Onion (*Allium cepa*) genotoxicity test

1. Purpose

The *Allium cepa* assay is an efficient test for chemical screening and *in situ* monitoring for genotoxicity of environmental contaminants. The test has been used widely to study genotoxicity of many pesticides revealing that these compounds can induce chromosomal aberrations in root meristems[1] of *A. cepa*. Pesticide residues can be present in fruit and vegetables and represent a risk for human health.

2. Test characteristic

The onion genotoxicity test provides for easy screening of chemicals or samples with genotoxic effects, especially to plants. First, usually root growth test is applied to find the EC50 value for root elongation. After that, sample is tested for chromosomal aberrations and/or micronuclei where EC50 value serves for the highest tested concentration.

3. Testing organism

The testing organism is common onion (*Allium cepa*), *e.g.* Stuttgarter variety. Other onion varieties can be also used. The onions should have a size of 15-22 mm and a weight of 2-4 g. If kept in cool (10-15 ºC) and dry onions can be used until a year after harvest.

4. Materials

3.1 Tested chemical, aquatic sample or water leachate

3.2 Positive control

methyl methanesulfonate, c = 10 mg/l

3.3 Negative control (dilution medium)

Dissolve following chemicals in distilled water or 2% DMSO in case of testing of water-insoluble chemicals (Tab. 1).

[1] Meristem is plant tissue consisting of undifferentiated cells whose function is to produce daughter cells and provide for plant growth. Meristems are usually located to plant tips – root tips, shoot tips (apical meristems) or places of future leaf bases *etc.* Meristematic cells are analogues to animal stem cells.

Tab. 1: composition of *Allium cepa* cultivation medium

Chemical	Concentration (mg/l)
$CaSO_4$ *	60
$MgSO_4$	60
$NaHCO_3$	96
KCl	4

* Dissolve $CaSO_4$ with heating and stirring before it is mixed with other salts!

Note: pH of cultivation and tested sample should be between 5.5 and 8. If needed, adjust the pH with 1M HCl or NaOH to 7.

3.4 Fixation and maceration solution

Use mixture of 45% acetic acid (9 parts) and 1M HCl (1 part)

3.5 Staining colour (orcein)

2% orcein dissolved in 45% acetic acid

5. Methods

5.1. Pre-treatment and exposition of testing organisms

Onions are grown in control medium for first 24 hours:

Put the individual onions onto 15-ml-Falcon tubes, filled with control medium. The base of the onion must reach the medium surface. Cover the tube-stand with aluminium to keep the onion roots in dark during growth. Incubate them at 25±1 °C in cultivator with light cycle.

After 24 hours prepare the positive control, sample concentrations and fresh cultivation medium. Use 6 onions per sample. Fill 150-ml-beakers with 100 ml of sample (or control medium) and cover the water surface with aluminium. Make 6 holes into aluminium cover and replace pre-grew plants as onions up the aluminium cover and roots inside sample in the beaker. Expose the plants for 48 hours at 25±1 °C in cultivator with light cycle.

Tab.2 Test conditions for onion genotoxicity test

Testing organism:	Onion (*Allium cepa*)
Parameter observed:	Micronuclei in interphase and chromosomal aberrations in anaphase-telophase root tip cells
Test conditions:	Stable temperature and light cycle 16 h/8 h (light/dark)
Replicates:	6 plants per concentration
Volume of tested sample:	100 ml
Temperature:	(25 ± 1)°C
Exposition length:	24 h pre-treatment in control water, 48 h of exposition
Illumination:	1000 - 2000 lux
Chemicals:	sample, cultivation medium, methyl methanesulfonate, DMSO, orcein, 45% acetic acid, 1M HCl
Instruments and equipment:	150-ml-beakers, 10-ml-tubes (glass and Falcon), analytical weighs, thermostat, pH-meter, pipettes, aluminium, cultivator, microscope, tweezers, microscopic slides, cover glass, cellulose

5.2. Maceration of the root tips and preparation for microscopy

After exposition exclude the plant with the poorest root growth. Use the rest 5 plants per sample for the microscopy. Cut 5 root tips per plant at a length of 10 mm and place them into 10-ml-glass tube with 2 ml acetic acid/HCl solution (see 3.4). Heat the root tips for 5 minutes at 50 ºC. Hereby, the root cells will become fixated and macerated.

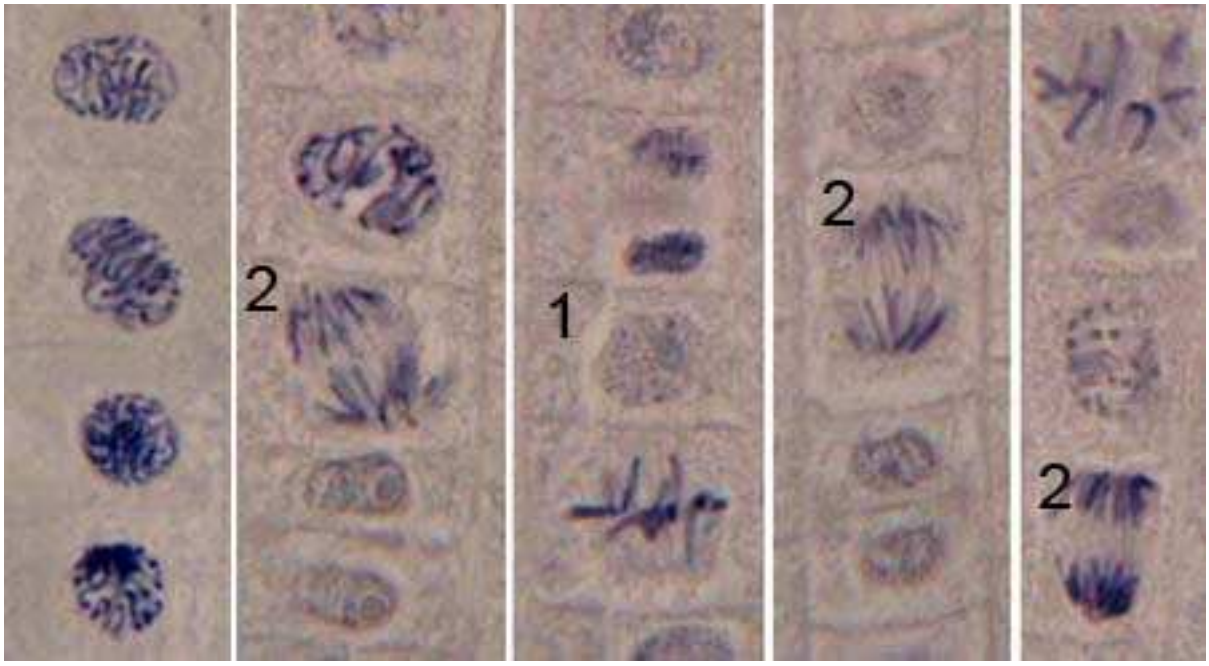
Thereafter, place the root tips on a microscope-slide on a black background and cut off the terminal tips (1-2 mm) for further preparation. Remove the rest of root material and liquid from the slide. Add 2 drops of orcein solution (see 3.5) and mix it with the roots properly by stirring and knocking with a stick of stainless steel (stirring spattle). Place a cover slip on the root cells. The staining procedure takes about 5-10 min. After that squash the cells by placing to layers of filtrate paper on the cover glass and pressing slightly down with thumb. Follow the microscopy immediately or fix the cover slip to the slide with clear nail varnish. Such a slide can be kept in the freezer fresh up to 2 months.

5.3. Microscopic examination

The microscopic analysis includes mitotic index, micronuclei presence in interphase cells and chromosomal aberrations in late anaphase and early telophase cells score. For better understanding, see following pictures (Fig. 1).

Interphase nucleus is compact, no individual chromosomes are seen. Cell is not prepared for division. Whereas in late anaphase – early telophase the nucleus division culminates.

Fig. 1: Interphase (1) and anaphase- early telophase cells (2)

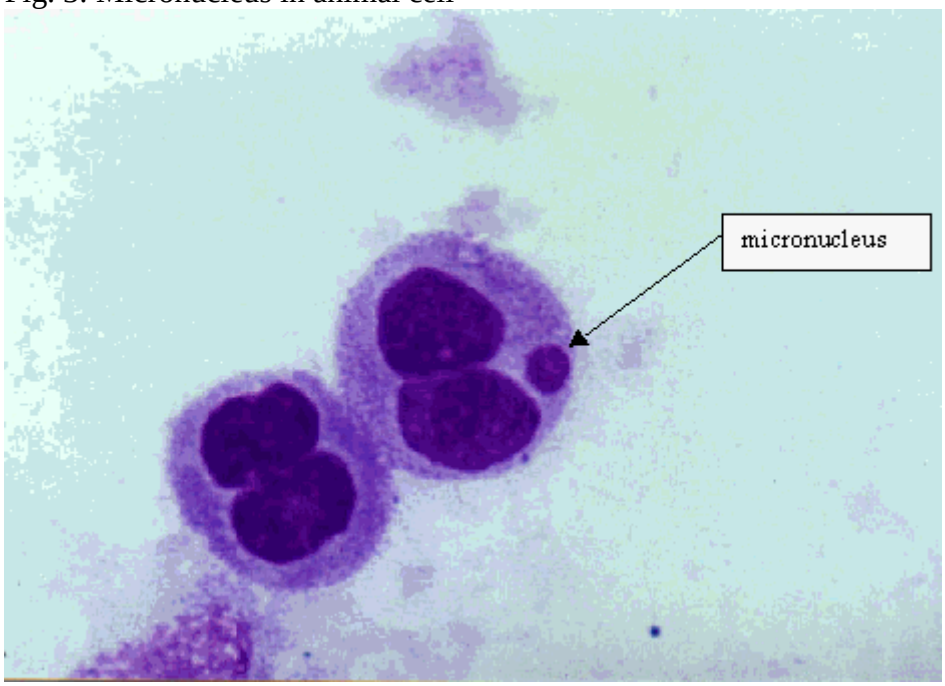


Mitotic index is found by counting all stages off mitotic cells (dividing cells) in 1000 cells. Chromosomal aberrations can be estimated only if mitotic index is above 10 per 1000. Then the aberrations are scored in first 100 cells in anaphase or telophase, when slides are scanned from right to left, up and down. For the laboratory occasion do not estimate the aberrations, only try to see if you can find some (chromosome fragments, bridges – Fig. 2).

Fig. 2: Two chromosomal bridges (signed with arrows)

In interaphase cells score the micronuclei presence. Micronuclei are the chromosomal fragments, covered by nuclear membrane (Fig. 3). 1000 interphase cells should be examined for the micronuclei. Count the mutated cells per 1000 cells.

Fig. 3: Micronucleus in animal cell



Compare the tested slides with negative control (no aberrations and micronuclei) and positive control slides (presence of aberrations and/or micronuclei).

6. Evaluation of the test result

Use the χ^2 test for statistical calculations of the test. Resolve whether the tested sample is genotoxic or not.

7. References

FERETTI, D., ZERBINI, I., ZANI, C., CERETTI, E., MORETTI, M., MONARCA, S. (2007): *Allium cepa* chromosome abberation and micronucleus tests applied to study genotoxicity of extracts from pesticide-treated vegetables and grapes. *Food Addit. Contam.* 24 (6): 561-572.

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