Šūnu bioloģijas metodes
1. seminārs 2014.

1.uzdevums. Izlasiet tekstu un apskatiet fotogrāfijas. (10 punkti)
Izplānojiet un prezentējiet eksperimenta plānu PCD analizēšanai tabakas lapās.

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2.2. Induction of apoptosis in meristematic cells of roots-tip in maize

Following the method of Katsuhara *et al.* [22], the seeds of maize were soaked in double-distilled water for 1 day, and transferred to the culture solution (4 mM KNO_3, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 1 mg/L ferrous citrate, pH 5.5) after germination and cultured in dark condition until the young root-tip developed into around 2 cm in length and then again transferred into 0.01-20 mg/mL IPC solution for treatments for 1-7 days, respectively.

2.3. Chromosome preparation and TUNEL in individual cells

The chromosome preparation and the *in situ* labeling followed the methods of Ning Shunbin *et al.* (1999) with modifications [23]. The root tips were cut at 2 mm in length in control and IPC-treated roots and immediately fixed in methanol : acetic acid (3:1) solution for around 3 h, then the root caps were cut off and discarded. After fully washing with dd-H_2O, the tips were enzymolysis with the mixture of 2% pectinase and 2% cellulase for around 3 h., the slide was then dried over flame. For *in situ* labeling, the Fluorescein-dUTP was substituted for Biotin-dUTP, and TdT enzyme (terminal deoxynucleotidyl transferase) for Klenow enzyme (TUNEL Detection Kit, purchased from Boehringer Mannheim Co., *In situ* Cell Death Detection Kit, Fluorescein). The reaction was carried out under 37℃ for 1 h. After labeling, the slides were washed in PBS for 3×5 min, and then counterstained with PI (propidium iodide), and observed under fluorescence microscope (BX60, Japan).

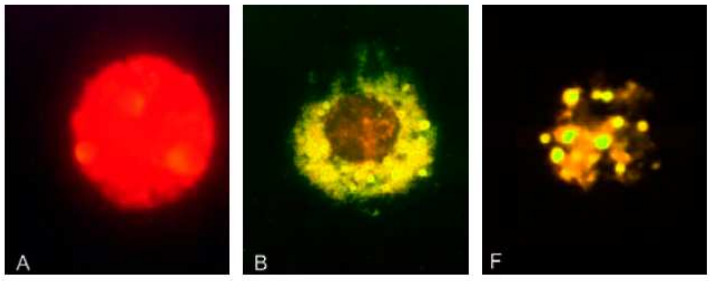
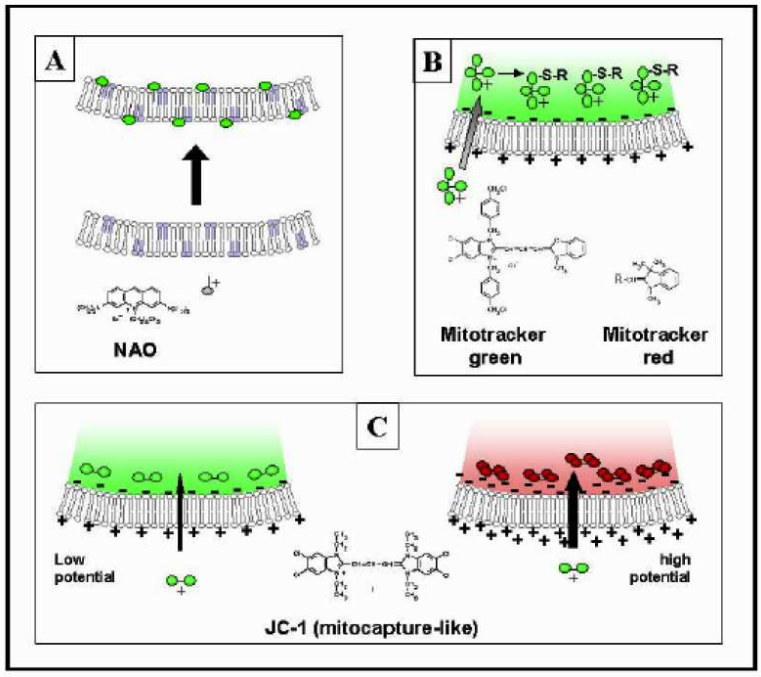


Fig.1 Nuclear morphological and biochemical change during IPC-induced apoptosis in individual meristematic cells of maize roots detected by TUNEL assay (×3300).
(**A**) Control, round, red with PI, no FITC fluorescence.
(**B**) Treated for 2d, DNA breaks are marked with FITC (yellowish green in color). Obvious nucleus condensation is not observed yet and nucleoli don't disappear.
(**F**) Treated for 5d. Nuclear substances degrades, very concentrated FITC fluorescence granules appear.

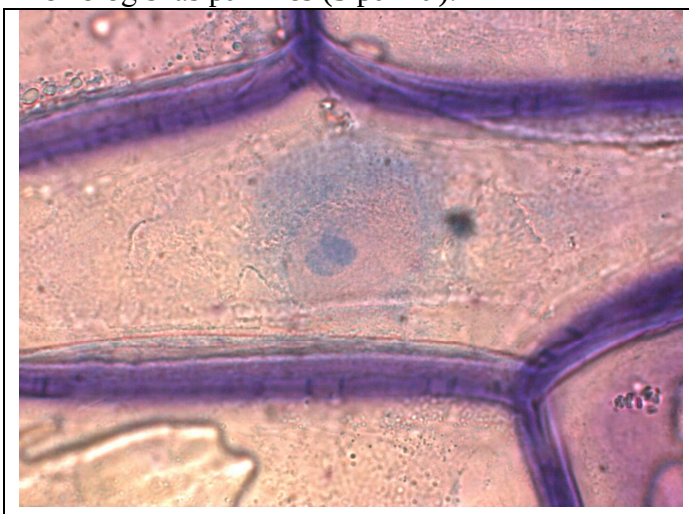
1. Priekšeksperiments
2. Eksperimenta varianti
3. Pozitīvā kontrole
4. Negatīvā kontrole
5. Fiksācija
6. Griešana
7. Krāsošana
8. Mikroskopēšana
9. Fotografēšana

2. uzdevums. Apskatiet attēlu.
a) Kuras no parādītajām krāsvielām varētu lietot, lai pierādītu, ka šūnā noris dzīvības procesi. Pamatojiet atbildi. (2punkti)
b) Vai visas trīs krāsvielas parādīs vienādas formas mitohondrijus? Pamatojiet atbildi. (3 punkti)

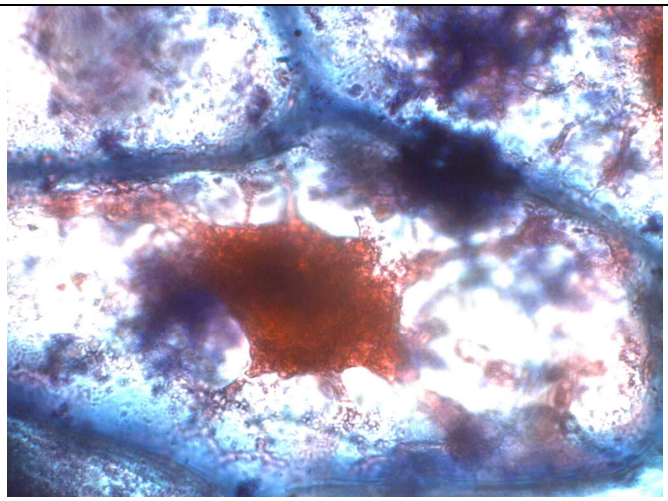


3. Kādas ir 70% etanola priekšrocības un trūkumi salīdzinot ar paraformaldehīda fiksāciju? (3 punkti)

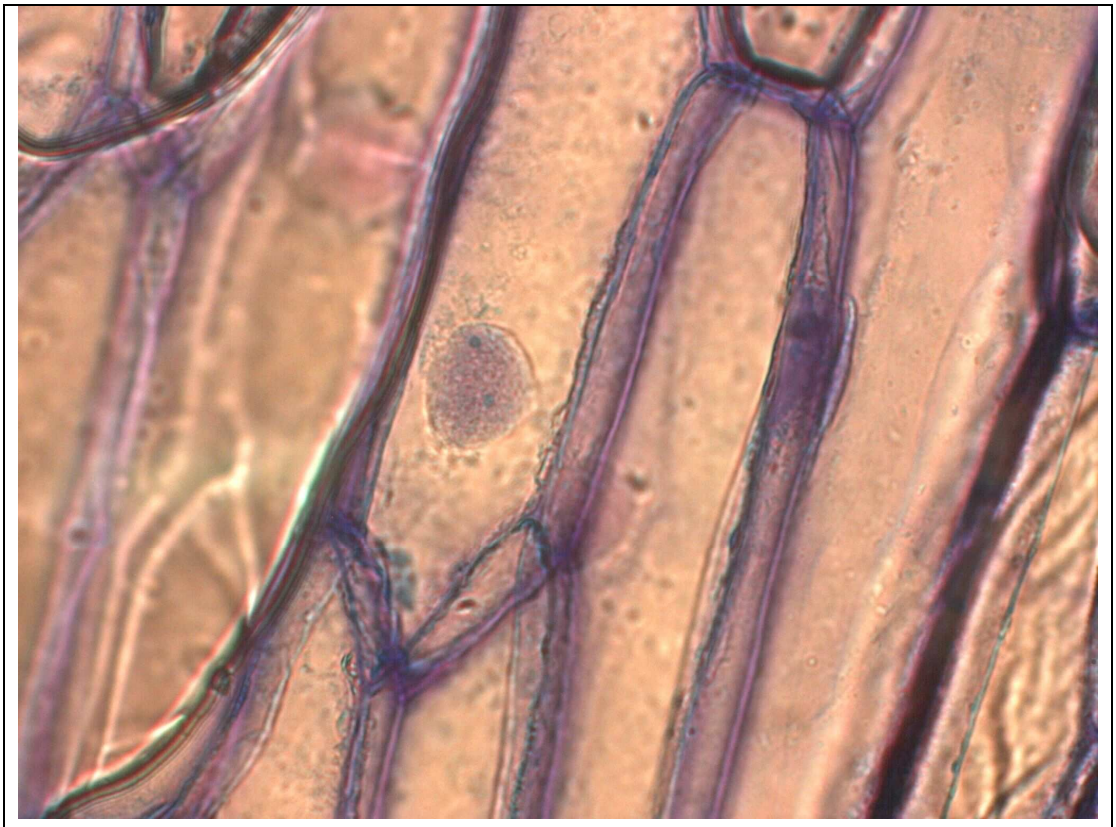
4. uzdevums. Salīdziniet a un b attēlus ar c attēlu. Nosauciet 5 apgalvojumus par pareizi vai nepareizi veiktām darbībām preparāta sagatavošanā, izmantojiet un nosauciet fotogrāfijās redzamās šūnu morfoloģiskās pazīmes (5 punkti).



a



b



c