

DISTANT HYBRIDIZATION FOR CROP IMPROVEMENT



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Introduction

Hybridization

Crossing between two genetically dissimilar parent is called hybridization.

Distant hybridization

Hybridization between individuals from different species belonging the same genus (interspecific hybridization) or two different genera of same family (intergeneric hybridization) is termed as distant hybridization and such crosses are known as distant crosses or wide crosses.

This because individuals used for hybridization in such cases are taxonomically more distantly related than different variety from the same species.

History

❑ Thomas Fairchild (1717):

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*).

❑ Karpechenko (1928):

An interesting intergeneric hybrid, *Raphanobrassica*, was produced.

❑ Rimpu (1890):

Produce the first intergeneric hybrid triticales which have greater potential than raphanobrassica.

Inter-specific hybridization:

- Ex. Nerica, an upland rice for Africa
 - *Oryza sativa* (Asian upland rice) : non-shattering , resistant to lodging, high yield potential
 - *Oryza glaberrima*(African rice): drought tolerant, disease resistant, weed-suppressing
- Nerica rice combines the best of both species.

African rice



Nerica rice



Asian rice



Intergeneric crosses

- Triticale, a new cereal created in the lab.
- Triticale, a cross(intergeneric cross) between wheat and rye , was produced by embryo rescue of the product of fertilization and a chemically induced doubling of the chromosomes.
- Embryo rescue become necessary when fertile offspring is never produced by an interspecific cross.



Triticale= Wheat x rye

Interspecific combinations of wheat and rye that produces hexaploid and octaploid triticales

Rye (*Secale cereale*) **x** **Bread Wheat (*Triticum aestivum*)**
Diploid Sporophyte = 14 Hexaploid Sporophyte = 42
haploid gamete: $n = 7$ triploid gamete: $3n = 21$

Rye ($n = 7$) + Wheat ($3n = 21$) = Triticale ($4n = 28$) $\xrightarrow{\text{colchicine}}$ Triticale ($8n = 56$)

Rye (*Secale cereale*) **x** **Durum Wheat (*Triticum turgidum*)**
Diploid Sporophyte = 14 Tetraploid Sporophyte = 28
haploid gamete: $n = 7$ diploid gamete: $2n = 14$

Rye ($n = 7$) + Wheat ($2n = 14$) = Triticale ($3n = 21$) $\xrightarrow{\text{colchicine}}$ Triticale ($6n = 42$)

Main features of Interspecific or Intergeneric hybridization

1. It is used when the desirable character is not found within the species of a crop.
2. It is an effective method of transferring desirable gene into cultivated plants from their related cultivated or wild species.
3. It is more successful in vegetatively propagated species like sugarcane and potato than in seed propagated species.
4. It gives rise to three types of crosses viz. a) fully fertile, b) Partially fertile and c) Fully sterile in different crop species.
5. It leads to introgression which refer to transfer of some genes from one species into genome of another species.
6. F1 hybrid between two genus are always sterile. The fertility has to be restored by doubling of chromosome through colchicine treatment.

- a) **Fully fertile crosses:** Interspecific crosses are fully fertile between those species that have complete chromosomal homology. Chromosome in such hybrids have normal pairing at meiosis and result the F1 plants are fully fertile.

Example. Cotton, Wheat, oat and Soyabean.

Cotton: There are four cultivated species of cotton Viz.,

G. Hirsutum and *G. barbadense* (Tetraploid, $2n=52$).

G. Arboreum and *G. herbaceum* (Diploid, $2n=26$).

- b) **Partially fertile crosses:** Interspecific crosses are partially fertile between those species which differ in chromosome number but have some chromosome in common. In such situation, F1 plants are partially fertile and partially sterile.

Example. Wheat, Cotton, Tobacco.

Cotton: $G.hirsutum(AADD)$ X $G.thurberi(DD)$

$2n=52$ | $2n=26$

F1(ADD)

13II+13I

Partially sterile or fertile, $2n=26$.

c) Fully sterile crosses: Interspecific crosses are fully sterile between those species which do not have chromosomal homology. Such hybrids can be made self fertile by doubling the chromosomes through colchicine treatment e.g. Tobacco, wheat, cotton, brassica and Vigna species.

Cotton: $G.arboreum(2n=26)$ X $G.thurberi(2n=26)$

|

F1 was sterile

Colchicine treatment → Fertile amphidiploid $2n=52$.

Problems associated with wide crosses

- ❑ The major problems associated with wide crosses are:
 - Cross Incompatibility
 - Hybrid Inviability
 - Hybrid Sterility
 - Hybrid Breakdown

CROSS INCOMPATIBILITY

- ❑ This is the inability of the functional pollen grains of one species or genus to effect fertilization in another species or genes.
- There are three main reasons of cross incompatibility viz.
 - I. Lack of pollen germination,
 - II. Insufficient growth of pollen tube to reach ovule and
 - III. Inability of male gamete to unite with the egg cell.
- These barriers are known as pre –fertilization barriers.
- This is overcome by employing different techniques like **reciprocal crosses, bridge crosses, using pollen mixtures, pistil manipulations, use of growth regulators etc.**

HYBRID INVIABILITY

- ❑ This refers to the inviability of the hybrid zygote or embryo. In some cases, zygote formation occurs, but further development of the zygote is arrested. In some other cases, after the completion of the initial stages of development, the embryo gets aborted.
- The reasons for this are:
 1. Unfavorable interactions between the chromosomes of the two species
 2. Unfavorable interaction of the endosperm with the embryo.
 3. Disharmony between cytoplasm and nuclear genes
- **Reciprocal crosses, application of growth hormones and embryo rescue** are the techniques that can be used to overcome this problem.

HYBRID STERILITY

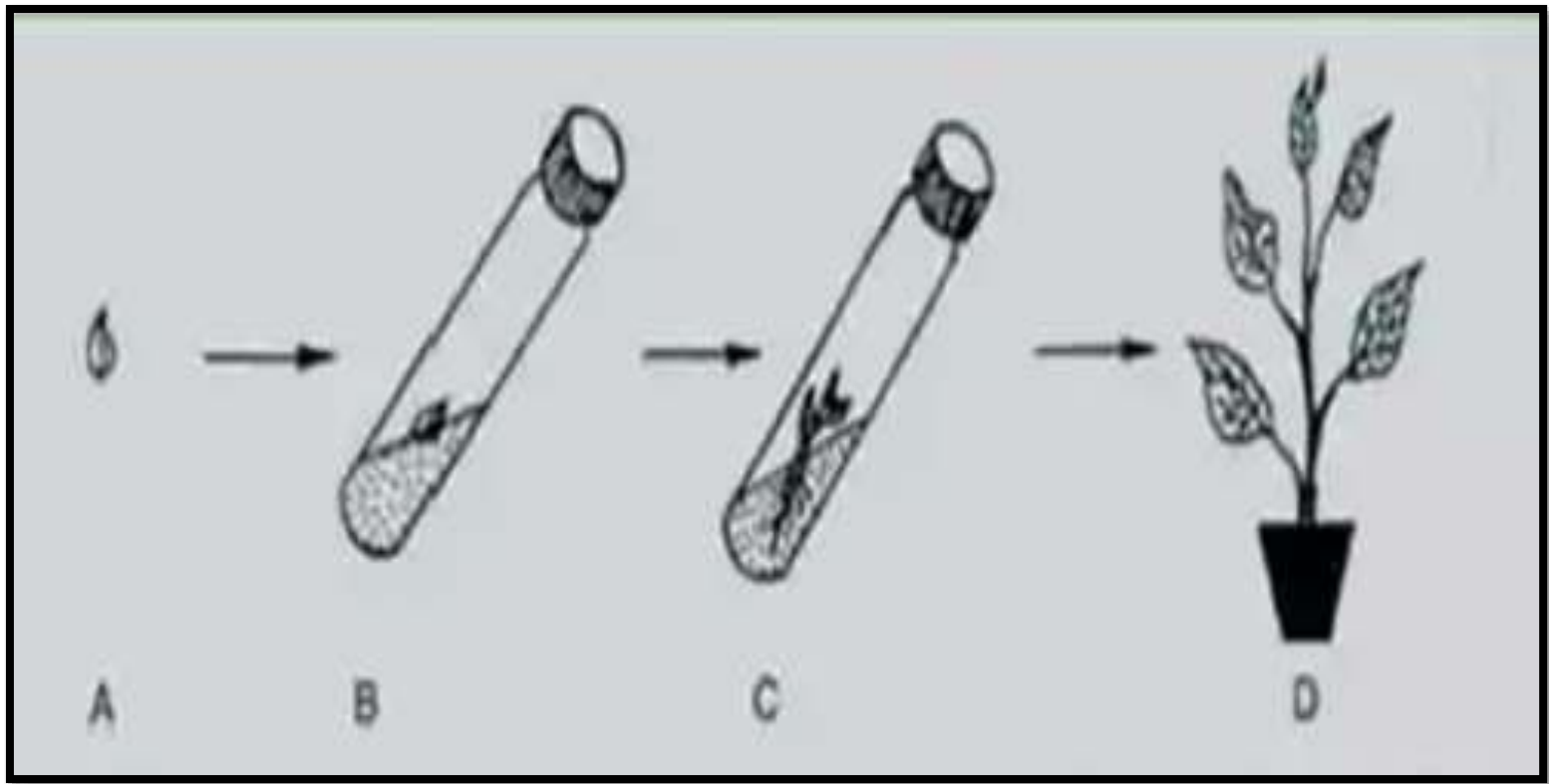
- ❑ This refers to the inability of a hybrid to produce viable offspring. This is more prominent in the case of intergeneric crosses. The major reason for hybrid sterility is the **lack of structural homology between the chromosomes of the two species**.
- This may lead to meiotic abnormalities like **chromosome scattering, chromosome extension, lagging of chromosome in the anaphase, formation of anaphase bridge, development of chromosome rings and chains, and irregular and unequal anaphase separations**.
- These irregularities may lead to aberrations in chromosome structure. Lack of homology between chromosomes may also lead to incomplete pairing of chromosomes.
- Sterility caused by structural differences between the chromosomes of two species can be overcome by **amphidiploidization using colchicine**.

HYBRID BREAKDOWN

- ❑ Hybrid breakdown is a major problem in interspecific crosses.
- When F1 hybrid plants of an interspecific crosses are vigorous and fertile but there F2 progeny is weak and sterile it is known as hybrid breakdown.
- So hybrid breakdown hinders the progress of interspecific gene transfer.
- This may be due to the structural difference of chromosomes or problems in gene combinations.

Embryo rescue

- **Embryo rescue :**
- **When embryos fails to develop due to endosperm degeneration, embryo culture is used to recover hybrid plants; this is called hybrid rescue.**
- **e.g; *H. vulgare* x *Secale cereale*.**
- **Embryo rescue generally used to overcome endosperm degeneration.**



Embryo culture.

(A) Proembryo dissected 3 to 5 days after pollination

(B) Proembryo culture on solid agar media

(C) Plantlet developing from embryo

(D) Plantlet transplanted into soil.

Embryo rescue in barley :

Hordeum vulgare Barley $2n = 14$

X

Hordeum bulbosum Wild relative
 $2n = 2X = 14$

Embryo Rescue

Haploid Barley $2n = X = 7$ H. Bulbosum
chromosomes eliminated

This technique was once more efficient than microspore culture in creating haploid barley

Wide crossing of wheat and rye requires embryo rescue and chemical treatments to double the no. of chromosomes triticales

Triticum durum (4X)
AABB

x

Secale cereale (2X)
RR

ABR F₁(3X): EMBRYO RESCUE

CHROMOSOME DOUBLING

HEXAPLOID TRITICALE (6X)
AABBRR

Limitations of embryo rescue :

- High cost of obtaining new plantlets .
- Sometimes deleterious mutations may be induced during the in vitro phase.
- A sophisticated tissue culture laboratory and a dependable greenhouse are essential for success.
- Specialized skill for carrying out the various operations are required.

TECHNIQUES TO MAKE WIDE CROSSES SUCCESSFUL

1. SELECTION OF PLANTS

The most compatible parents available should be selected for the crosses.

2. RECIPROCAL CROSSES

Reciprocal cross may be attempted when one parental combination fails.

e.g. Mung x udid- cross compatible and Udid x mung-cross incompatible

3. MANIPULATION OF PLOIDY

Diploidization of solitary genomes to make them paired will be helpful to make the cross fertile.

4. BRIDGE CROSSES

When two parents are incompatible, a third parent that is compatible with both the parents can be used for bridge crosses and thus it becomes possible to perform cross between the original parents.

e.g. Tobacco

-*Nicotiana repanda* x *N.tabaccum*– cross incompatible

-*Nicotiana repanda* x *N.sylvestris*- cross compatible

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

5. USE OF POLLEN MIXTURE

Unfavorable interaction between pollen and pistil in the case of wide crosses can be overcome to some extent by using pollen mixture.

6. MANIPULATION OF PISTIL

Decapitation of the style will sometimes prove helpful in overcoming incompatibility.

7. USE OF GROWTH REGULATORS

Pollen tube growth can be accelerated by using growth hormones like IAA, NAA, 2,4-D and Gibberellic acid.

8. PROTOPLAST FUSION

When fusion of gametes fails, protoplast fusion of somatic cells can be attempted.

9. EMBRYO RESCUE

Hybrid zygotes formed by wide crosses may fail to grow in a number of cases. The zygotes are taken out and grown in *in vitro* medium to overcome this problem.

ROLE OF WIDE CROSSES IN CROP IMPROVEMENT

Wide crosses are generally used to improve crop varieties for disease resistance, pest resistance, stress resistance, quality, adaptation, yield etc. These crosses can even be used to develop new crop species. Techniques like alien addition and alien substitution may also be effective.

IMPROVING THE CROP PLANTS FOR

a). Disease and insect resistance

Crop	Character transferred	Species transferred from	Species transferred to
Cotton	Jassid resistance Blackarm resistance	<i>G.Tomentosum</i> <i>G.arboreum</i>	<i>G.Hirsutum</i> <i>G.barbadense</i>
Okra	Resistance to YMV	<i>Abelmoschus</i> <i>manihot</i>	<i>A. esculenta</i>
Groundnut	Resistant to leaf chewing insect	<i>Arachis monticola</i>	<i>A.hypogea</i>
Wheat	Rust resistance	<i>Agropyron</i>	<i>T. aestivum</i>
Tobacco	Resistant to mosaic virus	<i>N.repanda</i>	<i>N. tabaccum</i>

b). Improvement in quality

Crop	Character transferred	Species transferred from	Species transferred to
Cotton	Fibre length Male sterility	<i>G. Thurberi</i> & <i>G. Raimondii</i> <i>G. harkenssii</i>	<i>G. hirsutum</i> <i>G. hirsutum</i>
Potato	Starch content Frost resistance	Wild species <i>Solanum acaule</i>	<i>Cultivated Spp.</i> <i>S. tuberosum</i>
Tomato	Carotenoid content	<i>Lycopersicon</i> Wild Spp.	<i>L. esculentum</i>
Palm	Oil quality	Wild Spp.	Cultivated Spp.
Rice, Oat & Rye	Protein quality	Wild Spp.	Cultivated Spp.

C). Improvement in yield: This also been achieved through the use of wild Spp. in some crops e.g. Oat, Vigna, Arachis, Potato, Tobacco.

ALIEN ADDITION LINES

- ✓ These lines carries one chromosome pair from a different species in addition to the normal somatic chromosome complement of the parent species.
- ✓ When only one chromosome from another species is present, it is known as alien addition monosome.
- ✓ Alien addition have also been done in rice, sugar beet, cotton, brassicas.
- ✓ The main purpose of alien addition is the transfer of disease resistance from related wild species. e.g. Transfer of mosaic resistance from *Nicotiana glutinosa* to *N. Tabacum*.
- ✓ The alien addition lines have been developed in case of wheat, oats, tobacco and several other species.
- ✓ Alien addition lines are of little agricultural importance since the alien chromosome generally carries many undesirable genes. e.g. Reduced growth and short, broad leaves in addition to mosaic resistance.

ALIEN SUBSTITUTION LINES

- ✓ This line has one chromosome pair from a different species in place of the chromosome pair of the recipient species.
- ✓ When a single chromosome (not a pair) from different species in place of a single chromosome of the recipient species, known as alien-substitution monosome.
- ✓ Alien –substitution line have been developed in wheat, cotton, tobacco, oats, etc.
- ✓ In case of tobacco, mosaic resistance gene N was transferred from the *N. Glutinosa* to *N. tabacum* line had 23 pairs of *N. tabacum* chromosomes and one pair (chromosome H)of *N. glutinosa* chromosomes.
- ✓ The alien substitution show more undesirable effects than alien additions and as a consequence are of no direct use in agriculture.

Limitations of Distant hybridization

1. Incompatible crosses
2. F1 sterility
3. Problems in creating new species
4. Lack of homoeology between chromosome of the parental species
5. Undesirable linkages
6. Problems in the transfer of recessive oligogenes and quantitative traits
7. Lack of flowering in F1
8. Problems in using improved varieties in distant hybridization
9. Dormancy

Achievements

- Hybrid varieties:
 - Upland cotton – **MCU-2, MCU-5, Khandwa1, Khandwa2** etc are derivatives of interspecific hybridization.
 - Hybrid between Pearl millet x Napier grass- Hybrid Napier which is very popular for its high fodder yield and fodder quality e.g. **Jaywant and Yashwant**
 - Interspecific hybrids in cotton- **Varlaxmi, Savitri, DCH-32, NHB-12, DH-7, DH-9** etc.
 - **Prabhani Kranti** variety of bhindi.



Thank you..