

## Session-17

Transfer of individual whole chromosome -alien addition lines-alien substitution lines;  
Apomixis:

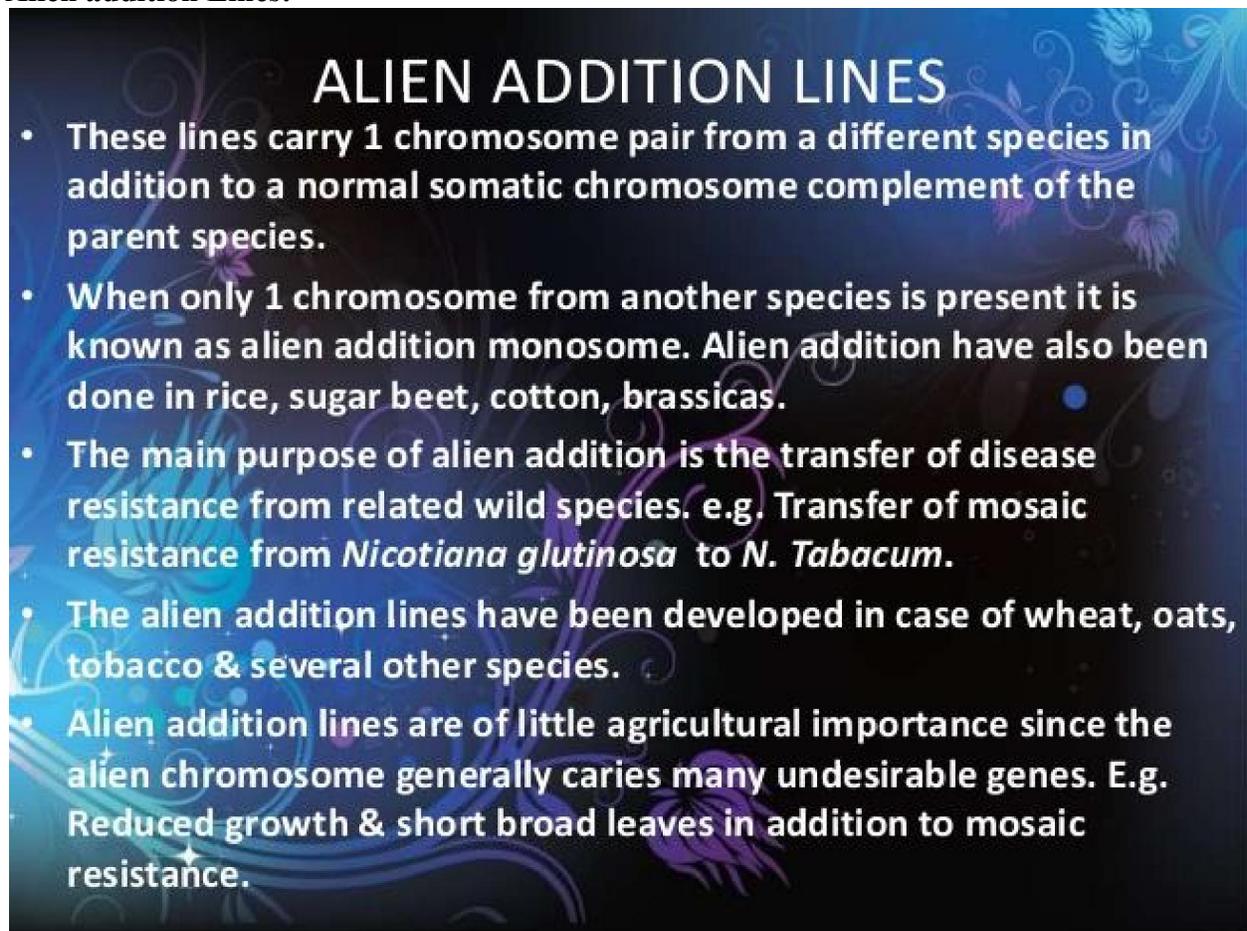
**1. Alien addition lines:** Carries one chromosome pair from a different species in addition to somatic chromosome complement. For Eg. Disease resistance in Wheat, oats, tobacco

**2. Alien substitution lines:** replacement of one chromosome pair of cultivated species with the chromosome pair of wild donor species.

**3. Introgression of genes:** Transfer of small chromosome segments with desirable genes from wild species into cultivated species. Introgression of genes from one species into another species by back cross method is known as introgressive hybridization.

Eg. Disease resistance, improving quality and Wide adaptation.

### **Alien addition Lines:**



## ALIEN ADDITION LINES

- These lines carry 1 chromosome pair from a different species in addition to a normal somatic chromosome complement of the parent species.
- When only 1 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 & several other species.
- Alien addition lines are of little agricultural importance since the alien chromosome generally carries many undesirable genes. E.g. Reduced growth & short broad leaves in addition to mosaic resistance.

Interspecies hybridization and backcrossing is a means to transfer desirable genes from one species to another in breeding programs of higher plants. Monosomic alien addition lines

(MAALs) can be produced via addition of single chromosome of an alien donor species to the entire chromosome complement of the recipient species. Monosomic alien addition lines (MAALs) are valuable for elucidating the genome structure and transferring the useful genes and traits in plant breeding. Backcrossing of MAALs to the recipient parent results in plants containing short overlapping introgressions, which cover the entire donor genome. These introgression lines can be used as vectors of alien genomic libraries in a recipient genetic background. In addition, a complete set of MAALs also serves as a library of the donor genome dissected into individual chromosome entities, which facilitates high-throughput marker allocation to individual donor chromosomes, and marker assignments and syntenic relationships can be compared between the donor chromosomes and the respective orthologous recipient chromosomes. Meanwhile, MAALs can be used to study the introgression mechanism and the pairing status of homologous chromosomes.

**Alien substitution lines:** Alien Substitution takes place when chromosome(s) of another species replace the own chromosome(s) of a species. Alien substitutions may be obtained from alien addition lines. However, monosomic lines are used most commonly. Monosomic lines can be maintained without too many difficulties in polyploids, because the genomes are better balanced. Monosomic plants produce some eggs that are nullisomic. These recipients are then crossed with a donor species. In the  $F_1$  the chromosome absent in the parent nullisomic will appear as a monosome of the donor. These monosomic individuals are repeatedly backcrossed (6–8 times) with the recipient, until in some individuals all the chromosomes of the donor are eliminated, except that particular monosome. Selfing this monosomic substitution line results in a disomic substitution that has the same chromosome number as the euploid line from which the nullisomic arose, but one pair of the chromosomes will represent the donor. Alien substitution lines have been developed in wheat, cotton tobacco, oats, etc • In case of tobacco, mosaic resistant gene N was transferred from *N. glutinosa* to *N. tabacum* line had 24 pairs of *N. tabacum* chromosomes & 1 pair of *N. glutinosa* chromosomes. • The alien substitution show more undesirable effects than alien addition & as a consequence are of no direct use in agriculture.

### **Chromosome Painting:**

The term chromosome painting was first used by Pinkel et al. in 1988 that describes direct visualization, using in situ hybridization, of specific chromosomes in metaphase spreads and in interphase nuclei. Chromosome painting, coupled with fluorescence in situ hybridisation (FISH), is now used routinely to enhance the identification of chromosomal rearrangements such as chromosomal translocations, the assignment of breakpoints/deletions, and the determination of the origin of extra chromosomal material. In short chromosome painting helps in the identification of both numerical and structural **chromosomal** aberrations in human pathology with high sensitivity and specificity. As a result, chromosome painting has rapidly become an established routine procedure in cytogenetic investigations.

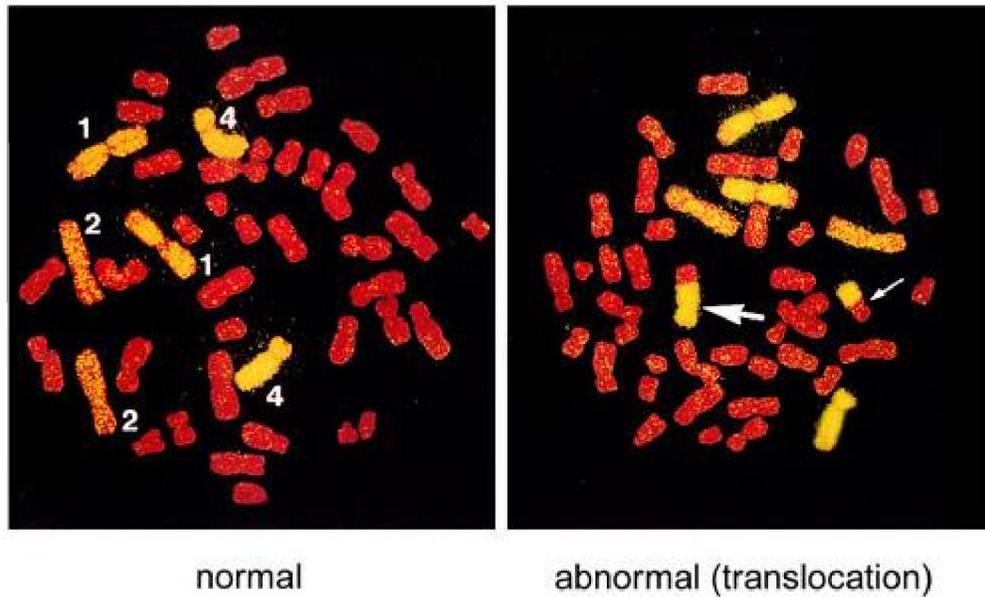


Figure. An example of FISH-treated metaphase chromosomes. Here, chromosomes 1, 2, and 4 were labeled yellow with FISH and the other chromosomes were stained red. Translocations between yellow and red chromosomes are detected. The left picture represents a normal cell (the numbers in the figure indicate chromosome numbers) and the right picture is an example of reciprocal translocation with two bi-color chromosomes (indicated by two arrows).

In this way, if a part of a painted chromosome (yellow, for example) had undergone an exchange with another, non-painted chromosomes (stained red), it is possible to detect the aberration (termed a reciprocal translocation) because the aberrant chromosome contains both yellow and red segments. Usually, a pair of bi-colored chromosomes can be detected in one metaphase because two chromosomes typically exchange a part of their DNA.

#### **Procedure of chromosome painting:**

1. The usual starting material for the production of a chromosome paint is to isolate each human chromosome.
2. DNA from these chromosomes was amplified either by polymerase chain reaction (PCR) or enzymatic digestion and molecular cloning into a bacterial vector.
3. These amplified DNA fragments are labeled with appropriate fluorescent (light-emitting) dyes such as fluorochrome (fluorescein isothiocyanate (FITC-dUTP) or biotin-dUTP and allowed to hybridize (attach) to metaphase chromosomes or interphase nuclei. The fluorescent-labeled DNAs will attach to the analogous chromosomes from which they were derived. (DNA fragments with the same base sequences have the characteristic of attaching to each other.)
4. **Confocal laser scanning microscopy:** Visualisation of the labelled DNA with a rabbit anti-FITC antibody followed by a goat anti-rabbit FITC conjugated antibody or enzyme conjugated affinity reagent systems such as streptavidin-HRP (horse radish peroxidase)

results in the painted chromosome being strikingly identified in metaphase spreads and interphase nuclei.

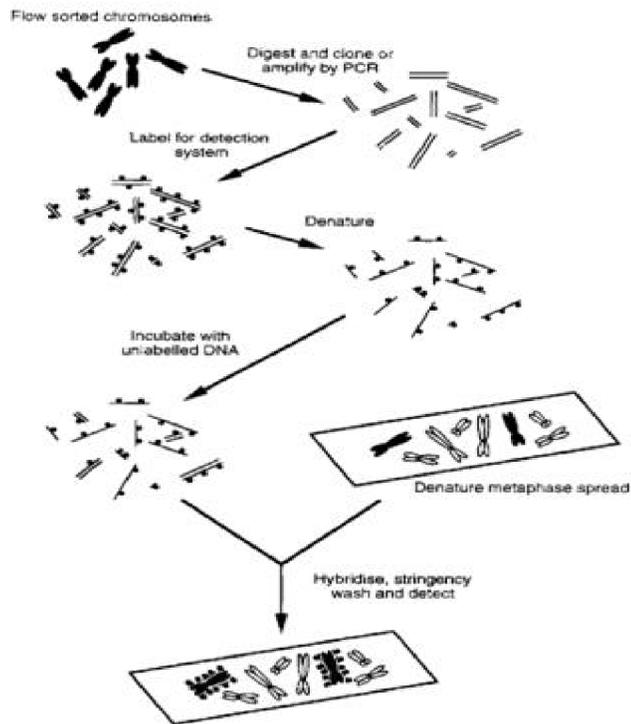


FIG. 1. General principles of chromosome painting.