possible to use the long-styled species as female by cutting back its style before pollen is applied. This technique can only be successful in species, such as maize, in which the shortened pistil remains receptive to pollen.

Chemical Treatment of the Pistil or Pollen. The compounds in the stigma or pollen that prevent pollen tube growth in an incompatible mating have been removed by application of an organic solvent to the stigma or the pollen. Pollen tube growth may be so slow in an interspecific mating that the egg dies or the

flower aborts before the sperm nuclei reach the ovary. Application of growth regulators has been used to promote more rapid pollen tube growth or to increase the length of time the pistil remains viable.

Large-Scale Matings. The frequency of successful pollinations can be extremely low for interspecific matings. Techniques for making a large number of matings have been used to obtain a limited number of seeds. Emasculation has been avoided or minimized by the use of female parents that are self-incompatible or that exhibit genetic or cytoplasmic-genetic male sterility. Rapid emasculation procedures that minimize, but do not eliminate, self-pollination are particularly useful when a genetic marker can be used to differentiate self-pollinated and hybrid offspring. Efficient pollination procedures are helpful to increase the number of matings.

Protoplast Fusion. It may be possible to use protoplast fusion to produce hybrids that cannot be obtained through sexual fertilization. The ability to fuse protoplasts, culture hybrid cells, and regenerate plants is necessary for utilization of this technique.

Lack of Hybrid Seed Development. Successful fertilization in an interspecific mating may not be followed by normal embryo or endosperm development. A number of causes for abnormal seed development have been suggested (Hadley and Openshaw, 1980): (a) Undesirable interactions between genes of the two species may interfere with cell division and differentiation. (b) There may be an unfavorable interaction in zygotic cells between the cytoplasm and nuclear genes. (c) The genetic relationship between embryo, endosperm, and maternal tissue may not be favorable. (d) The number of ovules fertilized may not be adequate to prevent flower and fruit abortion.

Techniques have been developed to overcome the problem of inadequate hybrid seed development. The success of a technique depends on the biological basis of the problem, knowledge of which may be unavailable to the breeder. As a result, each of the following techniques may have to be attempted to overcome the obstacle.

Parent Selection. Undesirable interactions between genes associated with seed development may be avoided by utilizing as parents an array of genotypes of diverse origin from the two species of a mating.

Reciprocal Crosses. An unfavorable interaction between nuclear and cytoplasmic factors in zygotic cells may prevent normal embryo development. The cytoplasm of the species crossed may differ in their capacity to support development of the hybrid embryo. Making reciprocal crosses between a number of

genotypes of each species increases the opportunity for favorable nuclear-cytoplasmic interactions required for hybrid seed formation.

Reciprocal crosses also provide a means of overcoming undesirable interactions among the embryo, endosperm, and maternal tissue. In a cross between species with different genomes and chromosome numbers, the developing embryo has one set of genomes from each species, the endosperm has two sets of genomes from the female and one set from the male, and the seed coat and other maternal tissues have two sets of genomes from the female. The differential contribution of the male parent to the three tissues may result in unacceptable dosage effects for factors related to the coordination of seed development. The number of chromosomes in the endosperm and maternal tissue depends on the species used as the female. Reciprocal crosses between species provide the best opportunity to obtain a favorable relationship between the tissues of developing seed.

Prevention of Flower and Fruit Abortion. The retention of flowers and fruit after fertilization may depend on the number of ovules in an ovary that contain developing seed. The frequency of successful fertilizations from an interspecific mating may be too low to stimulate the processes necessary for ovary development. Loss of the flower or young fruit may occur so early in seed development that rescue of the embryo is not possible. Growth-promoting substances have been applied to flowers to delay abortion at least long enough to obtain an embryo that could be cultured artificially (Hadley and Openshaw, 1980).

A technique used to provide adequate seed set for normal ovary development is to obtain self-pollinated and hybrid seed in the same fruit. This has been accomplished by applying to the stigmas a mixture of pollen from the female and male parents. Another technique used on flowers with multiple stigmas is to pollinate some of the stigmas with self-pollen and others with pollen from the male parent.

Embryo Culture. When a hybrid seed does not develop to maturity, it may be possible to aseptically transfer its embryo to an artificial medium and regenerate plants directly from embryoids or indirectly from callus tissue. Culture conditions that permit successful embryo culture have been identified for several plant species. In barley, embryo culture is used to obtain haploid plants in a cultivar development program that involves interspecific crosses between wild and cultivated barley (Chap. 27).

Inadequate Growth of Hybrid Plants. Hybrid plants obtained from an interspecific cross may lack sufficient vigor to flower and produce mature seeds. The lack of vigor may be caused by unfavorable interactions between genes of the two species within the nucleus or undesirable interactions between nuclear and cytoplasmic factors. Various techniques have been used successfully to overcome this barrier to interspecific hybridization.

Parent Selection. The use of a number of different genotypes as parents from each species provides an opportunity to identify genetic combinations that favor adequate hybrid growth.

Reciprocal Crosses. Lack of adequate hybrid growth due to an unfavorable nuclear-cytoplasmic interaction may be overcome in a reciprocal cross.

Grafting. A weak hybrid plant may be grafted to a normal individual from one of the species used in the cross. The rootstock of the normal individual may provide the growth substances necessary to obtain flowers, mature seeds, or both on the hybrid.

Hybrid Sterility. A frequent difficulty with interspecific hybridization is partial or complete sterility of the hybrid plant. There may be female sterility, male sterility, or both. The most common cause of hybrid sterility is inadequate chromosome pairing during meiosis. When meiosis occurs in an individual with diploid chromosomes for each genome, homologous chromosomes pair during prophase I and one member of each pair moves to separate poles during anaphase I (Chap. 2). After anaphase II, four nuclei are formed that contain a complete haploid set of chromosomes for each genome. If chromosome pairing does not occur, chromosomes line up independently at the equatorial plate. The number of chromosomes that move to each pole during anaphase I can be considered a chance event. If a complete set of chromosomes from each genome is not present in a nuclei after meiosis, the cell may be inviable. Consider an interspecific cross between a diploid species with five chromosomes in genome A and a diploid species with five chromosomes in genome B. The F_1 hybrid would have five A and five B chromosomes. When the chromosomes fail to pair, the nuclei formed by meiosis may have zero to five of the chromosomes of genome A and zero to five of genome B. The probability that a nucleus would have a complete set of each genome is small, the result of which is a high percentage of non-functional gametes. The probability that a functional female and male gamete would unite during self-pollination to form a viable seed is equal to the product obtained by multiplying the frequencies of functional male and female gametes. Consequently, little if any seed set would be expected from self-pollination.

A common technique used to overcome sterility caused by lack of chromosome pairing is to induce chromosome doubling in the hybrid. If the number is doubled, each chromosome would have a homologue with which to pair at meiosis, one chromosome of each genome would be present in the haploid nuclei, and functional gametes would result. If cells of a hybrid with two nonhomologous genomes A and B underwent chromosome doubling, the chromosome makeup of doubled cells that entered into meiosis would be AABB, normal chromosome pairing would occur, and viable gametes would be produced.

Inadequate Growth and Fertility of Hybrid Progeny. Unacceptable performance of the progeny of hybrid plants in the F_2 and later generations can impede the transfer of genes between species. Hybrid breakdown occurs when F_1 plants of an interspecific cross are vigorous and fertile but their F_2 progeny lack vigor and may be sterile. Two causes of hybrid breakdown have been suggested (Hadley and Openshaw, 1980): (a) Homozygous dominant alleles at multiple loci may favor plant development in one species, whereas homozygous recessive alleles at the same loci may favor plant development in another species. The F_1 of a cross between the species would be heterozygous and vigorous, but segregation occurring in the F_2 generation would break up the favorable combinations of dominant or recessive alleles. F_2 plants that did not have a dominant allele at each locus or that were not homozygous for all recessive alleles would lack vigor and might exhibit sterility. (b) Small structural differences that do not affect chromosome pairing in the F_1 hybrid may exist in the chromosomes of the two species. Recombination between the chromosome segments during meiosis could lead to the production of chromosome deficiencies or duplications in the gametes of the F_1 hybrid. Hybrid breakdown would occur if the aberrant gametes were viable, but the aberrancies had deleterious effects on the F_2 generation.

REFERENCE

- Hadley, H. H., and S. J. Openshaw. 1980. Interspecific and intergeneric hybridization. pp. 133–159. In W. R. Fehr and H. H. Hadley (eds.), *Hybridization of crop plants*. American Society of Agronomy, Madison, Wis.