PLANT KARYOLOGICAL TECHNIQUES

karyotype

- The number and appearance of chromosomes in the nucleus of a eukaryotic cell.

- complete set of chromosomes in a species or in an individual organism and for a test that detects this complement or measures the number.

Pretreatment

- well-spread,
- clear,
- condensed chromosomes

Pretreatment

- Increases the number of metaphase cells
- Constricts the chromosomes longitudinally
- Clarifies the constrictions
- Increases the viscosity of the cytoplasm
- Allows easy penetration of fixative

Microtubule-inhibiting pretreatment substances

- colchicine,
- 8-hydroxyquinoline,
- p-dichlorobenzene,
- ice cold water,
- α-bromonaphthalene

FIXATION

- Fixed at the point where it was at the time of fixation.
- Precipitate the chromatin are used in chromosomal studies
- Usually used in squash and smear preparations

COMMONLY USED FIXATIVES

- Mercuric Chloride ($HgCl_2$)
- Potassium Dichromate ($K_2Cr_2O_7$)
- Chromic Acid (H_2CrO_4) or Chromium Trioxide (CrO_3)
- Osmium Tetroxide (OsO_4)
- Ethanol (C_2H_5OH)
- Acetic Acid (CH_3COOH)
- Propionic Acid (C_2H_5COOH)
- Chloroform ($CHCl_3$)

Fixing Mixtures

- Flemming’s Weak Fluid
- Flemming’s Strong Fluid
- Carnoy’s Fluid
- Navaschin’s Fluid

Staining

1) Carmine
2) Orcein
3) Crystal Violet
4) Hematoxylin
5) Fuchsine (Fuschin)

The Feulgen Reaction

- Schiff’s Reagent/Fuchsine Reagent

Squash and Smear Preparation

- Smears are formed when fresh tissue is spread over a slide and no treatment is necessary.
- Treatment with acids is necessary for the dissolution of the middle lamella which will leave the tissue softer. This tissue is then pressed under a cover slip till the desired spread is reached.

Permanent Slides

Fluorescence *in situ* hybridisation (FISH)

1) *Tissue collection:*
2) *Fixation:*
3) *Enzyme digestion*
4) *Protoplast isolation*
5) *Hypotonic treatment of protoplasts*
6) *Cleaning and fixation of the protoplasts*
7) *Protoplast dropping:*
8) *Treatment of chromosomes for in situ hybridization*