Topic – Cytological Staining Techniques

Course- M.Sc. 4th Semester Botany

Paper- MBOTEC-2 (Cytogenetics and Crop Improvement)

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Cytological staining techniques

Chromosomes are physical basis of heredity. In eukaryotes, they are localized inside the nucleus. Here we are concentrating on viewing of chromosomes in higher plants.

Preparation of mitotic slides

In ***Vicia faba*** -

Vicia faba is a member of family Fabaceae

To prepare the root tips samples for staining, they will need to be pretreated in paradichloro benzene. Then the root tips will need to be fixed in 1:3 aceto alcohol.

Pretreatment of roots tips

Purposes

1. To arrest dividing cells at mitotic metaphase
2. Preventing the formation of microtubule
3. Chromosomes are more contracted and shorter making chromosome counts easier

Steps of pretreatment-

Cut roots when they are 1.5 to 2cm long at 10:30 am.

↓

Remove root cap by gentle brushing

↓

Place the root tip in glass vials that contain saturated solution of pardichlorobenzene

↓

Place the glass vial in refrigerator for 1.30 - $3\frac{1}{2}$ hour

↓

Get the glass vial containing root tip

↓

Wash the root in fresh water

↓

Lightly dry the root on blotting Paper

Fixation of root-

Purpose-

1. Immediate death of tissues
2. It also causes coagulation and precipitation of proteins to change the refractive index of the chromosome
3. It prevents bacterial growth and decomposition of root tissue.
4. It will cause the chromatin to precipitate and make the chromosome visible.

5. It helps with the adherence of acidic stain on the chromosome.

Steps of fixation-

Pretreated roots
↓
Fixed in 1:3 (Aceto alcohol)
↓
Add 1 or 2 drops of ferric chloride as mordant (for intensifying the stain)

Staining –

Place the fixed root in watch glass
↓
Add 2% acetocarmine
↓
Heat on spirit lamp discontinuously for 15 minutes
↓
Now roots will be black

Take 1 stained root on a slide
↓
Detach 2 mm root tip from root with the help of blade
↓
Add 2 drops of acetocarmine stain on top of root tip
↓
Put cover slip on soaked root tip
↓
Again heat the soaked root tip for few seconds
↓
Squash the root tip on each slide pressing straight down
↓
Observe and record mitotic metaphase
↓
count chromosome number and determine chromosome length

Acetocarmine staining

Carmine is a basic dye that is prepared from the insect *Coccus cacti*. Dissolve 2gm carmine in l00 cc of 45% glacial acetic acid. Boil the solution and cool it. Filter into dark bottles and store at 4^0C. This solution can be stored for a long time.

Staining can be intensified by adding ferric chloride ($FeC1_26H_20$)

Meiotic study-

Flower buds are suitable material for meiotic study.

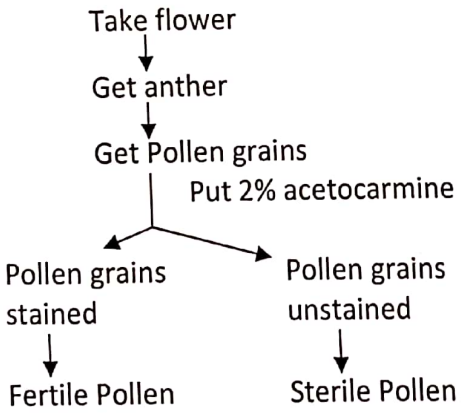
Pre-treatment is not required for flower bud.

Select suitable size of flower buds of a species (Say *Phlox drumondii*) and fix them directly in fixative (1:3 acetoalcohol) at 10 am to 10. 30am . Add 1 or 2 drops of ferric chloride as mordant. Store the fixed material in refrigerator.

At the time of meiotic study, take the fixed flower bud. Tease it and excise the anther. Heat the anther in 2% acetocarmine. Squash it on a slide after putting 1 or 2 drops of acetocarmine and materials covered by cover slip.

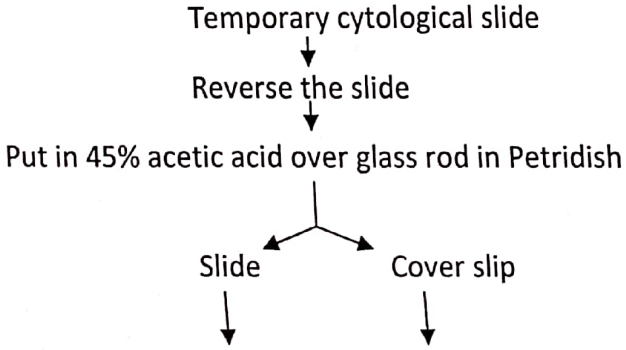
Study the meiotic stages. Focus on meiotic metaphase-I for calculating chiasma frequency.

Pollen grains fertility test –



Calculate Pollen grains fertility and sterility

For making permanent cytological slide



Place 1:1 (acetic acid and n-butanol)

↓

Again place them in n-butanol

↓

Mount in euparol.

Now you have 2 slides from single cytological slide