

28. Preparation of root tip squash

Onion (*Allium sp.*) is very useful for root tip preparation in the study of different stages in mitosis.



Preparation of onion root tip

1. Select a large onion bulb with undamaged disc-like stem.
2. Under a running tap, gently rub the stem to remove old, withered roots and adhering soil particles.
3. Place the bulb on a glass jar of suitable size filled with tap water so that the base is completely immersed in water.
4. Change the water in the jar twice daily.
5. When the adventitious roots have grown to 2-3 cm long, cut and fix the distal 1-2 cm of the roots in a vial containing freshly prepared fixative such as acetic alcohol.
6. Shake the contents of the vial. Replace the used fixative with equal volume of freshly prepared fixative. Allow it to stand at room temperature for 24-48 hours.
7. After 24-48 hours pour off the fixative, and add 70% alcohol to the vial and shake.
8. Replace with 70% alcohol, repeat twice.
9. The roots are now ready for making squash preparations.

Note

1. Onion bulbs may not sprout if the temperatures are too warm, e.g. during summer.
2. The adventitious root tips should be collected at about noon because the peak hours of mitotic activity for onion are from 1.00 p.m. to 2.00 p.m.

and 11.00 p.m. to midnight.

3. Properly fixed roots can be stored for 2-3 months at low temperature without any impairment of the quality of squashes made from them. If they are not to be used immediately, they can be stored in a refrigerator.

Procedures for making squash

1. Near one edge of a clean slide, place a root tip and excise the terminal 2-3 mm portion. Discard the proximal part of the root.
2. Place a drop of aceto-orcein stain in the middle of the slide and transfer the terminal portion of the root to it with forceps.
3. Use a spear needle and a scalpel to tease the root tip into a number of small pieces.
4. With the aid of a 'masher', crush the root tip tissue into a thorough pulp so that no large pieces are observable on the slide. If necessary, add another drop of stain but avoid excess.
5. Drain off all the stain which sticks to the masher on the slide, to minimise the loss of cellular material from the slide.
6. Place a cover slip over the suspension of cells. Gently tap the cover slip with the rubber end of a pencil or the wooden end of a dissecting needle to spread the material. Care must be taken not to move the cover slip.

CAUTION Avoid too much pressure as this will break the cover slip.

CAUTION The cover slip, while flattening the cells, must retain its original position on the slide and should not be displaced, even slightly. If this happens, the preparation will be marred.
7. Place the slide over a steam bath or warm it over a hot plate until the stain at the cover slip edges begins to show signs of drying off.

8. Remove the slide from the steam bath and further flatten the cells by applying firm pressure from above while the slide is being held between the folds of a mat made from sheets of tissue paper. This will also expel the excess stain from under the cover slip, as well as spreading the chromosome content of dividing cells in an even layer.
9. This slide may be used for observation for about 30 minutes without sealing. Its usefulness can be extended to 2-3 days if the slide is sealed off by nail varnish. The initial stain may intensify upon storage.
10. When examining the preparation, correct lighting is important and green light is most beneficial. Very good squash preparations can be examined with an oil-immersion lens.

Note

1. The above method does not require a long period of fixation. Alternatively, the plant material can be fixed for 24 hours in Carnoy's fixative. The fixed material is then stained by warming it in aceto-lacmoid + HCl stain for five minutes. HCl is important in aiding the penetration of the stain by macerating the tissues. Lack of fixation sometimes results in rather bubbly chromosomes, which occasionally appear double. In such case, care is required in interpreting the preparations.

Preparation of Reagent

1. Acetic alcohol

Acetic alcohol is prepared by mixing 1 part glacial acetic acid and 3 parts absolute ethanol. It should be mixed just before using.

2. Aceto-orcein stain (1% solution in 45% acetic acid)

- Introduce 1 g of orcein into a flask.
- Add about 55 cm³ of boiling water into the flask to dissolve the stain.
- Add about 45 cm³ of glacial acetic acid into the flask and shake for thorough mixing.
- Filter and store in a glass stoppered dark bottle in a refrigerator.

3. Carnoy's fixative

Carnoy's fixative is prepared by mixing 1 part glacial acetic acid, 1 part chloroform and 1 part absolute ethanol.

4. Aceto-lacmoid Stain

Aceto-lacmoid stain is prepared by mixing 0.2 g lacmoid, 3.3 g orcein and 100 cm³ glacial acetic acid. Add two drops of 1M HCl to a watch-glassful of the stain before use.