Hybridization - objectives and types of hybridization

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Abstract

Plant breeders have relied primarily on the genetic variability produced by sexually crossing plants within the same species. However, the variability within species populations is insufficient, necessitating the use of hybridization techniques to exploit desirable traits of interest in distantly related or even unrelated plants. There are two types of hybridization: sexual and somatic. Sexual hybridization, also known as wide or distant hybridization, is the process of combining two genomes from different parental taxa via pollination, either naturally or artificially. Somatic hybridization is the fusion of somatic cells rather than gametes, and it is highly dependent on the ability to obtain viable protoplasts and eventually differentiate them in vitro to whole plants. Hybrids can have either positive or negative consequences. One of the advantageous characteristics of hybrids that have been exploited is heterosis, which can occur as a result of dominance, over-dominance, or epistasis. The negative effects include sterility, pollen tube arrest, and embryo abortion. To address these issues, chromosome doubling, the use of hormones such as 2, 4-Dichlorophenoxyacetic acid (2, 4-D), and embryo rescue have been used to treat sterility, pollen tube arrest, and embryo abortion, respectively. Following the development of hybrids, various hybrid identification techniques, such as the use of molecular and morphological markers, cytogenetic analysis, and fluorescent in situ hybridization, were used to test them. The use of hybridization techniques in plant improvement remains a critical tool for crossing species barriers and utilising important attributes in unrelated crop plants that would not have been possible using traditional plant breeding techniques.

Keywords: Cybrids, hybrids, inbred line, polyembryony, protoplast, pure line, sterility, segregation.

I. Introduction

Hybridization is the natural or artificial process of creating hybrids by crossing two individuals from genetically different populations. This process does not alter the genetic content of organisms, but rather generates new combinations of genes that may have desirable characteristics or phenotypes. This technique also avoids issues such as sexual incompatibility, polyembryony, and male or female sterility that are common in traditional sexual crossing. Hybridization is used in crop improvement for one of the following reasons. To begin, a variable plant population must be created in order to select hybrids with a desirable combination of characteristics from within these populations. Second, combining certain desirable characteristics in specific crops into a single individual, or third, exploiting and utilising hybrid varieties. Whatever the breeder's intention, the overall goal of hybridization when two genetically different plants are brought together in the first filial generation is always to create genetic variation.

Hybridization techniques are classified into two types: sexual and somatic. Sexual hybridization, also known as wide or distant hybridization, produces hybrid combinations within specific taxonomic distances. Sexual hybridization techniques have been used to produce better and new crops over time, such as triticale, a crop species produced in 1875 from a sexual cross between wheat (Triticum vulgare) and rye (Secale cereale) [Maclntyre et a., 1973]. However, wide/distant hybridizations of individuals from various species and even genera have occurred. Inter-specific hybridization occurs when two species from the same genus cross, whereas inter-generic hybridization occurs when two individuals from different genera cross. These types of crossing are significant because they break down species barriers for gene transfer, allowing for the transfer of genomes from one species to another, resulting in phenotypic or genotypic changes in the progeny.

Somatic hybridization on the other hand results when somatic cells are fused instead of gametes. This technique unlike sexual hybridization is done *in vitro* and requires specific handling of the materials to be fused. Precisely, somatic hybridization is done via protoplast fusion and it has become an important tool for ploidy manipulation in plant improvement schemes, allowing researchers to combine somatic cells from different cultivars, species, or genera, resulting in novel allotetraploid and autotetraploid genetic combinations [Grosser et al., 2011]. After the successful establishment of plant protoplast strategy was realized, first by fusing the protoplasts of *Nicotiana tabacum* and *Nicotiana glauca*. In the gramineae family, the first ever somatic hybrid plantlet was a protoplast fusion of rice (*Oryza sativa* L.) and barnyard grass (*Echinochloa oryzicola*), which was done in 1987. This technique can facilitate conventional breeding, transfer of genes such as disease resistance genes, rapid growth rate genes, more product formation rate genes, drought resistance genes and heat or cold resistance genes, from one species to another, and cultivar development by bypassing some problems associated with conventional sexual hybridization including sexual incompatibility, nucellar embryogenesis, and male or female sterility

II. SOMATIC HYBRIDIZATION

Plant protoplasts can be prepared by treatment of plant cells with specific lytic enzymes which remove the cell wall. Protoplast fusion is a physical process during which two or more protoplasts come into contact with each other in the presence of fusion-inducing agents like polyethylene glycol (PEG). This is an inexpensive and rapid mechanism whereby two genetically different protoplasts, isolated from somatic cells, are fused to obtain parasexual hybrid protoplasts containing heteroplasmic cytoplasm and two fused parent nuclei. Protoplasts of a variety of plants can be fused using PEG, and the hybrid products will regenerate cell walls and divide.

II.I CLASSIFICATION OF SOMATIC HYBRIDS

Somatic hybrids can be classified into three types: symmetric somatic hybrids, asymmetric somatic hybrids, and cytoplasmic hybrids (cybrids) based on how they are developed. Symmetric somatic hybridization refers to the combination of nuclear and cytoplasmic genetic information of both parents. Asymmetric somatic hybridization is incomplete, with the loss of some cytoplasmic or nuclear DNA, and this type of hybridization has been used to introduce fragments of the nuclear genome from one parent (donor) into the intact genome of another one (recipient). Cybrids harbor only one parental nuclear genome and either the cytoplasmic genome of the other (non-nuclear) parent or a combination of both parents. Both symmetric and asymmetric fusion experiments can generate these three types of somatic hybrids. With the development of somatic hybridization technology, many new avenues have been adopted to create somatic hybrids. The evolution of such techniques is continuing, as recently obtained asymmetric hybrids in sunflower via microprotoplast fusion with partial chromosome transfer from the micronuclear parent.

II.II SOMATIC FUSION METHODS

The two primary somatic fusion methods are polyethylene glycol (PEG) induced fusion and electrofusion [Belete et al., 2018]. PEG induced fusion is advantageous in that it does not require special equipment, low cost, and high frequency of heterokaryon formation. Electrofusion relies on two different electrical pulses. Protoplasts are brought into intimate contact during the first pulse called di-electrophoresis; and the second pulse is a very short burst of intense direct current, which results in membrane fusion. Electrofusion has the advantages of convenience, no cell toxicity, and high frequency heterokaryon formation.

**II.III METHODS TO PRODUCE CYBRIDS**

Because of the associated increase in ploidy level and the combining of all nuclear encoded traits of both parents, symmetric hybrids frequently have no economic value. Because one or more traits can be added while maintaining cultivar integrity, cybridization is a more appealing option for crop improvement (just as with genetic transformation). Three methods are routinely used to create cybrids.

Asymmetric Fusion

Asymmetric fusion of irradiated donor protoplasts with destroyed nuclei and recipient protoplasts with metabolically inhibited organelle genomes by iodoacetate (IOA) can result in hybrids. As a result, the heterokaryons combine vital cytoplasm from the donor parent with the recipient parent's intact nucleus, resulting in asymmetric hybrids or cybrids. In addition to donor-recipient asymmetric hybridization, cybrids can be created by IOA treatment of one parent (or irradiation of one parent) while leaving the other parent intact. Previously, researchers created cybrids by fusing mesophyll protoplasts from a chlorophyll deficiency mutant Lycopersicon peruvianum var. dentatum with gamma-irradiated mesophyll protoplasts from L. esculentum.

**Cytoplast Isolation and Fusion**

Cytoplast-protoplast fusion was first observed between Nicotiana tabacum and Nicotiana plumbaginifolia protoplasts. Currently, two methods for removing nuclear DNA are used: cytochalasin B treatment and discontinuous percoll/mannitol gradient ultracentrifugation. This method can also be used to create cybrids by transferring organelle-encoded traits. For example, isolated cytoplasts using this method. Because there were many nucleated protoplasts, the cytoplast/protoplast fraction was then irradiated, and they successfully transferred a desirable male-sterile cytoplasm into cabbage.

Symmetric Fusion

Aside from asymmetric fusion and cytoplast-protoplast fusion, higher plants can spontaneously produce cybrids through intraspecific, interspecific, or intergeneric symmetric hybridization. This is a common occurrence in some species, particularly tobacco and citrus. Cybrids with carpelloid stamens were obtained through interspecific symmetric somatic hybridization in tobacco (Nicotiana tabacum and N. suaveolens). Citrus cybrids can occasionally be produced as a byproduct of standard symmetric somatic hybridization procedures.

II.IV SELECTION SCHEMES FOR SOMATIC HYBRIDIZATION

To achieve successful somatic hybrid regeneration, the hybrid products must be chosen from the unfused and homo-fused protoplasts. A good selection system eliminates the time-consuming task of identifying somatic hybrids among a large number of regenerated calli or plants. Several schemes for somatic hybrid selection have been devised. Selective media; metabolic inhibitors, complementation systems such as chlorophyll deficiency complementation, auxotroph complementation, resistance markers and double mutants, individual selection and culture, and use of the green fluorescent protein (GFP) marker gene are among these schemes.

The GFP gene has recently been used as a marker to select somatic hybrids. It is derived from the aquatic jellyfish Aequorea victora, and when expressed by living cells, it emits a stable and distinct green fluorescence with no cofactors or substrates other than oxygen. As a result, transgenic plants that express the GFP gene have recently been used as a parent in somatic hybridization. GFP's potential as a somatic hybridization marker was first demonstrated in a somatic fusion experiment using a transgenic citrange plant expressing GFP as a parent.

III. SEXUAL HYBRIDIZATION

Sexual hybridization is an important tool to plant breeders which enables the transfer of desirable traits from one species to another. The steps of sexual hybridization involve a series of events which include germination of the pollen, pollinating the maternal taxa with pollen from the paternal taxa, growth of the pollen tube, fertilization, embryo and endosperm development and seed maturation.

III.I TYPES OF SEXUAL HYBRIDIZATION

Intergeneric and interspecific hybridization are the two main types of sexual hybridization. Interspecific hybridization is the cross-fertilization of two species, whereas intergeneric hybridization is the cross-fertilization of two genera, resulting in offspring with phenotypic and genotypic traits of both parents, thereby encouraging genetic diversity and evolution. The primary benefits of hybridization include disease resistance, increased fitness, higher yield, and the development of new improved crop varieties.

Hybridization is the mating or crossing of two diverse genotypes or plants or lines by dusting pollen from the male parent genotype onto the stigma of the female parent genotype flowers. It is crucial to avoid self-pollination as well as accidental cross-pollination in the female parental flowers. At the same time, pollen from the desired male parent must reach the stigma of female flowers for successful fertilization. The seeds and offspring produced by hybridization are referred to as hybrid or F1. Progeny of F1 produced by selfing or intermating of F1 plants, and subsequent generations are called Segregating generations. The term cross is often used to denote the products of hybridization, i.e. the F1 as well as the segregating generations.

III.III Objective of hybridization

The chief objective of hybridization is to create genetic variation. When two genotypically different plants are crossed, the genes from both the parents are brought together in F1. Segregation and recombination produces many new gene combinations in F2 and the later generations, i.e. the segregating generations. The degree of variation produced in the segregating generations would, therefore, depend on the number of heterozygous genes in the F1. This still, in turn, depends upon the number of the genes for which the two parents differ. If the two parents are closely related, they are likely to differ for few genes only.

The aim of hybridization may be the transfer of one or few qualitative characters, the improvement in one or more quantitative characters, or use of F1 as a hybrid variety. These objectives are briefly discussed below.

**Combination breeding**

The primary goal of combination breeding is to transfer one or more character traits from other varieties into a single variety. These traits could be controlled by oligogenes or polygenes. The character intensity in the new variety is either comparable to or lower than in the parent variety from which it was transferred. In this approach, a variety's yield is increased by correcting flaws in the yield contributing traits, such as tiller number, grains per spike, and test weight for disease resistance. Backcross method was designed for combination breeding, and the pedigree method often serves the same purpose. The genetic divergence between parents is not a major consideration in combination breeding. What matters is that one of the parents has a robust enough connection to the character(s) under transfer, while the other parent is generally a popular variety.

**Transgressive breeding**

Transgressive breeding aims to improve yield or contributing characteristics which occurs when plants in an F2 generation outperform both parents in one or more characters. Such plants are produced by the accumulation of plus or favourable genes from both parents, which must combine well and preferably be genetically diverse, i.e., quite different. This way, each parent is expected to contribute different plus genes which when brought together by recombination give rise transgressive segregant. As a result, the intensity of character in the transgressive segregant, i.e., the new variety, is greater than that in either of the parents. The pedigree method of breeding and its modifications, particularly the population approach, are designed for the production of transgressive segregants.

**Hybrid Varieties :** In most self-pollinated crops, F1 is more vigorous and higher yielding than the parents. Wherever it is commercially feasible, F1 may be used directly as a variety. In such cases, it is important that the two parents should produce an outstanding F 1.

The plants or lines involved in hybridization may belong to the same variety, different varieties of the same species, different species of the same genus or species from different genera. Based on the taxonomic relationship of the two parents, hybridization may be classified into two broad groups :

1. Intervarietal and

2. Distant hybridization

**1.Intervarietal Hybridization :** The parents involved in hybridization belong to the same species ; they may be two strains, varieties or races of the same spicies. It is also known as intraspecific hyrbidization. In crop improvement programmes, intervarietal hybridization is the most commonly used. In fact, it is so common that it may often appear to be the only form of hybridization used in crop improvement. an example would be crossing of two varieties of wheat, rice or some other crop. The intervarietal crosses may be simple or complex depending upon the number of parents involved.

**Simple Cross:** In a simple cross, two parents are crossed to produce the F1. The F 1 is selfed to produce F2 or is used in a backcross programme, e.g., A X B ^ F1 (A X B)

**Complex Cross:** more than two parents are crossed to produce the hybrid, which is then used to produce F2 or is used in a backcross. Such a cross is also known as convergent cross because this crossing programme aims at converging, i.e., bringing together, genes from several parents into a single hybrid. A few examples of convergent cross are described in Fig. 7.i. As

Three Parents (A, B, C)

A X B

▼

F1 (A X B) X C

Complex hybrid (A X B) X C X D

FOUR Parents (A, B, C, D)

A X B C X D

▼ ▼

F1 (A X B) F1(C X D)

F1 (A X B) X F1 (C X D)

▼

Complex hybrid [(A X B) X (C X D)]

Eight Parents (A, B, C, D, E, F, G, H)

A X B C X D E X F G X H

▼ ▼ ▼ ▼

F1(A X B) X F1 (C X D) F1 (E X F) X F1 (G X H)

F1 [(A X B) X (C X D)] X F1 [(E X F) X (G X H)]

▼

Complex hybrid [(A X B) X (C X D)] X [(E X F) X (G X H)]

Fig.1. Complex crosses involving 3, 4 and 8 parents.

Crop improvement advances as crop varieties accumulate more and more beneficial genes. This would result in more similarities among even unrelated varieties. Given this, it is reasonable to expect that complex crosses will become increasingly important in the future. Complex crosses are now commonly used in the breeding of highly improved self-pollinated crops such as wheat and rice. Complex crosses would become routine in the near future in the improvement of other self-pollinated crops as their level of improvement increased.

**2. Distant Hybridization:**

When crosses are made between two different species or between two different genera, they are generally termed as distant hybridization (or) wide hybridization

History

Thomas Fairchild 1717 was the first man to do distant hybridization. He produced an hybrid between two species of *Dianthus* *Dianthus caryophyllus (*Carnation ) x *D. barbatus (*Sweet william). **Inter generic hybrid** produced by Karpechenko, a Russian Scientist in 1928. *Raphano brassica* is the amphidiploid from a cross between Radish *(Raphanus sativus)* and cabbage *(Brassica oleraceae).* Triticale was produced by Rimpau in 1890 itself. Triticale is an amphidiploid obtained from cross between wheat and rye. Another example is *Saccharum* nobilisation involving three species.

Crosses between different species of the same genus or different genera are illustrations of distant hybridization. Interspecific hybridization occurs when two species of the same genus cross; however, intergeneric hybridization occurs when two species of different genera cross. In general, the goal of such crosses is to transfer one or a few easily inherited characteristics, such as disease resistance, to a crop species. Interspecific hybridization is sometimes used to create a new variety, e.g., Clinton oat variety was developed from a cross between Avena sativa x A. byza ntina (both hexaploid oat species), and CO 31 rice variety was developed from the cross Oryza sativa var. indica x O. perennis. Almost al the present-day sugarcance varieties have been developed from complex crosses between Saccharum officinarum (noble canes), S. barberi (Indian canes) and other Saccharum species, e.g., S. spontaneum (Kans.). Crossing Indian Cotton (Gossypium arboreum) with American cultivated Cotton has resulted in increased fibre length; many improved varieties have resulted from such crosses. Intergeneric hybridization can also be used to create new crop species, such as Triticale, which is derived from a cross between Triticum sp. and Secale cereale (rye). Wild species frequently provide genes that are not found in cultivated species. Many of the genes for rust resistance in wheat, for example, are derived from closely related wild species. Distant hybridization is likely to become more important in the correction of specific crop species defects. In many cases, wild species can provide valuable 'yield genes' to cultivated species.

**Pre-requisites for hybridization**

Breeder should have clear knowledge about the following before taking up hybridization.

1. Requirements of the tract
2. Local conditions i.e. soil, climate, Agronomic practices and market requirements
3. Existing varieties of crops both local and introduced
4. Facilities like funds, land, labour and equipment
5. Plant material i.e. germ plasm
6. Objectives : Well set objectives and planning

Hybrids are the first generation (F1) of a cross between two genetically dissimilar pure lines, open pollinated varieties, or clones. The majority of commercial hybrids are F1s resulting from two or more pure lines (tomato, rice, Jowar) or inbred lines (maize, sunflower, castor etc.)

**Pure line:** It is the progeny of single self-fertilized homozygous plant.

**Inbred line:** It is a near homozygous line obtained by continues inbreeding in a cross­pollinated crop followed by selection.

**Single cross:** when two inbred lines or pure lines are crossed to produce the Fl hybrid it is known as single cross.

**Double cross:** when two single crosses are crossed the resulting hybrid population is known as double cross.

**Three-way cross:** It is a cross between a single cross and an inbred to give hybrid population.

**Top cross:** when an inbred is crossed with an open pollinated variety it is known as an inbred variety cross or a top cross. The purpose of top cross is to estimate the GCA of the inbred line crossed with OPV. When the cross is made to assess the combining ability it is known as test cross. A test cross may be made with an inbred (for SCA), hybrid, synthetic or OPV (for GCA). The common parent used in the test cross is known as tester and the progeny derived from these crosses are known as test cross progeny.

**Polycross:** It is the progeny of a line produced through random pollination by a number of selected lines.

**Varietal cross:** when two open pollinated varieties are mated it is known as varietal cross or population cross.

**History of hybrids:** Hybrids were first commercially exploited in maize because the yielding ability Of OPV could not be improved by mass selection or progeny selection. In 1878 Beal had shown that certain varietal crosses showed substantial heterosis and he suggested that such varietal hybrids might be used as varieties.

Shull proposed a method for producing single cross hybrids in maize in 1908. He proposed that inbreds be created from OPV through continuous self-fertilization. The superior hybrids produced by combining inbreds should then be crossed to produce single cross hybrids. Shull's scheme could not be commercialised for the following reasons:

1. There were no outstanding inbred lines available to produce hybrids with higher yields than OPV.
2. Because the female parent was an inbred, the amount of hybrid seed produced per acre was low (30-40% of OPV), making hybrid seed costly.
3. Because the male parent was also an inbred, pollen production was low. As a result, more land was to be planted beneath the male parent. As a result, the hybrid seed became more expensive.
4. Because it was produced on an inbred line, the hybrid seed was underdeveloped. The seeds were irregular, undersized, and had poor germination, necessitating a higher seed rate.
5. The price of hybrid seed was expensive.

The last four limitations were overcome by Jones's double cross scheme proposed in 1918. Because the female and male parents are single crosses in a double cross, seed and pollen production is abundant, seed quality and germination are high, and the cost of hybrid seed is low.

Burr Learning Hybrid was the first double cross maize hybrid developed at the Connecticut Agricultural Experimental Station and grown in Connecticut in 1921.

**Development of Hybrid: Breeding for hybrids involves three steps:**

1. **Development of Inbred lines**
2. **Evaluation of inbred lines**
3. **Commercial utilization of the crosses for seed production.**

**1. Development of inbred lines:** Continuous self fertilisation of a cross-pollinated species produces inbred lines. Inbreeding of an OPV causes many deficiencies such as loss of vigour, reduced plant height, plants becoming susceptible to lodging, insects and pests, and the appearance of many other undesirable characters. After each selfing, desirable plants are chosen and self or sib pollinated. It usually takes 6-7 generations to achieve near homozygozity. Selfing or sibbing can be used to keep an inbred line going. The goal of inbreeding is to keep desirable traits homozygous in order to maintain them without genetic change.

The original selfed plants is generally referred as S0 plant and the first selfed progeny as S1 second selfed progeny as S2 as so on. The technique of inbreeding requires careful attention to prevent natural crossing. The inbred lines are identified by numbers, letters or combination of both. In India inbred lines are developed and released through co-ordinate maize improvement scheme and are designated as CM (Co-ordinate maize), CS (Co-ordinate sorghum) etc.

CM-100-199 - Yellow flint

CM-200-299 - Yellow Dent

CM-300-399 - White Flint

CM-400-499 - White Dent

CM-500-599 - Yellow

CM-600-699 - White

**2. Evaluation of inbred lines:** After developing an inbred line, it is crossed with other inbreds and its productivity in single and double cross combinations is assessed. The ability of an inbred to pass on desirable traits to its hybrid offspring is referred to as combining ability. GCA stands for the average performance of an inbred line in a series of crosses with other inbred lines.

Specific combining ability (SCA) is the excess performance of a cross over and above the expected performance based on the parents' GCA. As a result, GCA is a characteristic of parents, whereas SCA is a characteristic of crosses or hybrids.

The inbreds are evaluated in following way.

1. **Phenotypic evaluation;** It is established on the phenotypic performance of inbreds. It works well for characters with a high GCA who are highly heritable. Inbreds who perform poorly are rejected. Inbred performance is evaluated in replicated yield trials, and inbreds that perform poorly are discarded.
2. **Top Cross test:** Inbreds selected based on phenotypic evaluation are crossed to a tester with a broad genetic base, such as an OPV, a synthetic variety, or a double cross. Planting alternate rows of the tester and the inbred line, with the inbred line detasselled, is a simple way of producing top cross seed in maize. The inbred seed is harvested and represents the best cross seed. Top cross progeny performance is evaluated in replicated yield trials, preferably across locations and years. Approximately half of the inbreds are eliminated based on the top cross test. This brings the number of inbreds down to a manageable level for the next step. Top cross performance provides a trustworthy estimate of GCA.
3. **Single cross evaluation:** Only by testing the performance of single cross combinations can outstanding single cross combinations be identified. To test for SCA, the remaining inbred lines after the top cross test are generally crossed in a diallel or line x tester mating design. Plants from a single cross are completely heterozygous and homogenous, and they are uniform. Superior single crosses regain the vigour and productivity lost due to inbreeding and can be more vigorous and productive than the original open pollinated variety. The performance of a single cross is evaluated in replicated yield trials across years and locations, and the best single cross is identified and released as a hybrid where single cross seed production is commercially feasible.

In case of maize the performance of single cross is used to predict the double cross performance.

Number of Single crosses with reciprocals = n(n-l)

Number of single crosses without reciprocals = n(n-l)/2

Prediction of the Performance of Double Cross Hybrids

Four inbred parents are involved in a double cross hybrid. In supposition, the breeding value of these four parental inbreds will determine the potential of the double cross. As a result, the performance of a double cross hybrid can be predicted using any of the four methods suggested by Jenkins based on the procedure for estimating the breeding value of inbreds (1934). Beginning with the simplest procedure, these are:

1. Top-cross testing (one cross per inbred) to determine each inbred's breeding value (total 4 top-crosses per double cross).
2. The mean of the four non-parental single crosses involved in the (AXB) X (CXD) double cross, namely (AXC), (AXD), (BXC), and (BXD) (total 4 non-parental single crosses per double cross).
3. Mean yield performance of all possible six crosses [n(n-1)/2], namely AXB, AXC, AXD, BXC, BXD and CXD (total six crosses per double cross).
4. The mean performance of each inbred in all possible single crosses where it occurs can be used to calculate the average progeny-performance of each inbred (n 1 crosses per inbred). For example, the average breeding value of the inbred A will be determined by the mean performance of AXB, AXC, and AXD. Similarly, the mean of AXB, BXC, and BXD will indicate the inbred B's potential, and so on. (total 12 crosses per double cross).

These procedures for predicting the performance of double cross hybrids have long been studied. According to the evidence, method (b), i.e. mean performance of non-parental single crosses, is the most adequate and effective, because there is a close correspondence between predicted and realised yields of double crosses in maize. Fortunately, the total number of crosses required to sample per double cross is also the smallest, making the testing programme much easier.

To avoid selfing, anthers must be removed prior to fertilisation when producing hybrid seed. Manually removing anthers is a time-consuming and labor-intensive process in almost all crops except maize and castor, which are monoecious. The following conditions must be met in order for large-scale hybrid seed production to be successful:

1. The presence of male sterility or self-incompatibility, which allows hand emasculation to be avoided.

2. There should be enough cross-pollination to produce a good seed set.

Male sterility is distinguished by the presence of non-functional pollen grains, whereas female gametes function normally. It occurs sporadically in nature as a result of mutations. MS is divided into three categories:

1. Genetic 2. Cytoplasmic 3. Cytoplasmic genetic

1. **Genetic male sterility**: GMS is primarily governed by a single recessive gene ms, but dominant genes governing male sterility, such as Safflower, are also known. MS alleles can arise spontaneously or be induced artificially. By crossing a GMS line with a heterozygous male fertile plant, a GMS line can be maintained. Such mating produces 50% m.s. & 50% MF plants

msms x Msms

(Male sterile) (Male fertile)

▼

msms : Msms (1:1)

(Male sterile) (Male fertile)

It is difficult and time consuming to identify the male fertile plants from the above progeny. As a result, GMS is rarely used in hybrid seed production.

It is used in Castor in the United States. It was previously used in Redgram in India, but is now used in Safflower.

Marker genes which are linked to male sterility/fertility can be used to identify the male fertile plants before flowering stage. In Maize, for example, there is a gene called pigmented hypocotyl (P) and green hypocotyl (P) that is closely related to the sterility locus.

P S - Pigmented & Sterile

P F - Green & Fertile

At seedling stage all the green plants are to be removed and pigmented plants are retained, as they are sterile.

**II. Cytoplasmic Male Sterility**: Two types of cytoplasms have been observed in crops such as maize, bajra, and sorghum. The first is normal cytoplasm, and the second is sterile cytoplasm that interferes with the formation of normal pollen grains. As a result of maternal inheritance, all offspring will be male sterile.

Because the F1 is male sterile, it cannot be used in crops where the seed is an economic component. As a result, its utility is limited to certain ornamental species or where a vegetative part is economically important. For example, onion, fodder jowar, cabbage, palak, and so on.

**III. Cytoplasmic Genetic Male Sterility System**: In this case of cytoplasmic male sterility, a nuclear gene for restoring fertility in the MS line has been identified. The dominant fertility restorer gene 'R' is found in certain strains of species or may have been transferred from a related species. Because this gene restores fertility in the MS line, it is known as the restorer gene. When restorer genes for cytoplasmic MS are discovered, they can be added to the CGMS system. If a thorough search is conducted, restorer genes can be found in all cases of cytoplasmic MS. Almost all seed crops use this system.

This system involves

1. A line in the genetic constitution of cytoplasmically determined MS plants.

2. Fertile offspring of the A line known as the maintainer line or the B line with the genetic constitution.

3. Restorer plants, also known as R lines in the genetic constitution, are used to restore fertility in commercial seed plots.

Transfer of Male Sterility from Exotic lines to Nature lines:

Most of the times the MS lines obtained from other countries may not be suitable to our condition. Examples are:

|  |  |  |
| --- | --- | --- |
| **Crop** | **Source of**  **cytoplasm** | **Drawbacks** |
| Maize | Texas Cytoplasm | Susceptible to *Helminthosporium* leaf blight |
| Sorghum | Combined kafir | Black glumes and chalky endosperm |
| Pearlmillet | Tift 23 A (Tifton) | Susceptible to Green ear & downy mildew |
| Rice | Wild abortive | Incomplete panicle exertion |
| Sunflower | *H.petiolaris H.gigantis* |  |
| Tobacco | *Microcephalan* | Reduced vigour in F1 hybrids |
| Wheat | *Aegilops caudata* | Susceptible to pistiloidy |

Due to these drawbacks, the well adapted local lines should be converted into male sterile lines. This can be done by repeated back crossing of the local lines to the exotic MS lines.

Transfer of Male Sterility to a New Strain

**Maintenance of Male Sterile Line or A line:** Because A line does not produce pollen, seed is not produced to sustain A line. It must be crossed with a fertile counterpart with similar nuclear genes and fertile cytoplasm, known as B-line.

**Production of Hybrid seed:** In order to induce fertility and seed development in the next generation, A-line must be kept as the female parent and the pollen parent must have the restorer genes. This type of line is known as restorer line and is denoted by the letter 'R'. The A and R lines should have different genetic constitutions and be able to provide maximum heterosis.

**Limitations in using Male Sterile Systems:**

1. The existence and maintainance of A, B, and R lines is time-consuming and difficult.

2. If exotic lines are not appropriate for our conditions, native/adaptive lines must be converted into MS lines.

3. Adequate cross pollination between A and R lines is required for good seed set.

4. Flowering should be synchronised between the A and R lines.

5. Sterility should be consistent across environments.

6. Fertility restoration must be completed or the F 1 seed will be sterile.

7. Isolation is required for the maintenance of parental lines as well as the production of hybrid seed.

**Hybridization procedure or steps involved in hybridization**

1. Choice or selection of parents
2. Evaluation of parents i.e. by selfing and studying the progeny
3. Emasculation
4. Crossing or pollination
5. Bagging & Labelling
6. Harvesting of F1 seed

From F2 onwards the generations are known as segregating generations and they may be handled either by pedigree method of Bulk method or backcross method for evolving new varieties.

1. Selection of parents

In most cases, the female parent will be a locally adapted one into which we can introduce the plus genes. Geographically diverse parents will be chosen in the case of intervarietal hybridization to produce superior segregants.

1. Evaluation of parents

Parents who are new to the area should be evaluated for their adaptability. They must also be evaluated to ensure homozygozity.

3. Sowing plan

If the flowering time is the same, both parents can be sown at the same time. Otherwise, staggered sowing is required. For each combination, the ovule parent is raised in rows in the centre of the plot, and the pollen parent is raised on the border.

**4. Emasculation and dusting**

The removal of immature anthers from a bisexual flower is known as emasculation. The method of emasculation varies depending on the crop. Hand emasculation and pollen dusting are common practises. The time of emasculation varies depending on the time of anthesis. For example, in rice, anthesis occurs between 7 and 10 a.m. in Coimbatore. So the emasculation takes place around 6.30 a.m., followed by pollen dusting.

5. Labelling and bagging

Immediately after hybridization, tie or keep a label with the parents' names and the date of crossing. Put on appropriate cover to prevent foreign pollen and contamination.

6. Harvesting and storage of seeds

The seeds are usually set 15-20 days after crossing. In the case of pulses, crossed pods are easily identified by the shrunken nature of the pod and the reduced seed set. Crossed seed harvesting must be done on an individual plant basis. Individual plant seeds should be collected and stored in appropriate containers with proper labelling.

**IMPACTS OF SEXUAL HYBRIDIZATION**

Heterosis

Heterosis is a hybrid phenomenon in which offspring outperform their parents in terms of yield, growth rate, biotic and abiotic resistance. As the genetic variation of the crossing parental taxa increases, so does heterosis. As extreme phenotypes such as superior fitness are selected in subsequent hybridization generations, further disruptions of the parental linkages will result in decreased fitness or increased fitness than the parental taxa. To demonstrate how heterosis occurs in hybrids, three models, dominance, overdominance, and epistasis concepts, have been proposed. Dominance is defined as the presence of recessive deleterious alleles in different loci of one parent that are masked by beneficial alleles from the other crossing parental taxa in the F1 hybrid. The concept of overdominance explains that at the loci controlling heterosis, the presence of a heterozygote genotype that is superior to both homozygous genotypes of the two crossing parents. Epistasis refers to the beneficial interaction of gene combinations within hybrids, which results in hybrid superiority. Other studies explain that heterosis is the outcome of multiple genetic occurrences caused by the concurrent effects of dominance, overdominance, epigenetics, and epistasis. However, research has shown that in some cases, heterosis can be caused by a single over-dominant gene [Chen et al., 2018]. Furthermore, F1 hybrids with increased expression levels outside the parental taxa range have been linked to heterosis by small interfering RNA and micro-RNAs [Goulet et al., 2017]. Interspecific hybridization between Oryza sativa japonica and Oryza sativa indica, for example, resulted in F1 hybrids with heterosis for spikelet fertility and harvest index. Furthermore, wheat and rye hybrids demonstrated a heterotic effect on yield due to greater spike density and biomass [Owuoche et al., 2003]. Furthermore, F1 hybrids of Zea mays and Tripsacum dactyloides showed higher salinity tolerance than their parents.

Sterility and Inviability

The main post-zygotic fertilization barriers to hybridization are sterility and inviability. They reduce gene flow, which causes less evolutionary consequences. However, when hybridization results in gene flow between species, evolutionary consequences emerge. The primary goal of hybrid sterility is reproduction isolation in order to inhibit gene flow and maintain species identity [Koide et al., 2018]. Low grain yield, failure to form grain, or pollen inviability are all symptoms of hybrid sterility. Inviability manifests itself as inviable seeds or weak and unfit germinated hybrids that are too fail to mature and survive.

Reduced fertility is caused by decreased gamete formation and chromosomal rearrangements in hybrids. Higher the divergence between the crossing parental taxa higher is the hybrid sterility [Edmands 2002]. Reduced fertility is more pronounced when the divergence between crossing parental taxa exceeds 4 million years [58]. This is due to a buildup of inter-locus incompatibilities between diverging populations.

The Dobzhansky-Muller model, which specifies that a genetic change due to divergence in loci in one population and a genetic change in the same loci in the second crossing population results in incompatibilities when the two genomes are hybridized, resulting in post-zygotic incompatibilities and thus infertility and inviability [Wang et al., 2012]. A cross between Sorghum bicolor and Saccharum officinarum yielded 53% fertility, while previous crosses yielded 0.13% fertility.

A cross between Avena sativa and Zea mays resulted in hybrids with partial fertility. Inviability was clearly demonstrated between Zea mays and Trypsacum dactyloides hybrids, with 80% of the F1 hybrid seeds failing to germinate. Another study of the same cross revealed that the hybrids had pollen fertility ranging from 0% to 50% . Hybridization can result in absolute inviability in certain crosses. Triticum durum and Aegilops umbellulata hybrid seeds, did not germinate .

Chromosome doubling can be used to overcome sterility in hybrids by using colchicine, Amiprophos-methyl, or pronamid treatment [Melchinger et al., 2016 ]. Because most infertility in plant hybrids is caused by chromosomes lacking a pairing partner during meiosis, doubling the parental sets of chromosomes ensures that pairing can occur within each set, allowing meiosis to proceed and thus the production of fertile gametes. The chromosome doubling technique produces amphidiploids, as seen in hybrids of Syringa vulgaris and S. pinnatifolia.

Hybrid Breakdown

At the second filial generation of hybrids, hybrid breakdown acts as reproduction isolation. This phenomenon is characterized by the development of sterility and inviability in F2 hybrids, whereas their parental filial generation is fertile and viable. This happens because the interaction of different loci during gene segregation is disrupted, resulting in incompatibility between the interacting genes after the first filial generation [Rose et al., 2000]. Previous research in F2 hybrids of Indica sp. and Japonica sp. revealed hybrid breakdown due to complementary recessive sterility genes between the genomes of two species in the hybrid [Li et al., 1997].

Arrested Pollen Tube Growth

Arrested pollen tube growth is a pre-zygotic reproduction isolation mechanism that limits gene flow between species by preventing zygote formation [Dickinson 2012]. Pre-zygotic barriers are frequently very strong in plants, contributing more to total reproductive isolation than postpollination barriers.

The slow and arrested growth of the pollen tube within the stigma of the crossing maternal taxa prevents ovule fertilization. This is evident in a cross between Zea mays and Sorghum bicolor, where the sorghum pollen tube growth was stopped and the sorghum pollen tube could not grow past the micropyle to fertilise the ovule. This barrier, however, can be overcome by supplementing pollinated parental taxa with auxin. Successful hybridization between Triticum estivum and Zea mays, for example, was accomplished by spraying the pollinated silk with 2, 4-D, which increased successful fertilization from 18.7% to 69.3% by increasing the number of pollen tubes growing down the pistil . Crosses of Triticum aestivum and Leymus arenarius were also supplemented with 2, 4-D to promote fertilization between the two taxa. Somatic hybridization, which involves protoplast fusion, is a commonly used technique for overcoming this impediment. Pollen tube arrest, for example, was overcome in a cross between Cucumis sativus and Cucumis melo by protoplast fusion, but successful hybridization is limited [Jin Feng and Adelberg 2000].

Embryo Abortion

A hybrid embryo formed in some crosses, maternal plants perceive it as foreign and abort it in a degeneration process characterized by embryo shrivelling [Liu et al., 2006]. Embryo abortion occurs when the hybrid zygote fails to develop during the early stages of cell differentiation. Furthermore, embryo abortion is associated with pollen donor and recipient parent asymmetry. However, a formed hybrid embryo can be saved using a tissue culture technique known as embryo rescue. This barrier is overcome by culturing the immature embryo prior to abortion by the maternal plant. This method was successfully used in an interspecific hybridization within the genus Leucadendro. In another study, embryo rescue was used to achieve an interspecific cross between wild and cultivated Vigna unguiculata. Furthermore, embryo rescue is used to overcome the reproduction barrier in chrysanthemum and Ajania przewalskii intergeneric hybridization [Deng et al., 2004].

2.3 Selection Schemes for Sexual Hybrids

Molecular markers involve amplification of specific amplified fragment length polymorphism (AFLP), rapid amplification of polymorphic DNA (RAPD) and single sequence repeat polymorphism (SSR) markers related to fertility restoration and specific ribosomal DNA sequences. Molecular markers are the most reliable for identification of hybrids due to their unlimited number in the genome in comparison to chemical profiling which is time-consuming and limited in predicting hybrid ancestry.

In most studies, the hybridity test entails usage of wide range of tests to determine true hybrids. A study involving Sorghum bicolor and Sorghum macrospermum hybrids involved determining true hybrids by evaluating the pubescence of the leaves of hybrid, which is a characteristic of Sorghum macrospermum, and for determining chromosome number, fluorescent in situ hybridization targeting the CEN38 marker, which is present in Sorghum bicolor but absent in Sorghum macrospermum, and specific amplification of the AFLP markers specific to each parent.

Secondary metabolite screening is a reliable hybridity test technique in which hybrids express secondary metabolites quantitatively and qualitatively different from their parents. To be more specific, hybrids may express novel secondary metabolites, some of the parental taxa's secondary metabolites in different quantities and qualities than their parents' secondary metabolites, or fail to express some of the parents' secondary metabolites completely [Orians et al., 2006]. As a result, hybrids typically have complex secondary metabolites inheritance patterns. The phenolic, terpenoid, alkaloid, isothiozyanates, and flavonoid compounds are the most commonly evaluated secondary metabolites, while flavonoid compound is the most commonly studied secondary metabolite due to its high variability and stability [86].

**Hybrids in self-pollinated crops - problems and prospects**

Exploitation of heterosis through F I hybrids has hitherto been the prerogative of cross- pollinated crops, chiefly due to their breeding systems favouring allogamy. However, possibilities of working for such a proposition have recently been realized in self-pollinated corps also. Indeed, exploitation of hybrid vigour in autogamous crops is easy and less time­consuming in that homozygous inbreds are already available. There is practically no difference with regard to hybrid breeding between self and cross-pollinated crops. But the prospects of hybrids in seIfers is dependent on three major considerations.

1. How high a heterotic effect can be gained under optimal production conditions.
2. In fact, a breeder's main concern is the magnitude rather than the frequency of occurrence of heterosis in crops. Thus the consideration is whether or not it is possible to obtain economically viable heterosis.
3. How much of the yield surplus due to high heterosis can offset the extra seed cost? In major self-pollinated crops like wheat, barley, rice, etc., the seed rate per unit area is exorbitant and hence the hybrid seed requirement is also more.
4. How efficient and effective is the mechanism of cross-pollination in seIfers? By nature, self-pollinated crops are shy pollinators with very poor pollen maneuverability (or movability to effect allogamy). Therefore, the efficiency (degree of allogamy) with which cross pollination can take place on a commercial scale is the true determinant of the success of a hybrid programme in seIfers.
5. Among self-pollinated crops, FI hybrids have been graduated into the farmer's field in barely, tomato, Sorghum (often-cross-pollinated) and wheat. Briggle (1963) presented a vivid account of heterosis in wheat. Work in rice is also most encouraging (IRRI, 1972).

Breeding Methods for handling of segregating generations - pedigree method, bulk method, back cross method and various modified methods

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