Hybridization - objectives and types of hybridization

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Abstract

Plant breeders have traditionally depended on the evolutionary divergence created by sexually crossing plants of the same species. However, within-species diversity is inadequate, necessitating the employment of hybridization methods to harness desired features in genetically distinct or even distant plants. Sexual and somatic hybridization are the two forms of hybridization. By pollinating two genomes from different parental taxa, sexual hybridization, also called broad or distant hybridization, can occur naturally or purposefully. which is strongly reliant on the capability to develop viable protoplasts and differentiate them into whole plants in vitro. Gametes are the reproductive cells that produce gametes. The results of hybridization have the potential to be both beneficial and harmful. One of the beneficial characteristics of hybrids that has been actively exploited is called heterosis. Heterosis may happen as a result of dominance, over-dominance, or epistasis, and it is one of the reasons hybrids have been so successful. One of the unfavourable results is sterility, as well as the inability to produce pollen tubes and termination of embryos. Chromosome doubling, the application of hormones like 2, 4-Dichlorophenoxyacetic acid (2, 4-D), and embryo rescue have been utilised, respectively, to address sterility, pollen tube arrest, and embryo abortion. After hybrids have been formed, they are evaluated using techniques such as fluorescence in situ hybridization, molecular and morphological markers, cytogenetic analysis, and molecular genetic analysis. When it comes to plant improvement, hybridization techniques continue to be an indispensable tool for overcoming barriers between species and making use of important characteristics in allied crop plants. These goals would not have been attainable through the use of traditional plant breeding practises.

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

I. Introduction

Crossing two genetically diverse individuals’ results in hybrids, which are produced by natural or artificial means. Rather than changing an organism's genetic composition, this mechanism develops novel gene combinations that may be useful for some traits. Furthermore, this procedure eliminates problems associated with traditional sexual crossing, such as incompatibility, polyembryony, and sterility. It is used for the following reasons to hybridize crops. For choosing hybrids with suitable traits among various populations, a variable plant population must be generated first. It is also possible to incorporate specific traits into individual crops, and create hybrids and deploy them.

The two types of hybridization methods are sexual hybridization and somatic hybridization. Wide or distant hybridization, another name for sexual hybridization, creates hybrid combinations within a certain taxonomic range. Over time, sexual hybridization techniques have been used to create better and new crop hybrids, such as triticale, a plant hybrid created in 1875 from a sexual cross between rye (Secale cereale) and wheat (Triticum vulgare) [Maclntyre et al., 1973]. However, individuals from different species and even genera have hybridized widely and distantly. Inter-specific hybridization happens when two species from the same genus cross, In contrast, inter-generic hybridization happens when two individuals from the same genus cross, These types of crossing are essential because they permit the transfer of genomes from one species to another, changing the progeny's phenotype or genotype.

On the other hand, somatic hybridization takes place when gametes and somatic cells unite rather than the other way around. In contrast to sexual hybridization, this process needs the components to be fused in vitro to be handled with extreme care and attention to detail. Somatic hybridization has become a powerful tool for manipulating ploidy in plant improvement schemes since the development of protoplast fusion. This has enabled researchers to combine somatic cells from a variety of cultivars, species, and genera, which has led to the creation of new genetic combinations that are both allotetraploid and autotetraploid. [Grosser et al., 2011]. By using this strategy, a lot of the issues that are common in conventional sexual hybridization may be avoided. These issues include sexual incompatibility, the formation of nucellar embryos, and sterility in either the male or female offspring. It may be put to use in traditional breeding, the transfer of genes from one species to another, as well as the generation of cultivars.

**II. SOMATIC HYBRIDIZATION**

The membrane of plant cells can be disintegrated by lytic enzymes to generate plant protoplasts. Two or more protoplasts fuse when they come in contact with fusion-inducing chemicals, such as polyethylene glycol (PEG). In parasexual hybrids, the heteroplasmic cytoplasm and nuclei of two different parent cells are fused in a cost-effective, instantaneous process involving two genetically distinct protoplasts. It is possible to fuse protoplasts from various plants with PEG, and the hybrid products will regrow their cell walls.

II.I CLASSIFICATION OF SOMATIC HYBRIDS

 There are three types of somatic hybrids: 1.Symmetric, 2. Asymmetric, and 3.Cytoplasmic hybrids (cybrids). The mixture of both parents' nuclear and cytoplasmic genetic information is referred to as symmetric somatic hybridization. Some cytoplasmic or nuclear DNA is lost in asymmetric somatic hybridization, but it has been used to move nuclear genome particles through one parent (the "donor") further into preserved genome of another parent (recipient). In a cybrid, one parent's nuclear DNA is combined with another's cytoplasmic DNA, or vice versa.

II.II SOMATIC FUSION METHODS

Fusion induced by polyethylene glycol (PEG) and electrofusion are the two most used somatic fusion techniques [Belete et al., 2018]. Union induced by PEG is preferable since it is low-cost, does not need specialised equipment, and results in frequent creation of heterokaryons. For electrofusion to occur, two separate electrical pulses must be applied. Di-electrophoresis, the first pulse, pulls protoplasts close together, and a very brief burst of high direct current, the second pulse, causes membrane fusion. Electrofusion's many advantages include low cytotoxicity, high heterokaryon generation, and simple operation.

**II.III METHODS TO PRODUCE CYBRIDS**

Cybrids are often created using three ways.

Asymmetric Fusion

Hybrids may form when irradiated donor protoplasts with damaged nuclei and recipient protoplasts with metabolically inhibited organelle genomes undergo an asymmetric unification driven by iodoacetate (IOA). To create asymmetric hybrids, or cybrids, heterokaryons join the functional cytoplasm of one parent with the unaltered nucleus of the other. The formation of cybrids is not limited to the donor-recipient method of asymmetric hybridization; it also occurs when just one parent is treated with IOA (or irradiated) while the other is left unaffected. Previous studies have fused mesophyll protoplasts from a mutant Lycopersicon peruvianum var. dentatum lacking chlorophyll with mesophyll protoplasts from gamma-irradiated L. esculentum to create cybrids.

**Cytoplast Isolation and Fusion**

The first fusion of cytoplasts and protoplasts was reported between Nicotiana tabacum and Nicotiana plumbaginifolia protoplasts. Currently, cytochalasin B treatment and discontinuous percoll/mannitol gradient ultracentrifugation are utilised to remove nuclear DNA. By transmitting organelle-encoded characteristics, this approach can also be utilised to make cybrids. This approach, for example, was used to isolate cytoplasts. Because there were a lot of nucleated protoplasts, the cytoplast/protoplast fraction was irradiated, and they were able to effectively transfer a male-sterile cytoplasm into cabbage.

**Symmetric Fusion**

Higher plants are able to create cybrids independently by the processes of intraspecific, interspecific, or intergeneric symmetric hybridization in addition to asymmetric fusion and cytoplast-protoplast fusion. This is common in many species, from citrus trees to tobacco plants. The tobacco hybrids with carpelloid stamens are the consequence of interspecific symmetric somatic hybridization (Nicotiana tabacum and N. suaveolens). Some citrus cybrids also emerge via the more typical symmetric somatic hybridization techniques.

II.IV SELECTION SCHEMES FOR SOMATIC HYBRIDIZATION

Somatic hybrid regeneration relies on selecting the hybrid progeny from among the unfused and homo-fused protoplasts. Identifying somatic hybrids among a group of calli or plants that have been successfully regenerated is a time-consuming process that may be simplified with a reliable selection approach. Different somatic hybrid selection techniques are now in use. Methods like green fluorescence protein (GFP) marker genes, resistance markers, and individual selection and culture are examples.

Recently, the GFP gene has been used to the detection of somatic hybrids as a diagnostic tool. This protein, found in the marine jellyfish Aequorea victora, produces a continuous, easily recognisable green light when produced in live cells with just oxygen as a cofactor. As a result, transgenic plants that produce green fluorescent protein have been employed as a parent in somatic hybridization. Somatic fusion was initially performed using a transgenic citrange plant expressing GFP as a parent, demonstrating the efficacy of GFP as a marker for somatic hybridization.

III. SEXUAL HYBRIDIZATION

Plant breeders rely heavily on the process of sexual hybridization, which allows for the transfer of desired characteristics from one plant to another.

III.I TYPES OF SEXUAL HYBRIDIZATION

Intergeneric hybridization and interspecific hybridization are the two primary forms of sexual hybridization. When two different species mate, this is called interspecific hybridization, while when two different genera mate, this is called intergeneric hybridization; both types of hybridization produce offspring with phenotypic and genotypic characteristics of both parents, which promotes genetic diversity and evolution.

III.III Objective of hybridization

The primary goals of hybridization are to increase genetic variability, to transmit one or more qualitative features, to enhance one or more quantitative characteristics, and to employ F1 as a hybrid variety. Those objectives are briefly explained in the text that follows.

**Combination breeding**

Combination breeding aims to produce offspring with traits from many parent cultivars. It is possible for such traits to be influenced both oligogenetically and polygenetically. The resultant variation has personality features that are either less pronounced than or on par with those of the parent strain. Enhancing a variety's yield by repairing errors in yield-contributing features including tiller count, grain per spike, and disease resistance test weight is possible using this method. Combination breeding often makes use of both backcrossing and pedigree analysis.

**Transgressive breeding**

 When plants in an F2 generation transcend both parents in one or more traits, this is called "transgressive breeding," and it's done with the intention of increasing yield or adding attributes. Such plants are the result of a combination of favourable genes from both parents, which should ideally mix well and be quite dissimilar genetically. Using this method, it is assumed that any parent may contribute novel accessory genes that, when recombined, can result in the development of novel, transgressive segregants. Transgressive segregants are the goal of the pedigree method of breeding and its variations, most notably the population approach.

**Hybrid Varieties:** F1 is more robust and productive than the parents in most self-pollinated crops. It is possible to use F1 as a variety in its pure form anywhere it is financially feasible.In such instances, it is critical that both parents generate an excellent F 1.

Hybridization may occur between plants of the same cultivar, among cultivars within the same species, between species within the same genus, or across genera. Based on the genetic similarity of the parents, hybridization may be classified into two broad types:

1. Intervarietal and

2. Distant hybridization

**1.Intervarietal Hybridization :** The parents of a hybrid are both members of the same species, although different varieties or races of the same spice. Intraspecific hyrbidization is another name for it. By far, the most common method used by such projects is intervarietal hybridization. Intervarietal crosses range from being quite simple to complex, depending on the number of parents used.

**Simple Cross:** Two parents are crossed to generate the F1 in a simple cross. The F 1 is either selfed to generate F2 or utilized in a backcross procedure, e.g., A X B ^ F1 (A X B)

**Complex Cross:** To develop the hybrid, greater than two parents are crossed, this is then utilized to generate F2 or employed in a backcross. This type of cross is also known as a convergent cross since the crossover program tries to converge, or gather together, genes from many parents into a single hybrid. Several examples of convergent cross are given in Fig 1. As

Three Parents (1, 2, 3)

1 X 2

▼

 F1 (1 X 2) X 3

Complex hybrid (1 X 2) X 3 X 4

FOUR Parents (1, 2, 3, 4)

 1 X 2 3 X 4

 ▼ ▼

 F1 (1 X 2) F1 (3 X4)

 F1 (1 X 2) X F1 (3 X 4)

▼

Complex hybrid [(1 X 2) X (3 X 4)]

Eight Parents (1, 2, 3, 4, 5, 6, 7, 8)

1 X 2 3 X 4 5 X 6 7 X 8

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 F1(1 X 2) X F1 (3 X 4) F1 (5 X 6) X F1 (7 X 8)

 F1 [(1X 2) X (3 X 4)] X F1 [(5 X 6) X (7 X 8)]

 ▼

 Complex hybrid [(1 X 2) X (3 X 4)] X [(5 X 6) X (7 X 8)]

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

Increases in agricultural yield occur when crop cultivars accumulate more and more useful genetic traits. So, it's reasonable to expect a higher degree of similarity amongst varieties that aren't closely related. That being the case, it stands to reason that tricky border crossings will become more common in the years to come. Self-pollinating crop improvement, including wheat and rice, is quickly adopting the use of complex crosses. Complex crosses will become increasingly widespread as the level of improvement of other self-pollinated crops increases.

**2. Distant Hybridization:**

Hybrids obtained by breeding two completely different species or genera are said to have resulted from "remote hybridization" or "wide hybridization."

History

Thomas Fairchild, in the year 1717, was the first person to attempt a distant hybridization. He crossed Dianthus caryophyllus (Carnation) with Dianthus barbatus (Sweet william) to produce a new species. In 1928, a Russian named  Karpechenko developed an intergeneric hybrid. The amphidiploid offspring of a radish (Raphanus sativus) and a cabbage (Brassica oleracea) hybrid is called Raphano brassica. In 1890, Rimpau started growing triticale. Amphidiploid Triticale is the result of a hybrid between wheat and rye. Saccharum nobilisation, which includes three different species, is another example.

**Pre-requisites for hybridization**

In order to successfully engage in hybridization, breeders need to have a comprehensive command on the following.

1. Tract needs

2. Local variables such as soil, temperature, agronomic techniques, and market demands

3. Existing crop cultivars, both indigenous and imported

4. Resources such as finances, land, labor, and equipment

5. Plant material (germplasm)

6. Goals: Well-defined goals and plan

The term "hybrid" refers to the progeny of a cross between two separate pure lines, open-pollinated varieties, or clones. F1 hybrids, which are the offspring of two or more genetically different parental lines, dominate the commercial hybridization industry (maize, sunflower, castor etc.)

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

**Inbred line:** It's a quasi homozygous line generated via continued inbreeding in allogamous 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 intention of top crossing is to determine the GCA of an inbred line crossed with OPV. When a cross is built to evaluate combining abilities, it is referred to as a test cross.

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

**Varietal cross:** A varietal cross or population cross occurs when two open pollinated varieties crossed.

**History of hybrids:** Hybrids were initially widely used commercially in maize since neither mass nor progeny selection could boost OPV's yielding capabilities. In 1878, Beal showed that heterosis was present in the offspring of some hybrid varieties, and he advocated that these hybrids be used as varieties.

**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**

Repeated self-fertilization within an allogamous species always results in inbred lines . Loss of vigour, shorter plants, increased susceptibility to lodging, pests, and other genetic abnormalities are only some of the negative effects of inbreeding among OPVs. After each selfing, the best plants are picked out and either pollinated by themselves or their siblings. It takes around seven generations for genetic diversity to approach homogeneity. Selfing or sibing may be used to keep an inbred line going. The goal of inbreeding is to prevent any genetic drift from occurring by keeping desirable traits homozygous.

**2. Evaluation of inbred lines:** The success of an inbred line is evaluated by crossing it with other inbreds, both once and twice, in both single and double cross circumstances. The "combining ability" of an inbred is its capacity to pass on favourable characteristics to its hybrid offspring. GCA quantifies the average reproductive performance of an inbred line over several generations.

Specific combining ability refers to a cross's performance over and above what would be expected based on the GCA of its parents (SCA). Thus, GCA is a feature that is passed down from one generation to the next, whereas SCA is a trait that is present only in hybrids. The inbreds are assessed as follows.

1. **Phenotypic evaluation;** It is based on the morphological competence of inbreds. It works effectively for characters with a high GCA, which are highly heritable. Poorly performing inbreds are discarded. Inbred effectiveness is investigated in repeatable yield experiments, and inbreds that underperform are eliminated.
2. **Top Cross test:** In this method, inbreds selected for desirable morphological traits are crossed with a tester from a diverse genetic background (e.g., an open pollinated variety [OPV], a synthetic variety [SYN], or a double cross]. Planting rows of the tester and the inbred line, with the inbred line detasselled, is a straightforward approach for getting top cross seed in maize. It is the inbred seed that is handpicked for its superior offspring. Top offspring from elite crosses are evaluated using repeatable yield tests, preferably from many locations and growing seasons. The top cross test eliminates around 50% of inbreds. That cuts down the number of inbreds to a manageable level for the next stage. The best predictor of GCA is top cross efficacy.
3. **Single cross evaluation:**

Only by analysing the efficacy of single cross combinations may exceptional single cross combinations be identified. Tests for SCA are often performed using a diallel or line x tester mating pattern on the remaining inbred lines after a top cross test. Single-cross offspring are completely heterozygous, homogenous, and reliable. The initial open-pollinated variety may have been weaker and less productive, but improved single crosses may restore that strength and efficiency. Once it becomes economically feasible to produce seeds from a single cross, researchers will conduct repeatable yield trials over several growing seasons and geographic locations to determine which single cross will provide the highest overall crop yield.

In the case of maize, we can anticipate how successful the double cross will be by analysing the results of the single cross.

Total single crosses with reciprocals = n(n-l)

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

Prediction of the Performance of Double Cross Hybrids

 A double cross hybrid is composed of four inbred parents. The breeding value of these four parental inbreds, it is assumed, will define the capability of the double cross. As a consequence, a double cross hybrid's performance may be anticipated by employing any of the four strategies proposed by Jenkins based on his methodology for assessing the breeding value of inbreds (1934). Starting with the most basic, they are:

1. a) Top-cross testing (one cross per inbred) to establish breeding value of each inbred (total 4 top-crosses per each double cross).
2. The average of the four non-parental single crossings used in the experiment. (1X2) X (3X4) double cross, namely (1X3), (1X4), (2X3), and (2X4) (total 4 non-parental single crosses/double crosses).
3. Average yield efficiency of all six possible crossings [n(n-1)/2], namely 1X2, 1X3, 1X4, 2X3, 2X4 and 3X4 (total six crosses per double cross).
4. The average performance of every inbred in all single crossings, where it occurs can be used to calculate the average progeny-performance of each inbred (n-1 crosses per inbred). For example, the average performance of 1X2, 1X3, and 1X4 will define the average breeding value of the inbred 1. Similarly, the mean of 1X2, 2X3, and 2X4 will reflect the potential of the inbred 2, and so on. (12 crossings total per double cross).

 These methods for forecasting the effectiveness of double cross hybrids have been investigated for a long time. Method (b), i.e. mean performance of non-parental single crossings, is the most appropriate and effective, according to the findings, because there is a tight correlation between projected and realised yields of double crosses in maize. Interestingly, the overall number of crosses necessary to sample every double cross is also the least, simplifying the testing method.

 When generating hybrid seed, anthers must be removed prior to fertilisation to prevent selfing. With the exception of maize and castor, which are monoecious, manually removing anthers is a time-consuming and labor-intensive operation in virtually all crops. To ensure the success of large-scale hybrid seed production, the following requirements must be met:

1. The presence of male sterility or self-incompatibility, which prevents hand emasculation.

2. Cross-pollination should be sufficient to achieve a robust seed set.

The existence of non-functional pollen grains differentiates male sterility, whereas female gametes operate appropriately. Mutations cause it to exist randomly in nature. MS is classified into three types:

1. Genetic MS 2. Cytoplasmic MS 3. Cytoplasmic genetic MS

 **1. Genetic Male Sterility**: GMS is mostly regulated by a sole recessive gene called ms, while dominant genes that control male sterility, like as Safflower, are also recognised. The appearance of MS alleles may be random or it may be the result of intentional breeding. Crossing a GMS plant with a heterozygous male fertile plant may help maintaining line. From this cross, we get half m.s. and half MF plants.msms / rfrf x Msms/ Rfrf

(Male sterile) (Male fertile)

 ▼

msms / rfrf : Msms/ Rfrf (1:1)

 (Male sterile) (Male fertile)

Finding the male fertile plants amongst the progeny is difficult and time consuming.As a result, GMS is hardly used in the production of hybrid seeds. It is employed in Castor in the U.s. It was traditionally utilized in Redgram in India, but which is currently utilised in Safflower.

**2. Cytoplasmic Male Sterility**: It has been shown that there are two distinct types of cytoplasm in plants including maize, bajra, and sorghum. The first is standard cytoplasm, while the other is infertile cytoplasm, which prevents healthy pollen grains from forming. All descendants will be male sterile due to maternal inheritance

Because the F1 is male sterile, it cannot be used in crops where the seed is a vital economic factor. As a result, its application is restricted to specific ornamental species or situations in which a vegetative element is commercially essential. For instance, onion, jowar, cabbage, palak, and so on.

 **3. Cytoplasmic Genetic Male Sterility System**: In this instance of cytoplasmic male sterility, a nuclear gene for reversing the condition has been identified and implemented in the CMS line. Some strains of a species carry the 'R' dominant fertility restorer gene, which may have been transferred from a closely related species. This gene is called the "restorer gene" because it helps the MS line fertile again. If and when restorer genes for cytoplasmic MS are discovered, they may be included into the CGMS framework. All cases of cytoplasmic MS may be treated if the underlying genes responsible for this process are found. To several extent, this method is used by all seed-producing plants.

This system involves

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

2. The A line's fertile offspring, sometimes called the maintainer line, or the B line's offspring who share the A line's genetic composition.

3. R lines (Restorer), are used to restore fertility in commercial seed plots.

Table1. Crops and their source of cytoplasm

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| **Crop** | **Source of** **cytoplasm** |
| *Zea mays* | Texas Cytoplasm |
| *Sorghum bicolor* | Combined kafir |
| *Pennisetum glaucum* | Tift 23 A (Tifton) |
| *Oryza sativa* | Wild abortive |
| *Helianthus annus* | *H.petiolaris H.gigantis* |
| *Nicotiana tabaccum* | *Microcephalan* |
| *Triticum aestivum* | *Aegilops caudata* |

Transfer of Male Sterility to a New Strain

**Maintenance of Male Sterile Line or A line:** Because it does not produce viable pollen, a line cannot produce seed. It can only reproduce by mating with a B-line counterpart, or a genetically identical individual with fertile cytoplasm.

**Production of Hybrid seed:** In order to promote fertility and seed development in the next generation, the A-line must be maintained as the female parent, and the pollen parent must carry the restorer genes. In this context, the letter "R" stands for "restorer line," a term for the kind of line in question. The best heterosis would come from a combination of A and R lines, each of which would have a unique genetic makeup.

**Limitations in using Male Sterile Systems:**

1. It is labor-intensive and time-consuming to create and maintain male-sterile (A), maintainer (B), and restorer (R) lines.
2. If exotic lines are not applicable to our situation, native/adaptive lines must be converted to MS lines.
3. A lot of pollination from both male sterile (A) and restorer (R) lines is required for good seed production.
4. Synchronizing the booting of male sterile (A) and Restorer (R) lines is an important step.
5. Fifth, uniformity in sterility standards is essential.
6. If fertility isn't restored, the F1 seed will be sterile.
7. Both the persistence of the parental lines and the establishment of hybrid seed depend on periods of isolation.

**Hybridization procedure or steps involved in hybridization**

1. Parental choice or selection
2. Parental evaluation, i.e. selfing and observing offspring
3. Emasculation
4. Crossing or pollination
5. Bagging & Labelling
6. Harvesting of F1 seed

 Following the second generation (F2), new cultivars may be developed via the pedigree method, the bulk method, or the backcross method, all of which are referred to as segregating generations.

1. **Parental choice or selection**

 The maternal parent is the most likely candidate for gene transfer because of its local adaptation. Geographically dispersed parents are more likely to produce high-quality offspring in the case of intervarietal hybridization.

 2. Parental evaluation

 Parents who seem to be unfamiliar to the region should have their adaptation assessed. They should also be screened for homozygozity.

 3. Sowing plan

Both parents can be sowed at the exact date if the blossoming time is similar. Alternatively, staggered planting is necessary. The ovule parent is elevated in rows in the plot's centre for each combination, and the pollen parent is raised on the periphery.

**4. Emasculation and dusting**

 Emasculation refers to the process by which immature anthers are removed from a bisexual flower. 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

 Knot or retain a label with the parents' names and the date of pollination promptly following hybridization. Place an appropriate brown paper bag/ butter paper  to stop foreign pollen and contaminants from rest.

6. Harvesting and storage of seeds

 It usually takes around 15 to 20 days after crossing for the seeds to be established. Smaller pod size and reduced seed production are classic symptoms when two pulse pods have been crossed. Crossed seeds may only be collected from individual plants. It's important to collect seeds for each plant individually, identify them, and store them in the correct containers.

 **IMPACTS OF SEXUAL HYBRIDIZATION**

**Heterosis**

The word "heterosis" refers to the occurrence in which the offspring of a hybrid species exhibit superior performance relative to its genetic parents. The incidence of heterosis rises as the number of parent taxa utilised in a cross increases. Three models have been developed to illustrate the emergence of heterosis in hybrids; these are the dominance, overdominance, and epistasis concepts. At an F1 hybrid, dominance is shown by deleterious recessive alleles in several loci from one parent being covered up by protective alleles from the other crossing species. The idea of overdominance arises at the locus regulating heterosis when a heterozygote genotype is present that is superior than the homozygous genotypes of both crossing parents. The epistatic interaction between genes in hybrids is responsible for hybrid competence.

**Sterility and Inviability**

One of the biggest problems with hybridization is that it often results in offspring who are sterile or otherwise unable to reproduce. There will be fewer evolutionary impacts because of the restriction of gene flow. However, evolutionary implications occur when hybridization results in interspecies gene flow. The main goal of hybrid sterility is to prevent offspring from reproducing with each other, which helps control gene flow and protects the purity of each species [Koide et al., 2018]. Low grain yield, inability to produce grain, and pollen inviability are all symptoms of hybrid sterility. Seeds that aren't viable or hybrids that fail to thrive after germinating are examples of inviability. Low fertility in hybrids is caused by problems with sperm and egg development and chromosomal rearrangements. More divergent parent taxa result in more infertile hybrids [Edmands 2002]. Crossing parental taxon divergence of 4 million years or more is associated with a significant decline in fecundity. This is associated with the buildup of incompatibilities between loci across genetically distinct populations.

 Post-zygotic incompatibilities cause reproductive problems and inviability [Wang et al., 2012], according the Dobzhansky-Muller model. These incompatibilities result from genetic alterations at the same loci in the second crossing population. The hybrid between Sorghum bicolor and Saccharum officinarum showed 53% fertility, far higher than the 0.13% fertility seen in prior crosses.

 Colchicine, Amiprophos-methyl, or pronamid treatment may be used to induce chromosomal doubling, which can be used to treat or prevent infertility in hybrids [Melchinger et al., 2016]. Duplicating or doubling the parental sets of chromosomes ensures that pairing may occur within each set, allowing meiosis and, ultimately, the generation of viable gametes to continue, while the absence of a partnering companion in meiosis is the most common cause of sterility in plant hybrids. As shown in hybrids of Syringa vulgaris and S. pinnatifolia, amphidiploids are created by the chromosomal doubling process.

Hybrid Breakdown

 In the second generation of hybrid offspring, hybrid breakdown acts as reproductive isolation. This phenomenon is characterised by the sterility and inviability of F2 hybrids developing while the paternal filial generation is fertile and viable. Because this gene-by-gene connection is broken up after the first filial generation, incompatibilities between associated genes arise [Rose et al., 2000]. A recent study of Indica sp. x Japonica sp. F2 hybrids revealed that the hybrids were breaking down because of the presence of recessive sterility alleles that complemented one another in the hybrid genomes. [Li et al., 1997].

Arrested Pollen Tube Growth

Reduced interspecific gene flow may be achieved using the pre-zygotic reproductive isolation approach of pollen tube arrest [Dickinson 2012]. Pre-zygotic barriers are often more stronger in plants than post-pollination barriers, and thus contributes more to total reproductive isolation.

The pollen tube's growth inside the stigma of a crossing maternal taxon is slowed or halted, preventing fertilisation of the ovule. Hybrids between Zea mays and Sorghum bicolor make this point abundantly clear since sorghum pollen tube growth is suppressed, rendering the sorghum pollen tube incapable of expanding beyond the micropyle and penetrating the ovule. However, this obstacle may be overcome by providing auxin to the pollinated parental taxa. To increase fertilisation rates from 18.7% to 69.3%, for instance, sprayed fertilised silk with 2, 4-D. This worked by increasing the number of pollen tubes growing along the pistil. Although successful hybridization is limited, pollen tube inhibition was overcome in a cross between Cucumis sativus and Cucumis melo by means of protoplast fusion. [Jin Feng and Adelberg 2000].

Embryo Abortion

Some types of hybridization result in the mother plant rejecting the developing embryo because it looks too different from the rest of the family. [Liu et al., 2006]. When the hybrid zygote is unable to grow during the initial stages of cell differentiation, this culminates in embryo abortion. Asymmetry in pollen donor and receiver parent  is also linked to embryo abortion. A created hybrid embryo, on the other hand, can be rescued via a tissue culture process called as embryo rescue. This impediment is removed by the mother plant cultivating the immature embryo prior to abortion. This approach was employed effectively in a Leucadendro interspecific hybridization. Another study employed embryo rescue to produce an interspecific hybrid of wild and domesticated Vigna unguiculata. Furthermore, embryo rescue is employed in chrysanthemum and Ajania przewalskii intergeneric hybridization to circumvent the reproductive barrier. [Deng et al., 2004].

Selection Schemes for Sexual Hybrids

The amplification of certain ribosomal DNA or amplified fragment length polymorphism (AFLP) sequences are two examples of molecular markers utilised in assisted reproductive technology (ART). The RAPD and SSR markers are two others. Molecular markers are the most reliable way for identifying hybrids since there are an endless number of them in the genome, in contrast to the restricted and time-consuming chemical profiling.

Most hybridity tests employed by scientists involve a range of approaches for assessing whether or not a particular organism is a true hybrid. Fluorescent in situ hybridization was used to identify chromosome sets in hybrids of Sorghum bicolor and Sorghum macrospermum by focusing on the CEN38 marker (which is present in Sorghum bicolor but absent in Sorghum macrospermum), and AFLP markers specific to each parent were amplified with great accuracy.

There is some evidence that hybrids may be consistently recognised by the production of secondary metabolites that are both quantitatively and qualitatively different from those produced by the parents. In particular, hybrids may express novel secondary metabolites, express some of the parental taxa's secondary metabolites in levels and quality different from the parental taxa, or fail to express some of the parental taxa's secondary metabolites at all [Orians et al., 2006]. This suggests that secondary metabolite inheritance patterns are often more nuanced in hybrids. Phenolic, terpenoid, alkaloid, isothiozyanate, and flavonoid molecules are examples of secondary metabolites that are both ubiquitous and stable.

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

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