EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
UNIVERSITAS MASARYKIANA BRUNENSIS
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ
TENTO PROJEKT JE SPOLUFINANCOVÁN EVROPSKÝM SOCIÁLNÍM FONDEM A STÁTNÍM ROZPOČTEM ČESKÉ REPUBLIKY

Topic 03: Mitosis – observation phases of mitosis and chromosomes
Cajal – Brožek double staining method of squash preparations

Mitotic division observation and karyologic study are mainly carried out on squash preparation of the root meristems. Different methods could be used for staining of these preparations, eg. acetocarmine, lactopropionic orceine, azureosinate (Giemsa staining) or basic fuchsine. Cajal-Brožek method is using basic fuchsine for the chromatin staining and pikroindigocarmine for the cytoplasm contra staining. Cyclamen red nuclear structures and blue-grey cytoplasm is the result of this staining method. Microtubules should be disorganized in case you plan observe single chromosomes. We prepare squash preparations of onion root meristems and compare control variant germinated in tap water with the structures after 5 hours of mitotic poison 8–hydroxichinolin.

Material: rooted bulbs of onion (*Allium cepa* L.)

0,2 M 8–hydroxichinoline, Carnoy fixation, maceration mixture conc. HCl: ethanol 1:1.

Samples: **1.** onion – bulbs rooted in tap water, roots immersed into 0,2M 8–hydroxichinoline for 5 hours
2. onion – control – bulbs rooted in tap water

Procedure:

1. **8–hydroxichinoline influence:** insertion of the roots into the 0,2M 8–hydrochichinoline for 5 hours (7.00 – 12.00) room temperature, control roots stay in tap water.
2. **Fixation**: segments of root are fixed for 2 hours in the mixture according to Carnoy (6:3:1): 70% ethanol 60 ml, chloroform 30 ml, concentrated acetic acid 10 ml.
3. **Washing:** 70% ethanol: 2 x 15 minutes
4. **Maceration**: root immersion for several minutes into the mixture HC1: ethanol (1:1, vol.), it is necessary to test appropriate interval for each plant material.
5. **Squashing of root tip:** on the microscopic slide covered with chrome-alum jelly with the aid of wet cellophane.
6. **Pick up** the cellophane after drying.
7. **Staining of the nuclear structures:** 15 minutes in saturated water solution of **basic fuchsine**
8. **Washing:** distillated water

9. **Staining of the cytoplasm:** pikroindigokarmine for 1 - 15 minutes. (100 ml of saturated water solution of indigokarmine + 50 ml saturated water solution of picric acid).
10. **Washing:** 70% ethanol
11. **Very quick dehydration**: 75%, 96%, 100%, 100% ethanol **2 minutes each slide**
12. **Transfer to xylene:** mixtures: 100% EtOH : xylen = 2:1, 1:1, 1:2, xylen, 2 minutes each.
13. **Mounting:** synthetic resin Eukitt® and cover slide

Evaluation:

1. quality of squash preparation
2. structure of the cells, possibility of the chromosome counting
3. quality of staining
4. efficiency (impact) of mitotic poison to the structure of microtubules
5. microphotography of the best preparations (after drying mounting medium)

Literature:

Hrubý K. (1933): Double Staining by the Cajal-Brožek Method. – Science **77**:352 –353.

https://en.wikipedia.org/wiki/Santiago_Ram%C3%B3n_y_Cajal

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