THE TiO_2-Pt AND TiO_2-Ag NANOCOMPOSITES EFFECT ON THE MITOTIC DIVISION

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Abstract. In paper the effect of a UV irradiation and of the TiO_2-Pt or TiO_2-Ag nanocomposites, applied alone or together, on the mitotic division in *Nigella damascena* L. (a tester radiobiological species). The investigations results, point out as the UV rays applied alone or together with TiO_2-Pt or TiO_2-Ag nanocomposites, affected the compaction degree of the chromatin fibers, the mitotic spindle division as well as the chromosomes integrity, induced diverse chromosome aberrations types. In the same time, the bimetallic TiO_2 nanocompozites presented a preferentiality of action on the NOR part of the chromosomes as well as on the heterochromatin regions, the both situated at the nucleus periphery. The BR index values, suggest as the TiO_2 bimetallic nanocomposites are not implicate in the reunion process of the broken end of the chromosomes.

Keywords: TiO_2-Pt or TiO_2-Ag nanocomposites; UV rays; chromosome aberration, *Nigella damascena*.

Rezumat. **Efectul nanocompozitelor TiO_2-Pt** şi TiO_2-Ag **asupra diviziunii mitotice.** In lucrare este prezentat efectul unei iradieri UV şi al nanocompozitelor TiO_2-Pt sau TiO_2-Ag aplicate singular sau împreună, asupra diviziunii mitotice la *Nigella damascena* L. (specie tester radiobiologic). Rezultatele investigaţiilor arată că radiaţii UV aplicate singular sau împreună cu nanocompozitele TiO_2-Pt, sau TiO_2-Ag au afectat gradul de compactare al fibrelor de cromatină, fusul de diviziune şi integritatea cromosomilor, inducând diferite tipuri de aberaţii cromosomiale. In acelaşi timp, nanocompozitele bimetalice TiO_2 au prezentat o preferenţialitate de acţiune asupra regiunii NOR a cromosomilor şi asupra regiunilor cu heterocromatina, ambele situate la periferia nucleului. Valoarea indicelui BR sugerează ca nanocompozitele bimetalice TiO_2 nu sunt implicate în reunirea capetelor rupte ale cromosomului.

Cuvinte cheie: nanocompozite TiO_2-Pt si TiO_2-Ag , radiaţii UV, aberaţii cromosomiale, *Nigella damascena*.

INTRODUCTION

Titanium was discovered with two hundred years ago, by William Gregor, in the ***ilmenit*** mineral rock. In environment it is a common element, his abundance being of 0.63% on Terra. Titanium posses a very good biocompatibility, being use initially in medicine as implant material (in dental and ostelogy), cardiac pacemaker, cardiac valves, a/o. Titanium formed at his surface a layer of about 2-10 nm TiO_2, which induce the resistance at corrosion. Under action of ***UV rays***, titanium can produce free radicals. For this rations, the recent researches are concerned for his use in the carcinogen tissues. His efficiency is depending on the size and nanoparticles, chemistry features of the surface, a/o. Also, titanium was doped with other metals (Au, Ag, Pt, a/o), their properties being enhanced. Also were obtained a new material, ***nanodevices***, hybrid between TiO_2 nanoparticles or TiO_2-Metal nanocomposites, with oligonucleotide (WOLOSCHAK et al., 2006).

LU et al. (1998), in experiments performed on Chinese hamster ovary-K1 cells, reported as TiO_2 induced sister chromatin exchanges and micronuclei, being affected the chromosome integrity. RAHMAN et al. (2002), in experiments performed on hamster fibroblasts, remarked that the ultra fine particles of TiO_2 induced the chromosome aberrations, as well as the apoptosis process. There are different results, regarding to the cytotoxicity of the TiO_2 (FERRIN et al., 1992; DONALDSON ET AL., 1998; OBERSDORFER, 2001, a/o.). The scientific literature is very poor with references about the TiO_2 effect on the vegetal cells ZHENG et al., 2005; CORNEANU et al., 2007).

MATERIALS AND METHOD

Nigella damascena L. (Fam. *Ranunculaceae*) is a tester species, used for cytogenetic and radiobiological investigations (MOUTSCHEN-DAHMEN (1966, 1968), GILLOT-DELHALE J. (1966), CORNEANU C.G., 1974, a/o). This species present a small chromosome number (2n=6), big and differentiate morphological between them and the synchronized mitotic divisions.

The titanium dioxide from atanase form, was doped with silver or platinum (1%), obtaining the nanocomposites of the TiO_2-Pt and TiO_2-Ag, atanase type. These were prepared at I.N.C.-D. Electrochemistry and Condensed Mater Timisoara after two protocols.

Radices of about 10 mm length, treated with an aqueous suspension of TiO_2-Pt nanocomposite particles (2 or 4 mg nanocomposites, with the particles of about 25-30 nm in diameter, suspended in 5 ml aqueous solution). After two hours from the treatment beginning, half of experimental variants were exposed at UV radiations emitted by a UV lamp (BLA-12 type, MEDICOR Budapest at the parameters: U=220 W, I=0.2 A, 50 Hz, 44 VA), and other half variant at solar light. The biological material was harvested at different stages in the first cell cycle (2 hours) and in the second

cell cycle after treatment (26 hours). Were analyzed the chromosome aberration in the meristematic cells (squash preparations, Carr stained).

RESULTS AND DISCUSSIONS

1. The percentage of the cell in division and of the phases of mitosis

In the meristem tops, were analyzed the all cells in mitotic division or in interphase: the chromatin fibers aspects, the spindle division and the chromosomes in different mitotic phases. In Table 1 is presented the percentage of the normal cells in mitosis, as well as the cells percentage in different mitotic division stages, under action of the UV rays, or solar light, at 2 hours after the treatment begin.

Table 1. The normal percentage cells in mitose and the percentage cells in different mitotic stages in the first mitotic cycle (at 2 hours after the treatment begin).
Table 1. Procentajul de celule normale în mitoza şi procentajul de celule în diferite stadii ale mitozei, în timpul primului ciclului mitotic (la 2 ore după începerea tratamentului).

Variant	Normal cells in mitosis (%)	Percentage cells in:				
		Prophase	Prometa-phase	Metaphase	Anaphase	Telophase
Solar light (2 hours)						
Control	99.4	41.7	2.5	19.7	16.7	19.4
TiO_2-Ag-1	76.2	43.0	5.2	24.3	17.4	10.1
TiO_2-Ag-S	87.7	38.7	1.6	17.6	16.2	25.9
TiO_2-Pt-1	93.9	40.5	3.3	21.7	14.5	20.0
TiO_2-Pt-S	92.0	46.0	1.7	25.0	16.1	11.2
UV rays (2 hours)						
Control	92.6	33.2	4.7	27.4	22.1	12.6
TiO_2-Ag-1	86.2	44.4	2.1	22.0	18.8	12.7
TiO_2-Ag-S	99.1	41.9	2.3	23.0	16.2	16.6
TiO_2-Pt-1	99.3	36.7	1.8	24.4	17.7	22.4
TiO_2-Pt-S	99.4	36.8	2.9	24.0	19.5	26.7

The UV rays alone reduced the cells number in mitotic division, instead the UV rays applied together with nanocomposites with TiO_2, enhanced the cell number in division. The presence of the TiO_2-Pt nanocomposite in the time of the UV irradiation (2 hours, at the parameters from experiment), enhanced the cells number in mitotic division, manifested a protecting effect against the UV rays action.

The analysis of the nanocomposites effect alone and together UV rays (2 hours), on the different mitotic phases, revealed a different action, depending on the mitotic phase and the metal component (Table 2). Thus, the nanocomposites of the TiO_2-Pt type manifest a protecting effect against the UV rays, in the all division stages.

Table 2. The percentage of normal cells in different mitosis phases (first mitotic cycle).
Table 2. Procentajul de celule normale în diferite faze ale mitozei (primul ciclu mitotic).

Variant	Prophase	Prometaphase	Metaphase	Anaphase	Telophase
Solar lighr (2 hours)					
Control	98.8	100.0	97.9	99.3	99.7
TiO_2-Ag-1	61.9	54.3	80.9	98.0	98.9
TiO_2-Ag-S	76.5	87.5	89.9	95.1	98.5
TiO_2-Pt-1	89.5	78.3	94.7	100.0	100.0
TiO_2-Pt-S	98.3	87.5	98.3	99.3	100.0
UV rays (2 hours)					
Control	90.0	85.1	90.5	98.4	100.0
TiO_2-Ag-1	81.8	61.5	80.9	96.6	100.0
TiO_2-Ag-S	99.7	93.8	98.1	100.0	99.1
TiO_2-Pt-1	98.6	100.0	100.0	98.7	100.0
TiO_2-Pt-S	98.8	100.0	100.0	99.2	100.0

In percentage normal cells in mitosis and the percentage cells in different mitoses phase in the second cell cycle, is presented in the Table 3. The values recorded in the solar light, are better in the second cell cycle, in comparison with the values recorded in the first cell cycle. Because UV rays presence, the normal cells in mitosis, recorded lower values.

Table 3. The normal percentage cells in mitosis and the percentage cells in different mitotic stages in the second mitotic cycle (at 26 hours after the treatment begin).
Table 3. Procentajul de celule normale în mitoza şi procentajul de celule în diferite stadii ale mitozei, în timpul celui de al doilea ciclu mitotic (la 26 ore după începerea tratamentului).

Variant	Normal cells in mitosis (%)	Percentage cells in:				
		Prophase	Prometa-phase	Mataphase	Anaphase	Telophase
Solar light (2 hours)						
Control	98.9	39.6	2.0	22.6	17.6	18.2
TiO_2-Ag-1	98.9	44.5	1.6	22.5	14.4	17.0
TiO_2-Ag-S	99.3	33.4	13.0	24.9	21.6	17.1
TiO_2-Pt-1	99.3	36.9	1.1	21.5	17.1	18.9
TiO_2-Pt-S	95.9	39.0	1.6		19.5	12.4
UV light (2 hours)						
Control	98.2	38.8	2.0	22.7	18.7	17.8
TiO_2-Ag-1	97.7	37.9	2.5	25.7	15.8	18.1
TiO_2-Ag-S	95.1	46.4	2.3	19.0	14.9	17.4
TiO_2-Pt-1	98.0	42.6	1.8	25.6	14.0	16.0
TiO_2-Pt-S	97.6	37.1	2.6	25.8	17.7	16.8

Also, between different experimental variants, were recorded a small differences in comparison with the values recorded in the first cell cycle. The nanocomposites, especially the TiO_2-Pt nanocomposites, were diminished the UV rays effect.

The percentage of the normal cells in different mitotic division stages, in the second mitotic cell cycle, reveal the protecting effect of the TiO_2-Pt nanocomposites, against the UV light (Table 4).

Table 4. The percentage of the normal cells in different mitosis phase (second mitotic cycle).
Table 4. Procentajul de celule normale în diferite faze ale mitozei (al doilea ciclu mitotic).

Variant	Prophase	Prometaphase	Matephase	Anaphase	Telophase
Solar light (2 hours)					
Control	98.7	100.0	97.7	99.0	99.5
TiO_2-Ag-1	99.4	88.2	98.9	100.0	98.9
TiO_2-Ag-S	99.0	100.0	100.0	98.5	100.0
TiO_2-Pt-1	98.7	100.0	99.4	100.0	100.0
TiO_2-Pt-S	93.2	87.5	98.1	96.9	98.9
UV light (2 hours)					
Control	97.6	100.0	99.1	97.8	98.8
TiO_2-Ag-1	94.9	100.0	98.9	100.0	99.5
TiO_2-Ag-S	94.4	96.3	91.6	97.2	99.0
TiO_2-Pt-1	99.2	80.0	97.2	97.5	98.5
TiO_2-Pt-S	97.8	100.0	99.7	97.7	99.0

The percentage chromosome aberrations in anaphase and telophase induced in the first cell cycle (Table 5) and in the second cell cycle (Table 6), recorded a small values, slightly in the second cell cycle.

In the second mitotic cycle, the percentage of the fragments and bridges recorded small values, but slightly upper in comparison with values recorded in the first mitotic cycle. The nanocomposites alone induced some chromosome aberrations, especially in the second cell cycle (a long time of action).

Table 5. The chromosome aberrations in *Nigella damascena* in the first mitotic cycle (after 2 hours)
Table 5. Aberaţiile cromosomiale la *Nigella damascena* în timpul primului ciclu mitotic (dupa 2 ore)

Variant	Fragments at 100 cells		Bridges at 100 cells		BR index	
	Anaphase	Telophase	Anaphase	Telophase	Anaphase	Telophase
Solar light (2 hours)						
Control	0.3	0.0	0.0	0.0	0.0	0.0
TiO_2-Ag-1	2.6	1.1	0.0	1.5	0.0	0.74
TiO_2-Ag-S	3.7	2.3	0.0	0.0	0.0	0.0
TiO_2-Pt-1	0.0	0.0	0.0	0.0	0.0	0.0
TiO_2-Pt-S	1.4	0.0	0.0	0.0	0.0	0.0

UV light (2 hours)						
Control	3.5	0.0	1.5	0.0	2.26	0.0
TiO_2-Ag-1	0.0	0.0	0.0	0.0	0.0	0.0
TiO_2-Ag-S	0.0	1.7	0.0	0.85	0.0	2.0
TiO_2-Pt-1	2.7	0.0	0.0	0.0	0.0	0.0
TiO_2-Pt-S	0.8	0.0	0.0	0.0	0.0	0.0

The analysis of the RB index values, not suggest that nanocomposites of TiO_2 (indifferent of the second metal, platinum or silver), present a role in the linked of the broken end of the chromosomes (Table 5, 6).

Table 6. The chromosome aberrations in *Nigella damascena* in the second mitotic cycle (after 26 hours)
Table 6. Aberațiile cromosomiale la *Nigella damascena* în timpul celui de al doilea ciclu mitotic (dupa 26 ore)

Variant	Fragments at 100 cells		Bridges at 100 cells		BR index	
	Anaphase	Telophase	Anaphase	Telophase	Anaphase	Telophase
Solar light (2 hours)						
Control	0.5	0.5	0.0	0.55	0.0	0.0
TiO_2-Ag-1	0.0	0.5	0.0	0.0	0.0	0.9
TiO_2-Ag-S	3.0	0.0	1.5	0.0	0.5	0.0
TiO_2-Pt-1	0.0	0.0	0.0	0.0	0.0	0.0
TiO_2-Pt-S	5.2	1.1	2.1	0.0	2.47	0.0
UV light (2 hours)						
Control	2.5	1.1	0.36	0.0	6.94	0.0
TiO_2-Ag-1	0.0	1.0	0.0	0.0	0.0	0.0
TiO_2-Ag-S	4.5	1.4	0.0	0.0	0.0	0.0
TiO_2-Pt-1	4.2	1.5	0.0	0.73	0.0	2.05
TiO_2-Pt-S	8.8	3.1	0.0	0.0	0.0	0.0

2. The types of chromosome aberrations and metabolic modifications

The used nanocomposites (especially TiO_2-Pt), affected the compaction degree of the chromatin fibers, inducting a particular aspect similar with the chromosome banding, as well as their integrity. As result of the cells analyzed in mitotic division, were evidenced two types of chromosome modifications: (a) metabolic modifications and (b) proper chromosome aberrations.

A. Metabolic modifications of the chromosomes:

The chromosome banding, determined by a different compaction degree in the eu- and heterochromatic regions of the chromosome; visible in prophase, prometaphase, metaphase and anaphase. Are affected especially the chromosomes with satellite;

Spindle mitotic inactivation, resulting figures similar with those from the colchicines action (C-mitosis); visible in the all mitotic division stages (from prophase to telophase); as result of this process, result autopolyploid cells;

Parallel disposed of the chromatin fibers, probably as result of some surface electrical phenomenon; visible in prophase, prometaphase and metaphase.

PCC (premature chromatin condensation), visible in prophase, prometaphase and metaphase;

DCC (delay chromatin condensation), visible in prophase, prometaphase and metaphase.

B. Proper chromosome aberrations

The nanocomposites of TiO_2-Pt and TiO_2-Ag type, induced chromosome aberrations: **minutes** and **acentric fragments** (visible from metaphase to telophase); **centric rings** and **acentric rings** (visible in anaphase and telophase), **bridges** and **arch** (in anaphase and telophase). All of this genetic material in not included in the nuclei in telophase is visible, in the next interphase as **micronuclei** (Fig. 4/16).

The chromosome aberrations of **minutes** or **acentric fragments** type, are produced by a one-hit, and affected a single chromosome. The chromosome aberrations of **centric rings** or **acentric rings** types, are produced by two-hit, which affectd the same chromosome (Casarett, 1968; Corneanu, 1971).

The chromosome aberrations of **bridges** and **arch** types, derivate from a dicentric chromosome. The dicentric chromosomes is obtained as result of the reunion of the end broken of two different chromosomes. When the two centromeres of the same chromosome, migrate toward oposide cells pole, result a chromosome aberration of **bridge** type. If the two centromeres of a dicentric, migrate to the same pole, result an aberration of **arch** type. The presence of these aberrations type was theoretical presented by Casarett (1968). Corneanu (1974, 1979) described this aberration type in *Nigella sativa* species, with long chromosomes, after the dry seeds exposed at a dose of 35 Gy, X-rays. In the Fig. 1, is visible a chromosome aberration of **arch** type (arrow).

In the Figs 1-4 are presented: ***acentric fragments*** and a aberration of ***arch type*** in anaphase (Fig. 1), ***bridge*** in anaphase (Fig. 2), ***acentric fragments*** and ***broken bridge*** in telophase (Fig. 3), ***micronucleus*** in the next interphase (Fig. 4).

CONCLUSIONS

The titanium dioxide from atanase form was doped with silver or platinum (1%), obtaining the nanocomposites of the TiO_2-Pt and TiO_2-Ag, atanase type.

The bimetallic nanocomposites (TiO_2-Pt and TiO_2-Ag, atanase type), were applied alone or together with UV irradiation at radicular meristem tissue of *Nigella damascena* (a radiobiological and cytogenetically tester specie), time of 2 hours. The cytogenetic effect was analyzed in the first cell cycle and in the second mitotic cycle (the normal cells in different mitotic stages, as well as the chromosome aberrations or metabolic modifications of the chromosomes.

The bimetallic nanocomposites (TiO_2-Pt or TiO_2-Ag), enhanced the cells number in mitotic activity in.

The UV rays, applied time of 2 hours (at the parameters from experiment), manifest a relative protecting effect over action of the nanocomposites (especially the nanocomposites of the TiO_2-Pt type) over the cell mitotic activity.

Thus, the nanocomposites of the TiO_2-Pt type, in variant of 2 hours treatment, manifest a protecting effect against the UV rays, in the all division stages.

In the second cell cycle after the treatment begin, the values recorded in the solar light are better, in comparison with the values recorded in the first cell cycle. In the UV rays presence, the number of the normal cells present in mitosis, recorded lower values. Between the different experimental variants, were recorded small differences, in comparison with the values recorded in the first mitotic cycle. The nanocomposites (especially TiO_2-Pt) were diminished the UV rays effect.

The percentage of the normal cells (presented in different mitotic division stages), in the second mitotic cell cycle, reveal the protecting effect of the TiO_2-Pt nanocomposites, against the UV light action.

UV rays applied alone or together with bimetallic nanocomposites affect: (a) compacting degree of the chromatin fiber; (b) spindle division activity; (c) chromosome integrity.

Bimetallic nanocomposites and /or UV-rays, manifest a preferential action at some chromosome level, especially on the chromosomes with NOR region (chromosomes with satellite), and at the heterochromatic regions level.

The fragments and bridges percentage recorded small values, but slightly upper in the second mitotic cycle. The nanocomposites alone induced some chromosome aberrations, especially in the second cell cycle (a long time of action).

The BR index value, suggest that TiO_2 nanocomposites are not implied in the reunion process of the broken end of the chromosomes.

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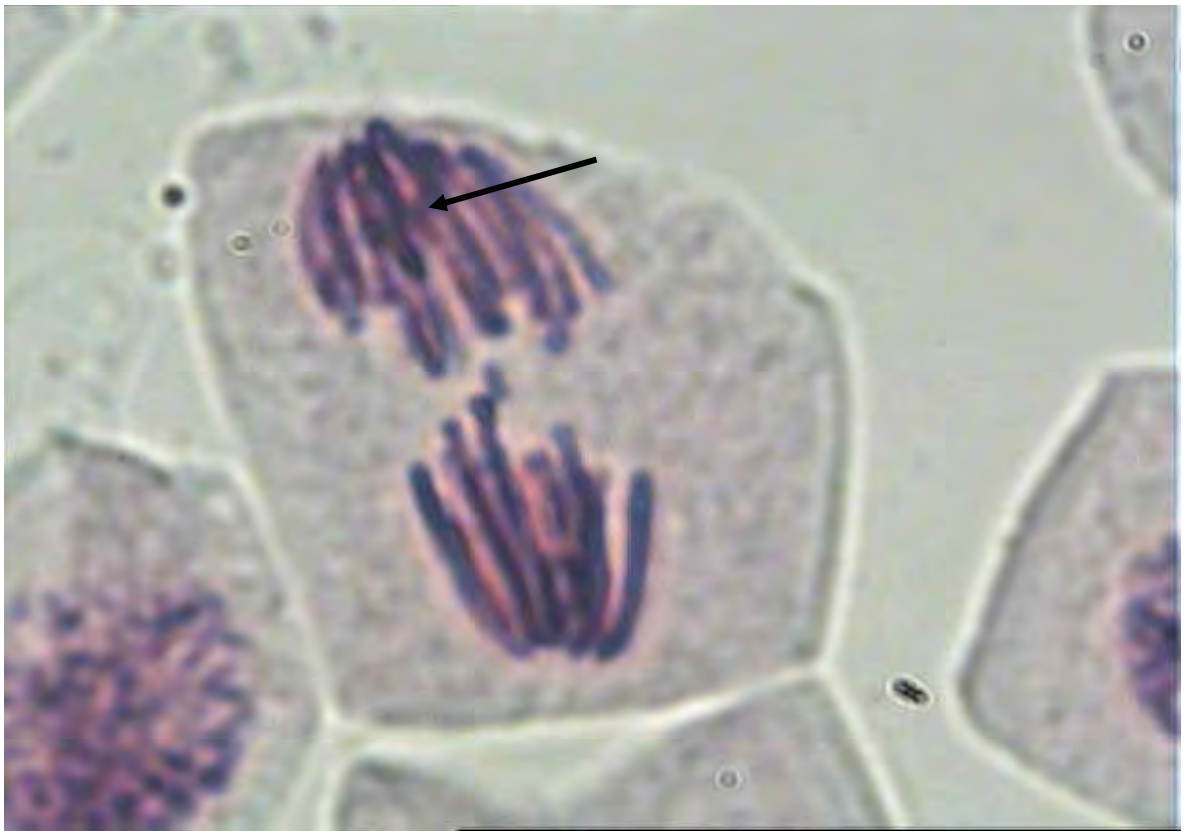

Figure 1. Acentric fragments and arch (arrow) in anaphase (*Nigella damascena* L.).
Figura. 1. Fragmente acentrice şi arch (sageata) în anafază (*Nigella damascena* L.).

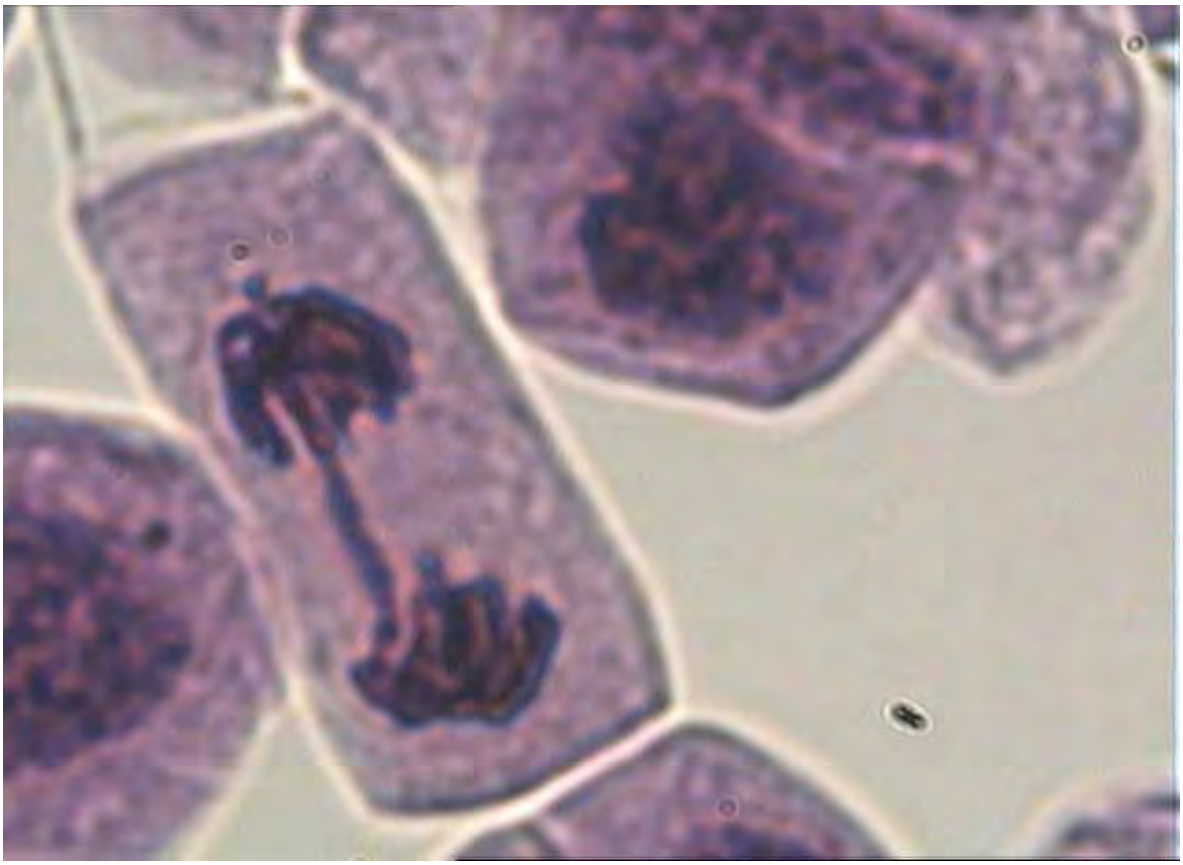

Figure 2. Bridge in anaphase (*Nigella damascena* L.).
Figura 2. Punte în anafază (*Nigella damascena* L.).

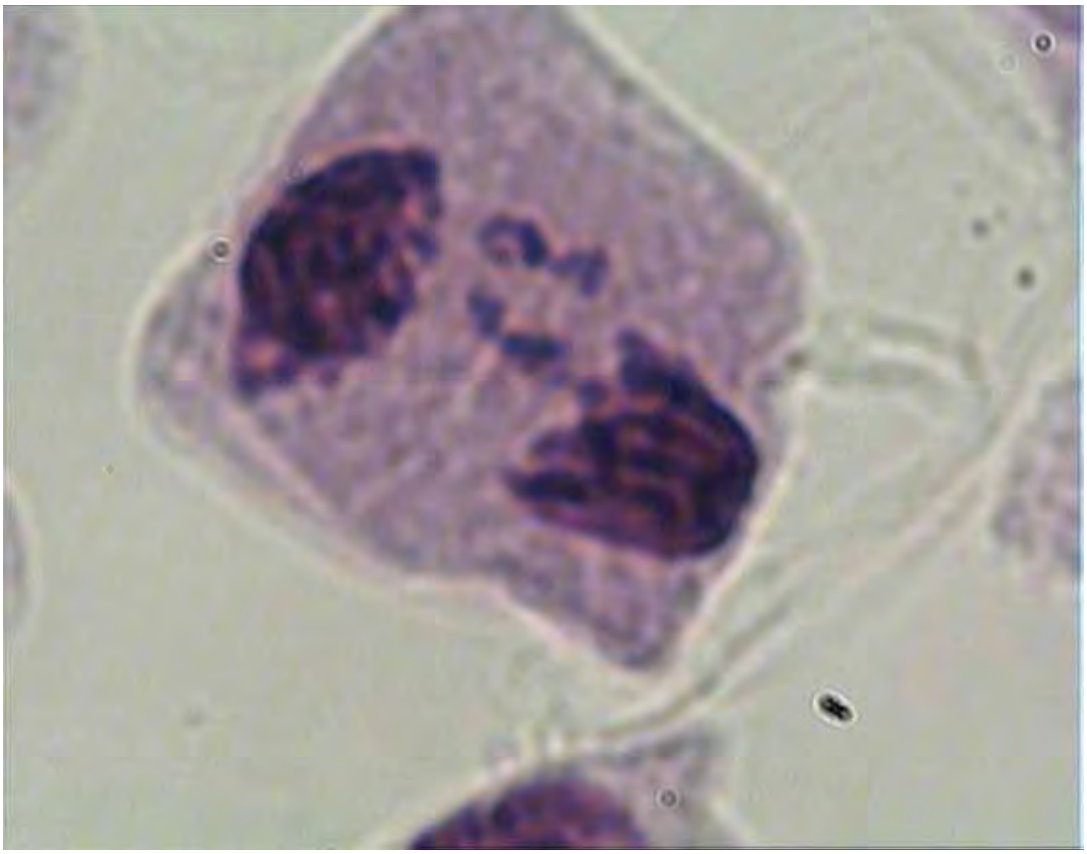

Figure 3. Broken bridge and acentric fragments in telophase (*Nigella damascena* L.).
Figura 3. Punte ruptă şi fragmente acentrice în telofază (*Nigella damascena* L.).

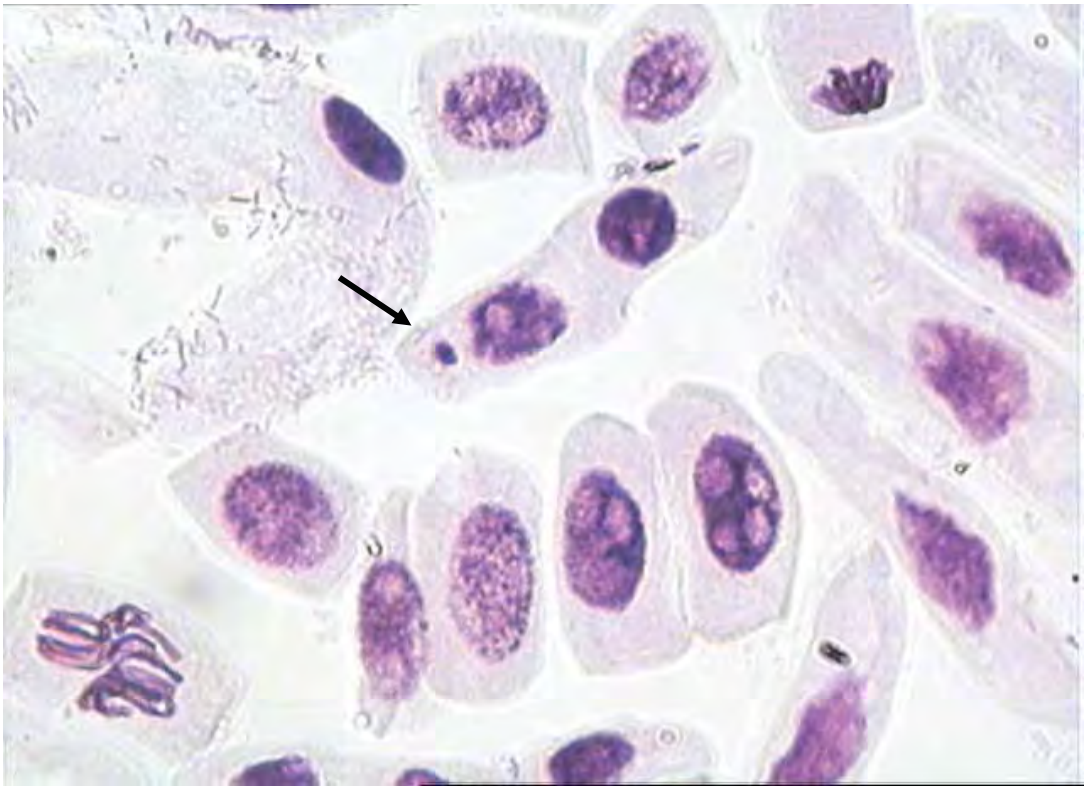

Figure 4. Micronucleus (arrow) in the next interphase (*Nigella damascena* L.).
Figura 4. Micronucleu (sageata) în interfaza următoare (*Nigella damascena* L.)