

Session-19

Fertilization barriers in crop plants at pre-and post -fertilization levels; In vitro techniques to overcome the fertilization barriers in crops; chromosome manipulations in wide hybridization; case studies-Production and use of haploids, dihaploids and doubled haploids in genetics and breeding.

Introduction: When individuals from two different species of the same genera are crossed, it is known as inter specific hybridization. Such hybrids vary from completely sterile to completely fertile and hence used in crop improvement. Eg. Inter specific hybridization :Eg. *Oryza sativa* x *O. perennis*. When individuals being crossed belong to two different genera, it is referred to as inter generic hybridization. Intergeneric hybrids are always sterile and cannot be commonly used in crop improvement. Eg. Triticale (Wheat x rye).

Hybridization between individuals from different species belonging to the same genus or two different genera, is termed as distant hybridization or wide hybridization, and such crosses are known as distant crosses or wide crosses.

History

- 1.The first distant hybridization; hybrid between carnation (*Dianthus caryophyllus*) and sweet william (*Dianthus barbatus*) by Thomas Fairchild in 1717 and the hybrid is called as fairchild's mule.
2. Most of the interspecific hybrids were of no agricultural value many interspecific hybrids particularly in case ornamentals, served as commercial varieties.
3. An interesting inter generic hybrid *Raphano brassica* was an amphidiploid cross between radish and cabbage but it was useless.
4. The first inter generic hybrid with a great potential was TRITICALE

Objectives:

1. To transfer some desirable character from wild relatives that are not available in cultivated varieties. Eg. Many disease resistance and, insect resistance genes, Wide adaptability : (i.e. drought-resistance, cold tolerance etc.), Quality improvement (Eg. Cotton (fibre) Tobacco (leaf), Yield improvement (Eg. Oats, Tobacco, Maize, S. cane) Other characters (Eg. CMS, Earliness, dwarfness morphological characters)
2. Exploitation of luxuriance (heterosis) in vegetatively propagated / ornamental crops. Prolonged vegetative period, Prolonged blooming period.
3. Creation of Novel genotypes: New species or F1 hybrids hitherto non – existent in nature.

BARRIERS TO THE PRODUCTION OF DISTANT HYBRIDS

1. Failure of zygote formation / Cross incompatibility
2. Failure of zygote development / Hybrid inviability
3. Failure of F1 seedling development / Hybrid sterility
4. Hybrid Breakdown: F1 is fertile and vigorous but F2 is weak and sterile.

A variety of mechanisms may be responsible for each of these three difficulties :

1. Failure of zygote formation / cross incompatibility: Inability of the functional pollens of one species or genera to effect fertilization of the female gametes of another species or genera is referred to as cross incompatibility. It may be due to – 1. failure of fertilization, because the pollen may not germinate. 2. Pollen tube is unable to reach to embryo sac and hence sperms are not available for fertilization. 3. Pollen tube may burst in the style of another species Eg. *Datura*. 4. The style of the female parent may be longer than the usual length of the pollen tube growth therefore the pollen does not reach the embryo sac. Eg. *Zea mays* and *Tripsacum* sp. 5. Pollen tubes of polyploidy species are usually thicker than those of diploid species. 6. When a diploid is used as female and a polyploidy as male, the polyploidy pollen tube grows at a slower rate in the diploid style than it would be in a polyploid style.

These barriers are known as pre-fertilization barriers.

2. Failure of zygote Development / Hybrid inviability: The inability of a hybrid zygote to grow into a normal embryo under the usual conditions of development is referred to as hybrid inviability. This may be due to: Lethal genes: some species carry a lethal gene, which causes death of the interspecific hybrid zygote during early embryonic development. Eg. 1. *Aegilops umbellulata* carries a lethal gene with 3 alleles against diploid wheats.

2. unfavourable interaction between the two parental genomes.

3. Chromosome elimination: In some cases of distant hybridization, chromosomes are gradually eliminated from the zygote. In a cross between *Hordeum bulbosum* x *H. vulgare* Chromosomes from one species (*H. bulbosum*) are successively eliminated from zygote due to mitotic irregularities. This bulbosum technique results in generation of haploids.

4. Incompatible cytoplasm: Embryo development may be blocked by an incompatibility between cytoplasm of the species used as female and the nuclear genes of the species used as male. Such an interaction, more generally, leads to hybrid weakness and male sterility in the hybrids or may some times leads to failure of embryo developments.

5. Endosperm Abortion: Seeds from a large number of distant crosses are not fully developed and are Shrunken due to poorly developed endosperm. Such seeds show poor germination, and may often fail to germinate. When the endosperm development is poor or is blocked, the condition is generally known as endosperm abortion.

Eg. 1. *Triticum x secale* – *Triticale*. In this case the endosperm aborts at a much later stage so that a small frequency of viable seed is obtained. 2. *Hordium bulbosum x H. vulgare* – the endosperm aborts at an early stage so that viable seeds are not produced. In case of endosperm abortion - embryo rescue culture is practiced.

3. **Failure of Hybrid seedling development / Hybrid sterility:** Hybrid sterility refers to the inability of a hybrid to produce viable off spring. Some distant hybrids die during seedling development or even after initiation of flowering. The mechanisms involved in the failure of seedling development most likely involve complementary lethal genes. Eg. 1. In cotton-certain interspecific hybrids appear normal, but die in various stages of seedling growth; some plants die at flowering. The main cause of hybrid sterility is lack of structural homology between the chromosomes of two species. Sometimes, sterility is due to small structural changes that cannot be detected during meiosis. Stebbins termed it as cryptic structural hybridity.

In vitro techniques to overcome the fertilization barriers in crops

Wild species are an important reservoir of useful genes for resistance to major diseases and pests, tolerance to abiotic stresses and cytoplasmic male sterility. However, several problems occur when transferring useful genes from wild species to cultivated rice. The barrier most commonly encountered is lack of crossability resulting from chromosomal and genic differences.

1. **Protoplast fusion:** The wide crosses can be obtained through protoplast fusion, when it is not possible to produce such crosses through sexual fusion.
2. **Embryo rescue:** This technique is being used widely to obtain viable interspecific or intergeneric hybrids. This is used when hybrid embryo is unable to develop due to endosperm degeneration. *H. Vulgare x Secale cereale*. In this technique proembryo is dissected 3-5 days after pollination, cultured on solid agar medium to get plantlets. The drawbacks are high cost of obtaining new plantlets, induction of new mutations in the invitro phase, requirement of sophisticated equipment and green house facilities, requirement of specialized skills in in vitro operation.

CHROMOSOME MANIPULATION IN DISTANT HYBRIDIZATION

- **Chromosome manipulation** - The term chromosome manipulation or chromosome engineering describes process or technologies in which chromosomes are manipulated to change their mode of genetic inheritance.
- **Distant hybridization** - a cross of two individuals belonging to different species (interspecific hybridization) or genera , it is called as intergeneric hybridization

TYPES OF CHROMOSOMES MANIPULATION

- ❑ Incorporation of a single or fragments of chromosome from a wild into the existing crops to enhance the genetic diversity
- ❑ Incorporation of an alien chromosome by chromosomes doubling in order to produce amphidiploids
- ❑ Elimination of an alien chromosome in order to induce haploids.

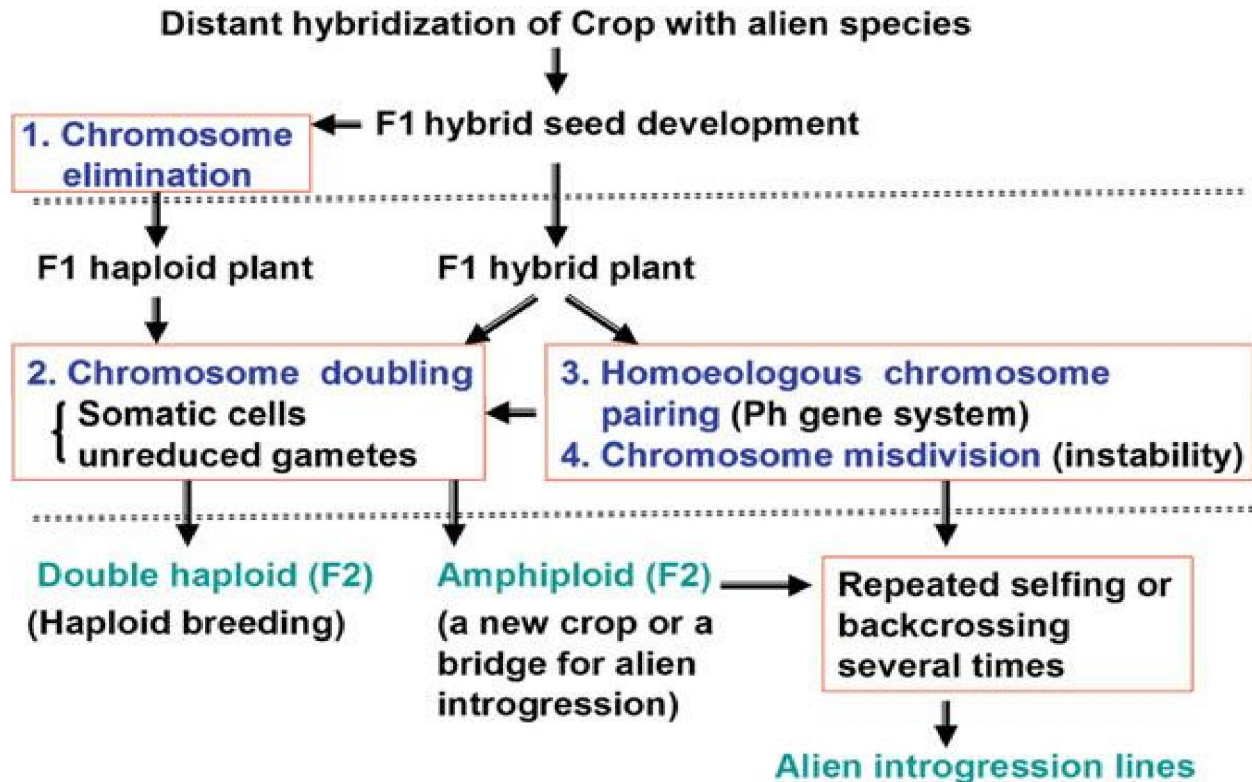


Fig. 1. Chromosome manipulation based on chromosome behaviors in F 1 hybrids

Chromosomes elimination and haploid crops: Chromosomes elimination mediated techniques are the method of choice for haploid production, for gene mapping and other breeding studies in cereals. Chromosome elimination is the degeration of one parental chromosome in F1 hybrid due to inactivation of kinetochore function. After chromosome doubling by colchicines treatment results in production of doubled haploids. For example, the generated F1 plants from the cross between wheat x job`s tears carries 21 chromosomes indicating the complete elimination of chromosomes of job`s tears. Colchicine treated F1 plants showed to carry 42 chromosomes (doubled haploid) i.e. the normal chromosomes of common wheat. Here Job`s tears can be used as a suitable pollen donor plant for haploid wheat production. In barley, haploid production termed bublbosum technique is the result of wide hybridization between cultivated barley (*Hordeum vulgare*, $2n=2x=14$) as the female and wild *H. bulbosum* ($2n=2x=14$) as the male.

After fertilization, a hybrid embryo containing the chromosomes of both parents is produced. During early embryogenesis, chromosomes of the wild relative (*H. bulbosum*) are preferentially eliminated from the cells of developing embryo, leading to the formation of a haploid embryo, which is due to the failure of endosperm development. A haploid embryo is later extracted and grown in vitro. The 'bulbosum' method was the first haploid induction method to produce large numbers of haploids across most genotypes and quickly entered into breeding programs. Pollination with maize pollen could also be used for the production of haploid barley plants, but at lower frequencies. Paternal chromosome elimination has also been observed after interspecific crosses between wheat (*Triticum aestivum*) and maize. After pollination, a hybrid embryo between wheat and maize develops but, in the further process, the maize chromosomes are eliminated so that haploid wheat plantlets can be obtained. Such haploid wheat embryos usually cannot develop further when left on the plant, because the endosperm fails to develop in such seeds. By applying growth regulator 2,4-dichlorophenoxyacetic acid in planta, embryo growth is maintained to the stage suitable for embryo isolation and further in vitro culture. The maize chromosome elimination system in wheat enables the production of large Haploids and Doubled Haploids in Plant Breeding. This 'bulbosum' method developed by Kasha and Kao in 1970 was first haploid induction method to produce large numbers of haploids across most genotypes and quickly adapted into breeding programs. The tetraploid female *S. tuberosum* (*Solanum tuberosum* L. ssp. *tuberosum*, $2n=4x$) produces an embryo sac containing one egg cell and two endosperm nuclei, all with the genetic constitution $n=2x$. After pollination with *S. phurea* ($2n=2x$), dihaploid ($2n=2x$) embryos can develop from unfertilized egg cells. The frequency of dihaploid seeds is low. Dihadloid potatoes can be used for breeding purposes, including alien germplasm introgression or selection at the diploid level, but such plants are not homozygous.

- **Chromosomes doubling and amphidiploids:** Chromosome doubling can be carried out through the treatment with antimicrotubule drugs, Colchicine (originally extracted from autumn crocus (*Colchicum autumnale*)).
- **Homeologous chromosomes pairing:** Bread wheat (*Triticum aestivum*) contains three closely related genomes: A, B and D. Chromosome pairing during meiosis in this allohexaploid is confined to strict homologues, despite the co-existence in the genome of homeologous chromosomes. Diploid-like behaviour results in 21 bivalents at meiotic metaphase I. In polyploid wheat, the diploid-like chromosome pairing is principally controlled by the pairing homeologous1 (*Ph1*) gene on the long arm of chromosome 5B via preventing homeologous chromosome pairing (HECP). The manipulation of this gene can be used to induce recombination between chromosomes that will not normally pair at meiosis. This has application in the introduction of new genetic information into wheat. That is, improving the efficiency of alien introgression development.

Advantages of chromosomes manipulation

- Increase genetic diversity

- Produce variability and varieties
- Production of gene introgression lines
- Overcoming barriers of distant hybridization

Limitation

- Limited knowledge on process of gene expression and intermediary metabolism
- Complex nature of DNA