Distant Hybridization

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Abstract

Distant hybridization, which can shatter species boundaries, boost genetic diversity, and blend the biological traits of existing species, refers to crossings between two distinct species, genera, or higher-ranking taxa. It is a crucial method for developing genetic diversity, viable strains, and superior traits in new strains and populations. Wide crosses play a crucial role in crop improvement because they are an effective tool for transferring genes from wild forms and relatives into cultivated cultivars for disease resistance and for enhancing various quality parameters in cultivated plant species. Wide crosses should have specific problems including cross incompatibility, hybrid inviability, hybrid breakdown, and hybrid sterility in addition to applications. To solve these problems, certain methods are employed, including pollen mixing, bridge crossings, ploidy level modification, and embryo rescue.

Keywords: *Distant hybridization, Chromosome manipulation, Limitations, Role, Crop improvement.*

I. INTRODUCTION

Crossing between two genetically dissimilar parents is called hybridization. While Hybridization between individuals from different species belonging to the same genus or two different genera of the same family is termed distant hybridization and such crosses are known as distant crosses or wide crosses. This is because individuals used for hybridization in such cases are taxonomically more distantly related than different varieties from the same species.

In areas of diversity, wild species are the results of natural evolution. They are neither controlled nor employed by people. Abiotic (physical and chemical) and biotic (living creatures, most pests, and illnesses) factors interact with populations of genetically diverse plants to cause evolution. The abundance of variation found both within and between species is the result of this. The species may cohabit in areas of high variety, but external and internal barriers created throughout the history of evolution keep them generally apart.

Plants that have been domesticated and grown under cultivation are relatively new. Man has improved their genetic and agronomic adaption to human requirements by manipulating and safeguarding them. Their genetic diversity is often constrained. As a result, they are susceptible to a variety of unfavourable conditions, as previous catastrophes in their past have demonstrated. Therefore, it follows that plant breeding must heavily rely on the riches from the gene centers. The first stage in starting a program to use wild species in plant breeding is to gather as many accessions of related species and genera as you can, either from gene banks that already exist or through expeditions to diversity hotspots. It is important to identify, categorize, correctly disseminate, and preserve the acquired material. It is necessary to use a mix of taxonomic, biochemical, cytological, genetic, or molecular techniques to ascertain the affinity between species and crops. Due to natural recombination, species with the highest compatibility to the crop can be employed for gene transfer most effectively. The comprehensive assessment of species for desired features is crucial. A thorough understanding of the obstacles that separate species and how to get beyond them is essential. Repeated backcrossing of wide hybrids to their parental species has also contributed to the evolution and speciation of some species by gene introgression, i.e., the infiltration of chromosomes or chromosome fragments from one species into another through repeated backcrossing of wide hybrids to their parental species (Anderson 1953; Stebbins 1971; Arnold 1997; Mallet 2007).

The first authentic record of a distant hybridization for crop improvement is the production of a hybrid between Carnation (*Dianthus caryophyllus*) and Sweet willian (*Dianthus barbatus*) which is done by Thomas Fairchild (1717). Karpechenko (1928) introduced the first intergeneric hybrid between Raphanus (radish) and *Brassica* (cabbages), which gave rise to Raphanobrassica. However, Triticale, a crop with more promise than Raphanobrassica, was created by Rimpu (1890) by crossing wheat and rye). (Allard 1960)

Types of Distant Hybridization

Distant hybridization is of two types, *viz*:

1. **Interspecific hybridization,**
2. **Intergeneric hybridization.**

I. Interspecific Hybridization

Interspecific hybridization is the term used to describe the crossing or mating of two distinct species belonging to the same genus. Interspecific hybridization, also known as intrageneric hybridization, occurs when two species belonging to the same genus mate.

When the desired characteristic of a crop cannot be found within the species, interspecific hybridization is performed. It is a useful technique for introducing favourable genes into domesticated plants from closely related domesticated or wild species. In vegetatively propagated species, such as sugarcane and potatoes, interspecific hybridization is more effective than in seed propagated species. Introgression, or the transfer of certain genes from one species into the genome of another, is a result of interspecific hybridization.

Takeda and Frey (1977) showed significant impacts of wild and primitive species on crop yields in oats, Yerk and Peloquin (1987) and Glendinning (1979) in potato, and W.D. Evans (1977) in strawberry. These impacts on polygenic features are most likely the result of gene interactions between genes from the crop and related species. Interspecific hybrid derivatives may have useful novel traits, including thornlessness in Ribes, branching ears in cereals, and unique forms and colours in ornamental plants. They are unexpected and, according to Rick (1979), may result from genic or cytoplasmic interaction or from latent variation in the parents.

Interspecific hybridization gives rise to three types of crosses, *viz.*

a. Fully fertile,
b. Partially fertile, and
c. Fully sterile in different crop species.

a. Fully Fertile Crosses

All interspecific crosses between those species that have total chromosomal homology are fully fertile. Such hybrids' chromosomes pair normally during meiosis, which results in completely fertile F_1 plants.

Fully fertile interspecific crosses have been seen between the following Cotton, Wheat, Oats, and Soybean species

i. Cotton

Gossypium hirsutum, G. barbadense, G. arboreum, and G. herbaceum are the four cultivars of cotton. *Gossypium hirsutum* and *G. barbadense* are members of the tetraploid group (2n = 52), while *G. arboreum and G. herbaceum* are members of the diploid group (2n = 26). Fully viable crosses may be obtained between the diploid species *G. arboreum* and G. *herbaceum* and the tetraploid species *G. hirsutum* and *G. barbadense*.

***G. hirsutum* (2n = 52) x *G. barbadense* (In = 52) → F_1 plants are fully fertile.**
***G. arboreum* (2n = 26) x *G. herbaceum* (2n = 26) → F_1 plants are fully fertile.**

ii. Wheat

There are several species of the hexaploid Wheat (2n = 42). Club wheat (*Triticum compactum*) and common wheat (*Triticum aestivum*) interspecific crosses are totally fertile.
***Triticum aestivum* (2n = 42) x *T. compactum* (2n = 42) → F_1 plants are fully fertile.**

iii. Oats

Oats come in two cultivatable varieties: white oat (*Avena sativa*) and red oat (*Avena byzantiana*). They are both hexaploid (2n = 42) species. These two species can produce totally viable hybrids.

***Avena sativa* (2n = 42) x *A. byzantiana* (2n = 42) → F_1 plants are fully fertile.**

iv. Soybean

Glycine max (cultivated soybean) is thought to have evolved from the wild species *Glycine Soja*. These two species have an annual 2n = 40 diploid genome. The other wild species are perennials. Crosses between *G. max* and *G. Soja* are totally fertile.

***Glycine max* (2n = 40) x *G. Soja* (2n = 40) → F_1 plants are fully fertile.**

b. Partially Fertile Crosses:

Interspecific crosses between species that differ in chromosomal number but share certain chromosomes are partially fertile. In such cases, the F_1 plants are partly fertile and partially sterile.

The following are reports of partially fertile interspecific crosses in wheat, cotton, and tobacco

i. Wheat

Wheat is classified into three species: diploid (2n = 14), tetraploid (2n = 28), and hexaploid (2n = 42). The offspring of the durum wheat (*T. durum*, 2n = 28) and common wheat (*Triticum aestivum*, 2n = 42) hybrid are only half fertile. Both of these species share chromosomes from the A and B genomes, making F_1 hybrids partially fertile. During meiosis, there are 7 univalents and 14 bivalents in F_1. In this cross, seeds are occasionally set.

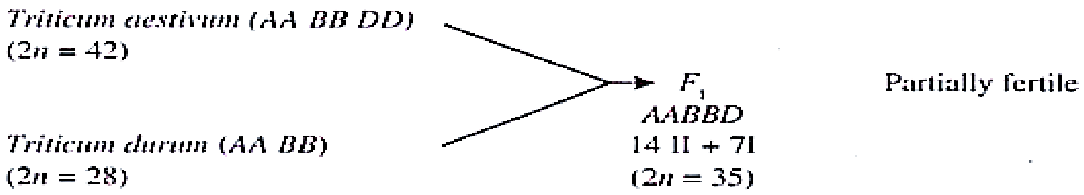

Fig 1: Partially fertile cross in Wheat

ii. Cotton

There are two different species of cotton: tetraploid (2n = 52) and diploid (2n = 26). Because these two species share D genome chromosomes, the hybrid between American cultivated cotton (*G. hirsutum*, 2n = 52) and American wild diploid (*G. thurberi*) is only partially fertile. Thirteen bivalents and thirteen univalents are produced during meiosis in F1. In this cross, seeds are occasionally set.

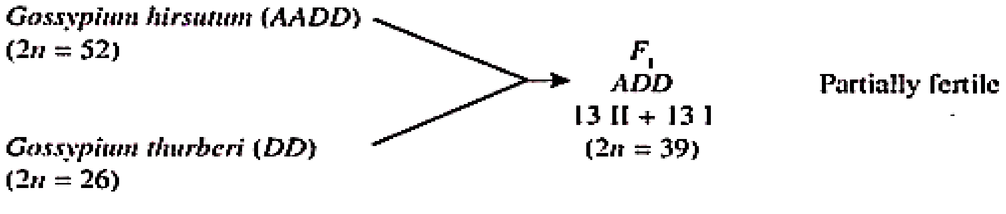

Fig 2: Partially fertile cross in Cotton

iii. Tobacco

There are three different species of tobacco: hexaploid (2n = 72), tetraploid (2n = 48), and diploid (2n = 24). Due to the shared T1 and T2 genomes in these two species, the hybrid between hexaploid wild tobacco (*Nicotiana digluta*) and common tetraploid tobacco (*N. tabacum*) exhibits partial fertility. In F_1, meiosis results in the creation of 12 univalents and 24 bivalents. In this cross, seeds are seldom rarely set.

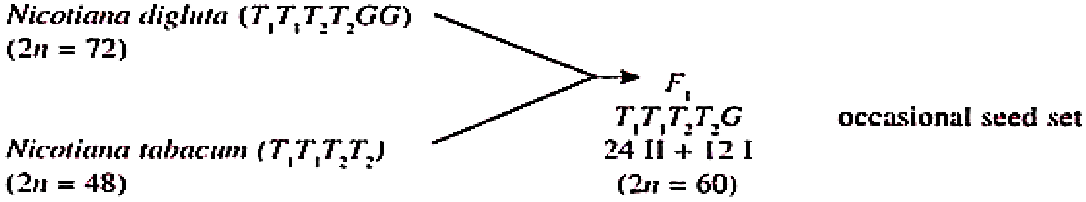

Fig 3: Partially fertile cross in Tobacco

c. Fully Sterile Crosses

All interspecific crosses between species that lack chromosomal similarity are completely sterile. Such species may or may not have a comparable chromosomal number. Chromosome homology does not allow for the pairing of two species' chromosomes during meiosis.

The F_1 plants are entirely self-sterile as a result. By tripling the number of chromosomes in these hybrids by colchicine treatment, they can become self-fertile. There have been reports of fully sterile F_1 hybrids in a variety of crops, including wheat, cotton, brassica, vignette, and tobacco.

i. Tobacco

Nicotiana sylvestris (2n = 24) and *Nicotiana tomentosa* (2n = 24) are two wild diploid species of tobacco that were crossed by Clausen and Goodspeed in 1928. The F_1 hybrid was completely sterile. Colchicine treatment of the F_1 plants resulted in a totally viable, 2n = 48 tetraploid that resembled farmed species (*N. tabacum*).

They created another hybrid between *N. paniculata* (2n = 24) and *N. undulata* (2n = 24), two more wild diploid species of tobacco. The F_1 was once again sterile. Colchicine treatment of F_1 produced viable amphidiploid (2n = 48) plants that resembled the farmed species *N. rustica*.

ii. Cotton

Gossypium arboreum, a cultivated diploid from Asia, and *G. thurberi*, a wild diploid from America, were crossed by Harland (1940). The F_1 was completely sterile. Colchicine treatment of F_1 plants produced viable amphidiploid (2n = 52) plants that resembled upland cotton (*G. hirsutum*).

iii. Brassica

Several people created interspecific crosses within the genus Brassica. Black mustard (*Musa nigra*), rapeseed (*B. campestris*), and cabbage (*Brassica oleracea*) were crossed three times (*B. nigra*). All three crossings resulted in sterile F_1 hybrids.

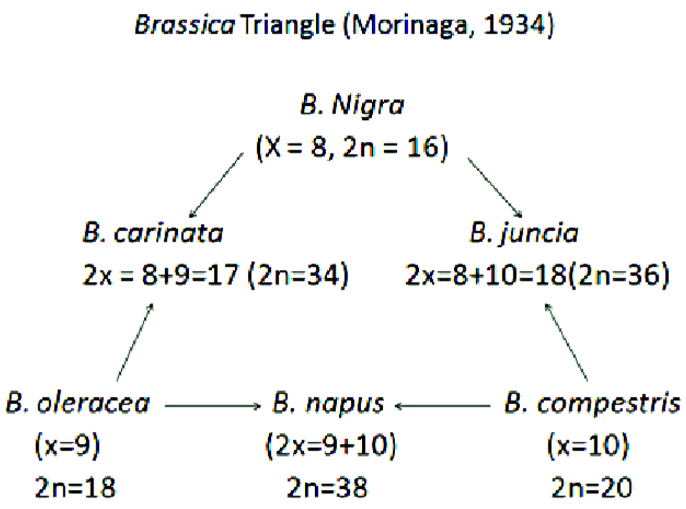

Fig 4: The treatment of F_1 plants with colchicine resulted in the production of fertile amphidiploid in each cross of brassica (Chahal and Goshal, 2003):

iv. Vigna:

Singh & Singh, 1975 and others created interspecific crosses between the green-gram (*Vigna radiata*, 2n = 22) and the black-gram (*V. mungo*, 2n = 22). The F_1 was completely sterile. Colchicine treatment caused the F_1 chromosome number to double, which led to the development of fertile amphidiploid.

II. INTERGENERIC HYBRIDIZATION

When two distinct genera within the same family cross, this is referred to as intergeneric hybridization. These crosses have a number of issues, which makes them rarely employed in crop improvement.

When the desired genes are absent in various species of the same genus, intergeneric hybridization is utilized. Using this strategy to introduce particular traits into farmed plants from related genera is uncommon in crop improvement programmes. Asexually propagated species have often employed intergeneric hybridization. F_1 hybrids produced by crossing two genera are always infertile/sterile. Through the use of colchicine, fertility must be restored by doubling the number of chromosomes. Some researchers employed intergeneric hybridization to create new crop species. E.g., *Tritical*.

Some examples of intergeneric hybridization are given below

i. Wheat-Rye Cross

Around 1890 in Sweden, Rimpau created the first intergeneric cross in the family Gramineae between bread wheat (*Triticum aestivum*, 2n = 42) and rye (*Secale cereale*, 2n = 14). The F_1 was sterile before colchicine therapy, which turned it fertile. Triticale was the name given to the amphidiploid (2n = 56) plant.

This combines the rye's hardiness with the grain quality and productivity potential of wheat. The finest illustration of the results of intergeneric hybridization in practise is triticale.

Nowadays, triticale is farmed commercially in nations like Argentina and Canada. Triticale has been enhanced and several variants have been made available for industrial farming. At CYMMIT in Mexico, research on Triticale is now being conducted.

ii. Radish Cabbage Cross

Karpechenko created an intergeneric hybrid of the Cruciferae family members radish (*Raphanus sativus*) and cabbage (*Bassica oleracea*) in 1928. The primary goal was to mix cabbage leaves with radish root. The F_1 was completely sterile. Colchicine treatment caused the number of chromosomes to double, leading to the generation of viable amphidiploids that Karpechenko named *Raphanobrassica*. However, the new species that was created in this way possessed useless traits like radish-like leaves and cabbage-like roots.

iii. Intergeneric Crosses in Sugarcane

In sugarcane, a number of intergeneric crosses have been created. Intergeneric hybrids with sugarcane have been created in eight genera (*Saccharum*). These species belong to the genera *Eccoilopus, Erianthus, Miscanthidium, Miscanthus, Narenga, Rapidum, Sclerostachya,* and Sorghum (Sweet Sorghums).

Male sterility makes it simple to create several intergeneric hybrids. Intergeneric hybrids with sugarcane, however, haven't contributed much to the creation of contemporary commercial cultivars. Intergeneric hybrids offer enormous promise for germplasm enhancement.

iv. Maize-*Tripsacum* Crosse

There have also been attempts at intergeneric crosses between maize and *Tripsacum*. In maize and *Tripsacum*, the number of basic chromosomes is 10, respectively. Crosses are effective in both ways, however because maize pollen can build a long pollen tube to reach the ovule, hybrids can be created more readily when *Tripsacum* is employed as the female parent.

Tripsacum pollen, on the other hand, are unable to generate lengthy pollen tubes to reach the maize ovule. To allow the *Tripsacum* pollen tube to reach the ovule, the length of the maize styles (ear silks) must be shortened when a reciprocal cross is made. Now, commercial crop development programmes use *Tripsacum* x diploid maize hybrid variants.

v. Intergeneric Crosses in Barley

Barley (Hordeum) has been interbred with several *Avena, Phleum, Dactylis, Alopercunis, Triticum, Lolium,* and *Festuca* species. A significant issue in these crosses was seed set.

It was discovered that applying 2, 4-D prior to pollination and then treating with gibberellic acid helped the aforementioned intergeneric crossings succeed. But none of these intergeneric unions helped barley cultivars emerge. They continued to be purely intellectual in nature.

Table 1. Difference between intergeneric hybridization and interspecific hybridization.

Particulars	Interspecific Hybridization	Intergeneric Hybridization
Parents involved	Involves two different species of the same genes.	Involves two different genera of the same family.
Fertility	Such hybrids vary from completely fertile to completely sterile.	Hybrids are always sterile.
Seed setting	More than intergeneric crosses.	Low
Use In crop Improvement	More than intergeneric crosses.	Less than interspecific crosses.
Release or Hybrid varieties	Possible in some crops.	Not possible.
Evolution of new crops	Not possible. But evolution of new species is sometimes possible.	Sometimes possible. Example is Triticale.

Scope for Wide Crosses in Crop Improvement

Wide crosses are typically employed to enhance crop types' quality, adaptability, yield, disease resistance, insect resistance, and stress tolerance. Even new crop species can be created using these crosses. Techniques like "alien addition" and "alien substitution" might also work.

Desirable genes for diverse traits of agricultural plants may come from wild species or wild genetic resources. Wide crossover is a successful strategy for using beneficial traits from wild species to enhance cultivated crop plants. Therefore, the importance of wild species and remote hybridization are related.

Distant hybridization has played significant role in:

1. Improving the crop plants for:
- Disease and insect resistance,
- Quality,
- Adaptation,
- Yield,
- Mode of reproduction, and
- Several other characters.

2. Developing commercial hybrids in some crops, and
3. Creation of new crops.

These aspects are briefly discussed below:

1. Character Improvement

a. Disease and Insect Resistance

Disease resistance has been transferred from wild species to cultivated ones through distant hybridization. For example, resistance to rust and black arm in cotton, mosaic virus, wildfire, black-fire, blue mould, black root rot, and sereh disease in sugarcane, late blight, leaf roll, and virus X in potato, rust and eye spot in wheat, and yellow mosaic virus in okra has been passed down from wild species of these crops into cultivated species.

The commercial cultivars of tomato now have bacterial cankar, bacterial wilt, Fusarium wilt, grey leaf spot, leaf moulds, Verticillium wilt, curly top virus, and mosaic virus resistance that was previously only present in wild species. Numerous insect pests and diseases of major horticultural crops, including citrus, rubber, grape, pistachio, and peach, have been eradicated via the use of wild root stocks.

Insect resistance has made little progress. Resistance to cotton Jassids and Boll Weevil, Peanut leaf chewing insects, and Strawberry aphids has been transmitted from wild species to cultivars.

Some examples are given below. (Table 1) (Harlan 1976; Singh 2010; Knott and Dvorak 1976).

Table 1: Resistance to diseases and insect transferred through interspecific hybridization in different crops

Crop	**Character transferred**	**Species transferred From**	**Species transferred to**
Cotton (Meyer 1974)	Jassid resistance Blackarm resistance	*G. Tomentosum* *G. arboreum*	*G. Hirsutum* *G. barbadense*
Okra	Resistance to YMV	*Abelmoschus Manihot*	*A. esculenta*
Groundnut	Resistant to leaf chewing insect	*Arachis monticola*	*A. hypogea*
Wheat	Rust resistance Yellow rust	*Agropyron* *T. spelta*	*T. aestivum*
Barley	Powdery mildew Powdery mildew	*Hordeum spontaneum* *H. bulibosum*	*Hordeum vulgare* *Hordeum vulgare*
Sorghum	smuts	*Sorghum bicolour*	*Cultivated sorghum*
Tobacco	Resistant to mosaic virus	*N. repanda*	*N. tabacum*
Sugarcane	Sereh disease resistance	*Saccharum spontaneum*	*Saccharum officinarum*
Sugarbeet	Cercospora leaf spot and curly top virus	*Beta patellaris*	*Beta vulgaris*
Potato	Late blight and leaf roll resistance	*Solanum denissum*	*Solanum tuberosum*
Tomato	Fusarium wilt, leaf mold, curly top.	*Lycopersicon pimpinellifolium*	*Solanum Lycopersicum*
Straberry	Resistance toward aphid	*Fragaria chiloensis*	Cultivated strawberry
Coffee	Leaf rust	*Cofea cabnephora*	*Coffee arabica*

(ii) Improvement in Quality

Wild species have been employed in various crops to enhance the quality of cultivated species. For instance, the use of their wild species in the hybridization programme has improved the protein content of rice, oats, and rye; the length of cotton fibre; the oil quality of oil palm; the carotenoid content of tomatoes; the starch content of potatoes; the quality of tobacco leaves; and the percentage of oil in oats.

To make maize more suitable for silage, teosinte has been employed. For improved green fodder in cultivated species, wild sorghum has been utilised. Wild tobacco has been used to lower the amount of nicotine in cultivated species, while wild tea has been used to enhance the flavour of cultivated tea.

Some examples are given below (Table 2) (Simmonds 1979; Stalker 1980).

Table 2: Resistance to diseases and insects transferred through interspecific hybridization in different crops.

Crop	Character transferred	Species transferred from	Species transferred to
Cotton	Fibre length Male sterility	*G. Thurberi & G. Raimondii* *G. harkenssii*	*G. hirsutum* *G. hirsutum*
Potato	Starch content Frost resistance	Wild species *Solanum acaule*	*Cultivated Spp.* *S. tuberosum*
Tobacco	Leaf quality	*Nicotiana debneyi*	*N. Tabacum*
Tomato	Carotenoid content	*Lycopersicon* Wild spp.	*L. esculentum*
Palm	Oil quality	Wild Spp.	Cultivated Spp.
Oat	High oil content	*Avena sterilis*	*A. sativa*
Rice, Oat & Rye	Protein quality	Wild Spp.	Cultivated Spp.

Through the utilisation of wild species, adaptation to varied environmental situations has been boosted. For instance, rye, wheat, onions, potatoes, tomatoes, grapes, strawberries, and peppermints, among other crops, have inherited their resistance to cold from wild species of these crops in Russia. Increased winter hardiness has been included into wheat from *Agropyron*.

Through the use of the wild species *Vitis amurensis* in the breeding programme, hardier grape plants have been generated. Cold tolerance has been inherited by sugarcane from American wild species. Using their wild species in the breeding programmes, it has been possible to develop crops with traits like drought tolerance in peas and wheat, salt tolerance in tomatoes, tolerance to calcareous soils, and photo insensitivity in Pennisetum.

(iv) Improvement in Yield

The incorporation of wild species into some crops has also increased yield. For instance, a cross between *Avena sativa* and *A. sterilis* resulted in an increase in oat production of between 25 and 30 percent over the normal parent. After 4 backcrosses, high producing transgressive segregants were produced.

Interspecific hybridization has been observed to increase yield in a number of crops, including tobacco, potato, vanilla, *zea*, ribes, and *arachis*. Utilizing *Nicotianci debneyi*, a wild plant, boosted tobacco yields. The utilisation of their wild species has boosted the yields of octaploid strawberries and sugarcane.

(v) Mode of Reproduction

When using wild species in hybridization programmes, the mode of reproduction might occasionally change. The most frequent modification to the mechanism of reproduction that comes from interspecific hybridization is male sterility. For the generation of hybrid seeds, cytoplasmic male sterility (CMS) is a cost-effective tool.

In wheat, cotton, barley, tobacco, potato, sunflower, and ryegrass, crosses between wild and cultivated species have been found to contain CMS. The CMS has been adapted to these crops cultivated species. Apomictic genes have been transmitted from wild Beta species to cultivated species as well as from maize — Tripsacum cross to maize. Cultivated rye has acquired the cleistogamy and self-fertility features of wild Secale (*secale cereale*).

(vi) Other Characters

Other beneficial traits have also been included into cultivated plants from wild species. For instance, bright red thin flesh and outstanding leaf texture in red peppers and dark green colour and texture in lettuce have been transferred from wild species. Derivatives of the hybrid *Triticum* x *Agropyron* have produced semi-dwarf wheat. Interspecific hybrids produced short-stipped oil palms. With soybean, earliness has been attained by the utilisation of wild species.

2. Hybrid Varieties

Improved hybrid cultivars have been created mostly in sugarcane, potato, and other forage crops by using wild species. The majority of modern cultivars of potato and sugarcane are interspecific hybrids. Commercial interspecific hybrids in cotton have been created at both the tetraploid and diploid levels, but only between cultivated species.

Interspecific hybridization is the source of certain upland cotton varieties, including MCU 2, MCU 5, Deviraj, Devitej, G 67, Khandwa 1, Khandwa 2, Badnawar 1, PKV081, Rajat, and Arogya. A hybrid of napier grass and pearl millet has been created, and it has gained a lot of popularity due to its great potential for fodder output and excellent quality of fodder.

3. New Crop Species

Polyploidy and distant hybridization can occasionally result in the development of new crop species. *Nicotiana digluta* was created by crossing Nicotiana tabacum with *Nicotiana glutinosa*. Triticale is an example of a novel crop that combines the best traits of two different species, *Triticum aestivum* and *Secale sereale*, through intergeneric hybridization.

Table 3: Some practical achievements of wide hybridization

S.N.	Crop and Achievements	Varieties/species developed
1.	Cotton (Varieties)	MCU 2, MCU 5, Deviraj, Devitej, Khandwa 1, Khandwa 2, Badnawar 1, Gujarat 67, PKV 081, Rajat, AKA 8401, Arogya, etc.
2.	Cotton (Hybrids)	Varalaxmi, Jayalaxmi, DHB 105, DDH 3, DH 7, DH 9, NHB 12, TCHB 213, HB 224, etc
3.	Sugarcane	Many varieties

4.	Potato	Many varieties
5.	Napier Grass	Hybrid Napier
6.	Evaluation of new crop species: 1. Wheat-Rye 2. Tobacco 3. Radish-Cabbage	 *Triticale* *Nicotiana digluta* *Raphanobrassica*

The main barriers to the use of distant hybridization include

1. Cross incompatibility,
2. Hybrid inviability,
3. Hybrid sterility, and
4. Hybrid breakdown.

These problems along with their remedial measures are discussed below

1. Cross Incompatibility

Cross incompatibility is the inability of the functional pollens of one species or genera to cause fertilisation of the female gametes of another species or genera. Cross incompatibility, in other words, is the inability of the male and female gametes to combine to produce a zygote in interspecific and intergeneric hybrids.

This is a major problem with distant hybridization. The three primary causes of cross incompatibility are the failure of pollen to germinate, the pollen tube's inability to develop enough to reach the ovule, and the failure of the male gamete to fuse with the egg cell. These are referred to as pre-fertilization barriers.

2. Hybrid Inviability

Fertilization and zygote formation both happen in some wide crosses. However, the zygote doesn't develop. Hybrid inviability is the failure of a hybrid zygote to develop into a typical embryo under typical conditions of development.

This may result due to three main factors

- Unfavourable interaction between chromosomes of two species,
- Disharmony between cytoplasm and nuclear genes and,
- Unfavourable interaction among embryo, endosperm and maternal tissues.

The following techniques may be useful to overcome the problem of hybrid inviability

The development of a zygote into a viable seed is more likely to occur under favourable conditions when the right parents are chosen, reciprocal crosses are made, and growth hormones are used. The embryo can be taken out and placed in the culture media if the endosperm is preventing the development of the embryo.

The embryoid cells in the culture media can be used to regenerate new plants. For several plant species, the embryo cultures have been discovered and produced. Therefore, using the embryo culture approach to prevent the production of hybrid zygotes is a successful solution.

3. Hybrid Sterility

Hybrid sterility is the primary issue in the majority of wide crosses. The inability of a hybrid to generate viable offspring is referred to as hybrid sterility. Compared to interspecific crossings, the issue of hybrid sterility in intergeneric crosses is more severe. Interspecific crosses range from fully fertile to fully sterile. Intergeneric crosses, however, are never fertile. Lack of structural homology between the chromosomes of two species is the primary factor in hybrid sterility.

The following meiotic abnormalities result from this decreased or absent chromosomal pairing

- Scattering of chromosomes throughout spindles during metaphase I.
- Extension of chromosomes into cytoplasm.
- Lagging of chromosomes during anaphase.
- Formation of Anaphase Bridge.
- Presence of ring and chain configurations.
- Irregular and unequal anaphase separation of chromosomes.

All of these meiotic abnormalities result in structural chromosomal alterations, including as deletions, duplications, translocations, and inversions, which prevent pollen from developing or cause it to form ineffectively. Sterility has occasionally been linked to chromosomal pairings that are entirely normal (genie sterility). Sometimes, chromosomal structural changes that are too tiny to notice during meiosis are the cause of sterility. It is known as criptic structural hybridity, according to Stebbins.

By giving the hybrid a colchicine treatment, the chromosomal number of the hybrid can be doubled, eliminating the sterility brought on by the structural differences between the chromosomes of the two species. Each chromosome will have a partner to mate with at meiosis following chromosomal doubling. Normal chromosomal pairing and the creation of functional gametes will result from this.

4. Hybrid Breakdown

For interspecific crosses, hybrid breakdown is a major problem. Hybrid breakdown occurs when F_1 plants from an interspecific cross are healthy and fertile but their F_2 offspring are weak and sterile. The interspecific gene transfer process is hampered by hybrid breakdown.

There are two main causes of hybrid breakdown, viz.

(i) Gene combination, and
(ii) Structural differences.

(i) Gene Combination

Sometimes, homozygous recessive alleles at the same loci prefer to exist in one species while homozygous dominant alleles on a number of loci prefer to exist in another species. Such species would produce a heterozygous, vigorous F_1 cross. Due to segregation and recombination in F_2, the advantageous combination of dominant and recessive genes is disrupted. The plants would be weak and infertile if neither a dominant allele nor homozygosity for all recessive alleles were present at each locus.

(ii) Structural Differences

The chromosomes of two species may have a few minor structural variations, but these do not alter chromosome pairing in F_1. Recombination between chromosomal segments during meiosis may produce gametes with deletions or duplications in these hybrids. Hybrid breakdown is caused by the deletions and duplications in the gametes.

Limitations of Distant Hybridization

Although distant hybridization has many beneficial uses for crop development, it has some limitations that have limited its widespread usage.

Some of the limitations are briefly discussed below

- Cross incompatibility, hybrid viability, hybrid sterility, and hybrid breakdown issues are linked to distant crossings. These issues provide a number of challenges for interspecific or intergeneric gene transfer.
- To make distant hybrids successful in various situations, a variety of special techniques must be used, including ploidy modification, pistil manipulation, chemical (growth regulator) treatment, bridge crossing, grafting, embryo culture, *etc*. As a result, this is a difficult task.
- In order to employ favorable genes from wild species through distant hybridization, unpleasant features are typically connected to certain desirable characters, which presents challenges. There have been a number of wheat chromosome addition and replacement lines generated, but none of them could be used for commercial production since they contained some undesirable genes.
- Sometimes, distant hybrids will combine useless traits like *Raphanobrassica* with undesirable traits like non-flowering, late maturity, and seed dormancy.
- It is particularly challenging to transfer traits governed by recessive genes in interspecific crosses.
- Character transmission during distant hybridization is more complicated than during intervarietal crossings.
- In F_1 hybrid absence of flowering.
- Linkage drag is an issue that results from undesirable linkages between genes of interest.

Techniques to Overcome the Limitations

1. **Selection of Plants:** For the crosses, the finest potential parents should be chosen.
2. **Reciprocal Crosses:** If one parental combination fails, a reciprocal cross may be attempted.e.g., Mung x Udid - cross compatible and Udid x mung - cross incompatible.
3. **Manipulation of Ploidy:** To make the cross viable, single genomes will need to be diploidized so that they are paired.
4. **Bridge Crosses:** When two parents are incompatible, a third parent which is compatible with both parents can be employed as a bridge parent, allowing the original parents to be crossed.
   e.g., Tobacco
   -*Nicotiana repanda* x *N. tabaccum*– cross incompatible
   -*Nicotiana repanda* x *N. sylvestris*- cross compatible

-*Nicotiana syivestris* x *N. tabaccum*- cross compatible

5. **Use of Pollen Mixture:** Using a pollen combination can help to some extent overcome the unfavourable interaction between pollen and pistil in wide crossings.
6. **Manipulation of Pistil:** Decapitation of the style may occasionally assist to overcome incompatibility.
7. **Use of Growth Regulators:** IAA, NAA, 2,4-D, and gibberellic acid are examples of growth hormones that can be used to speed up pollen tube growth.
8. **Protoplast Fusion:** When gamete fusion is unsuccessful, protoplast fusion of somatic cells might be attempted.
9. **Embryo Rescue:** Wide crossings can result in hybrid zygotes that in certain instances are unable to develop. To solve this issue, the zygotes are removed and developed in in vitro media.

It is possible to use an embryo rescue procedure to prevent embryo abortion.

Ex. *Hordeum vulgare* x *Secale cereale*. Degradation of endosperm can be overpowered through rescue of embryo (Allard 1960).

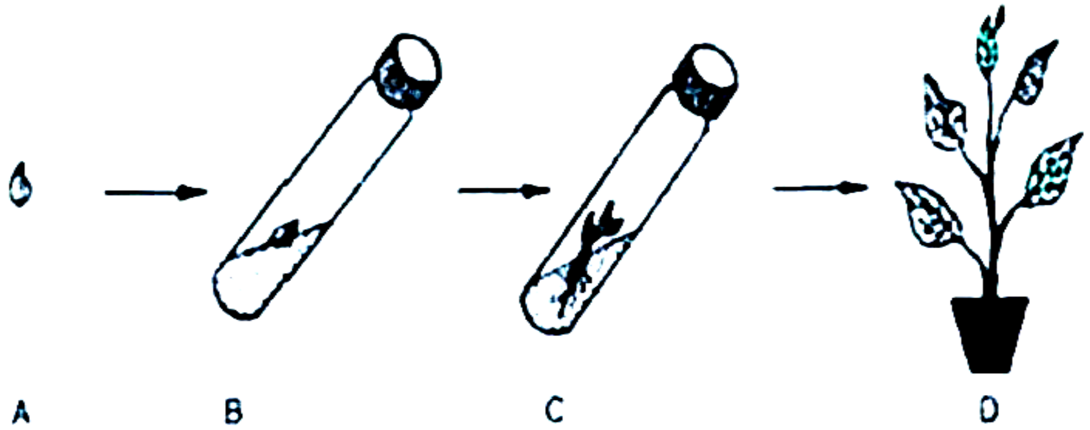

Fig. 1 Embryo rescue technique

Embryo rescue technique

A. Cutting of proembryo within 3-5 days of pollination.
B. Culturing of proembryo onmedia containing agar.
C. Collection of plantlets obtainedfrom the embryo.
D. Transplantation of the plantletin soil.

Constraints in embryo rescue

- Generation of new plantlets is not cost effective.
- Induction of mutations lethal innature in invitro phase.
- Well-equipped and established greenhouse and tissue culture lab are major requisite for distant hybridization. Also, Skillful persons are required for performing all the operations.

References

[1] Allard, R. W. (1960). Principles of plant breeding. John Wiley and Sons, Inc., New York.

[2] Takeda K, Frey KJ (1977) Growth rate inheritance and associations with other traits in backcross populations of Avena sativa x A. sterilis. Euphytica 26: 309-312

[3] Yerk G, Peloquin SJ (1987) Evaluation of wild species by use of haploid-wild species hybrids. In: 10th Trienn Conf EAPR, Aalborg, Denm, p 74

[4] Glendinning DR (1979) The potato gene-pool, and benefits deriving from its supplementation. In: Zeven AC, Harten AM van (eds) Proc Conf broadening the genetic base of crops. PUDOC, Wageningen, pp 187-194

[5] Evans WD (1977) The use of synthetic octoploids in strawberry breeding. Euphytica 26:497-503

[6] Rick CM (1979a) Potential improvements of tomatoes by controlled introgression of genes from wild species. In: Zeven AC, Harten AM van (eds) Proc Conf Broadening the genetic base of crops. PUDOC, Wageningen, pp 167-173

[7] Clausen RE, Goodspeed TH (1925) Interspecific hybridization in Nicotiana II. A tetraploid

[8] glutinosa-tabacum hybrid an experimental verification of Winge's hypothesis. Genetics 10:279-284

[9] Karpechenko GD (1928) Polyploid hybrids of Raphanus sativa L. x Brassica oleracea L. Z Indian Abstr Verer-Bughsl 39: 1-7

[10] Anderson E (1953) Introgressive hybridization. Biol Rev 28:280–307

[11] Stebbins GL (1971) Chromosomal evolution in higher plants. Addison-Wesley, Reading, MA

[12] Arnold MA (1997) Natural hybridization and evolution. Oxford University Press, New York

[13] Mallet J (2007) Hybrid speciation. Nature 446:279–283

[14] Meyer, V.G. (1974). Interspecific cotton breeding. Econ. Bot.28: 56-60.

[15] Simmonds, N.W. (1979). Principles of crop improvement. Longman, London and New York. Stalker, H.T. (1980). Utilization of wild species for crop improvement. Adv. Argon. 33: 111-147.

[16] Knott, D.R. and Dvorak, J. (1976). Alien germplasm as a source of resistance to diseases. Ann. Rev. Phytopath, 14: 211-235.

[17] Chahal GS, Gosal SS (2003) Principles and procedures of plant breeding – biotechnological andconventional approaches. Narosa Publishing House, New Delhi.

[18] Morinaga T (1934) Interspecific hybridization in Brassica. VI. The cytology of F1 hybrids ofB. Juncea and B. nigra. Cytologia 6:62–67.