8.10.

PRETREATMENT AND PRETREATING AGENTS :

PRINCIPAL :

The pretreating technique with some particular chemicals is applied to the materials in the study of chromosome morphology for the following reasons —

i) It increases the viscosity of plasma in turn arranged spindle mechanism.

ii) Cleaning the cytoplasm.

iii) Separation of middle lamella causing softening of the tissue.

iv) Bringing about scattering of the constriction region.

Pretreatment may also be needed to achive rapid penetration of fixative by removing undissolvable deposits on the tissues as well as for the study of spiral nature of chromosome. Pretreatment changes cytoplasmic viscosity and brings about destruction of spindle mechanism. It causes differential hydration and dehydration of chromosome with chromosomal constriction region will clarified. As it arranged metaphase stage at a high frequency so it is helpful for chromosome study.

Remarks : ..... Date : ..... Teacher's Signature .....

AGENTS :

A number of chemicals are used in the purpose of pretreatment. These chemicals are not universally applicable to all plant materials. A particular group of plants give better results in a particular chemical while others do not. The time of treatment and temperature are not fixed in all cases. Usually it is done at low tempera-ture because it ensures a slow and steady operation of the process of condensation and hydration of chromo-some where as they become shortened and straightened. Some common treatment chemicals are as follows —

Chemicals	Effective concen-tration in aqueous solution	Period of treatment	Temperature
1. Aesculine.	1. Saturated or half saturated.	1. 5 mins to 24 hours.	1. 4°C to 16°C.
2. Para di chloro benzene (PDB).	2. Saturated.	2. 3 1/2 hours.	2. 12°C to 16°C.
3. Oxyquinoline.	3. 0.0024 M.	3. 3 to 4 hours.	3. 12°C to 16°C.

Remarks : ..... Date : ..... Teacher's Signature .....

(3)

PARA DICHLORO BENZENE (1,4,DCB)

Very effective, particularly in plant with high number of chromosome. It has a much wide application in both long and short chromosome.

The drawback of this chemical in majority of cases that the period of treatment necessary atleast for three hours.

PAGE NO. 8.
DATE

(4)

FIXATIVES AND THEIR USES :

Fixation is a process by which tissues or their components are selectively fixed to a desire extent. The purpose of fixation is to kill the tissue without causing distortion of the components. It is a critical stage on chromosome study. It not only increase the visibility of chromosome structure but should also clarify the details of chromosome morphology. A fixative should have the following properties —

i) Coagulation of protein components and consequent participation causing a marked change in the refractive index of the chromosome helping them to appear as different bodies within the cell.
ii) It arrested the cell division.
iii) Check denaturation of protein.
iv) Precipitates chromatin matter.
v) Check bacterial autolysis i.e act of preservation.
vi) It increases the basophilia of chromosome, thus helps in staining.

Fixation may be done by chemicals of two types —

i) Metallic and
ii) Non metallic.

R.N. 256412 GOLDEN EAGLE Lab.

Remarks : ..... Date : ..... Teacher's Signature .....

Non metallic fixative has an advantage over metallic fixative in that sense no washing is require after fixation. Fixation may also be done at low temperature followed by drying of tissues.

ACETIC ALCOHOL :

A mixture of glacial acetic acid and absolute alcohol in different proportion (1:1, 1:2, 1:3) used in most cases.

PREPARATION OF STAIN :

The structure and behaviour of chromosome only after they are made visible under microscope in fixed material, stained with certain dyes. Stain may be defined as a chemical substance which may be coloured or colourless and capacity of combining specific cellular component by chemical combinations and that combina-tions must be rigid not easily washed out.

ACETO-ORCEIN :

Orcein is a deep purple dye obtained from the action of hydrogen peroxyde and ammonia on the colourless parent substances "Orceinol". 2g of orcein powder is gradually added to 100 c.c of 45% acetic acid boiling in a conical flask. It was steared all time. 2g of orcein powder produces 2% aceto-orcein solution.

ACETO-CARMINE :

Carmine is valuable chromosome stain. It is a basic dye whose active principal is carminic acid.

1000 c.c of 45% acetic acid in a conical flask, 2g of carmine powder was added. The solution shaken thoroughly. Then the solution was filtered in slightly warm state.

Remarks : ..... Date : ..... Teacher's Signature .....

(7)

SOURCE MATERIAL FOR CHROMOSOME STUDY:

The most convenient stage for studying chromosome morphology is during divisional cycle both mitosis and meiosis. Study of chromosome during meiotic divisional cycles in higher plants. The root tips, shoot tips and leaf tips are required. To count the number of chromosome metaphase stage is suitable.

Meiotic stage in higher plants may be studied from the pollen mother cells (P.M.C). The pollen grain is an excellent material for haploid meiotic complement analysis during the division into vegetative and reproductive nuclei.

SEPARATION AND CLEANING OF CELLS:

Separation is essential for plants to study meiotic stages. Due to compactness of tissue and the presence of middle lamella. In plants, heating or warming in dilute acid (HCl) serves the dual purpose of dissolving the middle lamella and cleaning of the cytoplasm.

Remarks : ..... Date : ..... Teacher's Signature .....

PREPARATION OF SLIDES:

PROCESSING:

After suitable fixation the tissue is proceeded for further study. Processing is done in two ways -

1. Smear
2. Squash. The first one is used during staining while the next one is done after staining.

SMEAR:

In smear the cells are directly spread over the slide. Prior to fixation in this process more treatment is necessary to secure cell suspension. Pollen mother cell from the anther ake more convinent object for smear.

SQUASH:

In squash speacial treatment is needed for the desolution of pectic salts of the materials causing the separation of cells. After passing through steps the soften bulb material or small tissues can be neatly squash on the slide by generally applying pressure or tapping with a needle over the cover glass. The best way to study mitotic chromosome is by squash at root tip.

These two processes have great advantage over sectioning in that the entire process is very rapid and much more suitable for critical observation.

Remarks : ..... Date : ..... Teacher's Signature .....

PREPARATION OF PERMANENT SLIDES:

The slides prepared by any of the methods previously described can not be kept for an indefinite period and require to be made permanent. The principle behind this process is dehydration of material followed by mounting in a medium which is sticky enough to stick the cover glass tightly to the slide and having a refracting index similar to the glass.

FOR SMEAR AND SQUASH:

The paraffin on the slide and the cover glass was carefully removed by a sharp plate taking precausion that the cover glass is not displaced during such operation. Then it was proceeded as follows —

A. The preparation was carefully inverted in a mixture of glacial acetic acid and ethyle alcohol (1:1) taken in cover petridish until the coven glass fell on the slide.

B. Both coven glass and slide were carefully run through absolute alcohol and n-butyle alcohol (1:1, 1:2, 1:3) keeping in each for 5 minutes.

C. Two changes were gaven to n-butyle alcohol as an interval of 10 minutes each.

Mounting was done in canada bulsome or upered or DPX or entellan for the slide a new coven glass and for the cover glass a new slide was gaven.

Remarks : ..... Date : ..... Teacher's Signature .....

Hydrolysis of root tips for mitotic study :

PROCEDURE :

i) Root tips were taken in a test tube along with 2-3 ml of HCl (N) kept in a waterbath in a temperature ranging from 58°c - 62°c for 10 minutes.

ii) After hydrolysis acid was poured down and root tips wash with water to remove the acid. After drying the root tips a few drops of stain was added and kept in dark at 10 - 12°c for one hour. The root tips then squash in 45% acetic acid and observed under microscope.