INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

TENTO PROJEKT JE SPOLUFINANCOVÁN EVROPSKÝM SOCIÁLNÍM FONDEM
A STÁTNÍM ROZPOČTEM ČESKÉ REPUBLIKY

Topic 06: Structure of lily anthers and pistils
B. Preparation of paraffin sections

Detailed study of plant organs enables stained serial paraffin sections prepared by means of a microtome, which is equipment that facilitate sectioning of thin sections of selected sample. Microtomes could be constructed either as rotary or sliding microtomes. Thickness of prepared sections depends on the character of sectioned object, often vary between 6 to 15 μm.

Plant material: Segments of lily anthers and pistils isolated from flower buds embedded to paraffin blocks at the year 2011 (see protocol 06_lily_2015_A)

Microtome Reichert, microscopic slides covered with chrome-alum jelly, blades, brush, diamond pencil, warm plate.

1. **Chrome-alum jelly (Pappas 1971)**
   100 ml of distilled water
   0.5 g jelly
   0.05 g potassium chromium sulphate

1. Dissolve sulphate in small amount of water (this solution will be added to the dissolved jelly later)
2. Mix jelly with the rest amount of water to swell, warm and dissolve it on the water bath.
3. Mix dissolved jelly with the sulphate solution and filter through the Whatman1 filter paper.
4. Immerse **perfectly degreased** slides – thin layer of chrome-alum jelly will cover the slide surface.
5. Dry slides in vertical position and store in dust free place. Covered slides could be stored for some time (Refrigerator storage is better for longer periods).

Notes:
Prepared solution of chrome-alum jelly could be stored at 5°C for 48 hours.
Longer storage is not recommended.

2. Procedure of paraffin sectioning:

1. Divide paraffin block with embedded oriented segments of samples with a razor blade on the strips and subsequently to paraffin block with one object inside.
2. Melt the base of the prepared paraffin block with the warmed scalpel and firm it to the wooden block, which will be fastened to the Reichert slider microtome. Paraffin oil could be used for good sliding of the block with special cutting blade.
3. Paraffin block will be trimmed to the shape of truncated pyramid with edges parallel with the blade edge. Single sections will be then connected to the straight paraffin ribbon.
4. Orient microtome clamp to obtain cross sections (not oblique) of our selected object.
5. Decide the thickness of sections and appropriate inclination of the blade.
6. Repeat sliding with the microtome blade and prepare sections, which are forming straight paraffin ribbon.
7. Paraffin ribbon will be manipulated with the wet brushes (be careful to the microtome blade) and transferred to the black paper, where we will cut segments of this rib on. The size of this ribbon segments will depend on the size of cover slip. Please have in mind that ribbon will be longer after warming.
8. Ribbon segments will be transferred to the water surface on the microscopic slide covered with chrome-alum jelly.
9. Warm plate is used for ribbon straitening. Dry sections are firmly attached to the surface of the microscopic slide and could be manipulated with the slides through the next steps of the histologic procedures. Such prepared and attached sections could be stored on dry and dust-free place for longer period.

Literature:

1. Kiernan, J. (1981): Histological and histochemical methods: Theory and practise. 1st Ed. Oxford: Pergamon Press, 344 s.
2. Pappas, P. W. (1971): The use of chrome alum-gelatin (subbing) solution as a general adhesive for paraffin sections. Stain Technol., 46: 121-124.