



EVALUATION OF THE CYTOGENETIC RESPONSE OF *ANTIRRHINUM MAJUS* L. UNDER INFLUENCE OF POLLUTED WATER

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ABSTRACT

The purpose of this paper is to evaluate the cytogenetic response of the *Antirrhinum majus* L. species treated with polluted water from the Jiu river, in which is discharged the wastewater from Oltenia region, Craiova city (Romania). The seeds of *A. majus* L. were exposed to action of the polluted water for 72 hours, after which the meristematic roots were processed for cytogenetic study, together with the control exposed to action of distilled water. The obtained results showed a strong mitodepressive effect of the polluted water, intensity of mitotic activity being decreased by 47.40% compared with the untreated control, which indicates its strong cytotoxic effect. Also, the occurrence of several types of cytological anomalies was observed, the most frequent being sticky chromosomes, ring-type chromosomes, C-mitosis and nuclear alteration. The results suggest the potential of *A. majus* L. to be used as a test plant for assessing and monitoring water quality.

KEYWORDS: *A. majus* L., polluted water, cytogenetic, mitodepressive.

INTRODUCTION

A. majus (common snapdragon) is a species of flowering plant belonging to the genus *Antirrhinum*. The plant was placed in the family *Plantaginaceae* and includes about 20 species with the chromosome number of $2n = 2x = 16$.^[15, 18] *Antirrhinum* has served as a model system for molecular and developmental genetics for the past three decades.^[10, 18] Many of the characteristics of *A. majus* made it desirable as a model organism; these include its diploid inheritance, ease of cultivation (having a relatively short generation time of around 4 months), its ease of both self-pollination and cross-pollination, and *A. majus*'s variation in morphology and flowering colour.^[15]

A. majus has also been used to examine the relationship between pollinators and plants. With debate as to the evolutionary advantages the conical-papillate shape of flower petals, with arguments suggesting the shape either enhanced and intensified the color of the flower or aided in orienting pollinators through sight or touch. The benefit that *A. majus* brought was through an identification of a mutation at the MIXTA locus that prevented this conical petal shape from forming.^[13]

As a model system in classical plant genetics, the genus *Antirrhinum* has been well studied, especially in gametophytic self-incompatibility, flower development biology, and transposon-induced mutation. In contrast to

the advances in genetic and molecular studies, little is known about *Antirrhinum* cytogenetics.^[33]

The intensity of the economic activities in a certain region invariably leads to the continuous pollution of the environment in general. Environmental protection issues are extremely complex and cover all sectors of activity: economic, social and political.^[2, 6, 7, 8, 27] With the modern and competitive lifestyle, all types of diseases are becoming a big issue for human health.^[14] A large share of the pollution of the waters of the Jiu River (Romania) is due to the economic activities in the SV Oltenia region.^[1] For example, in the Podari-Malu Mare area, are discharged the waters from the Isalnita Power Plant, Doljchim, Craiova municipality, as well as the waste water from the Podari platform.^[1] Doljchim Craiova and R.A. Craiova water are units with significant weight in the pollution of the Jiu river, which makes the water of the Jiu river downstream from the discharge of the two large units to be of category II quality.^[1]

The purpose of this paper is to evaluate the cytological response of the *A. majus* L. species treated with polluted water from the Jiu River in the Oltenia region (Romania) and to determine its potential to be used as a test plant for monitoring water quality.

MATERIALS AND METHODS

The seeds of *A. majus* L. were purchased from the central market of Craiova city. The polluted water samples were collected from the Jiu river, which crosses the neighboring region of Craiova city, at Podari platform area, coordinates^[19]: 44°15'N 23°47'E (Figure 1).



Figure 1. Map data Podari (Dolj County, Romania).

A. majus L. seeds were soaked in the Jiu water sample for 72 h, time required for the appearance of meristematic roots and their growth to lengths about 1.3-1.5 cm. The experiment was carried out in the laboratory, at room temperature ($\pm 22^\circ\text{C}$). After that, the roots were fixed in ethyl alcohol and acetic acid (3:1) for 24 h, hydrolysed for 5 min in HCl 1N and staining by the Feulgen-Rossenbeck method. It was squashed on the slide with cover slip and observed under optical microscope (model Kruss, manufacturing in Hamburg, Germany). Were established an untreated control variant and a treated variant (V1) with three replicates (R1-R3). For each replicates variant were made 5 microscopic preparations and were visualized 1000 cells per replicate. The mitotic index (MI%) and the index of the cytological anomalies (CA%) were calculated using the following formulae:

Mitotic index (MI%) = total number of cells in division / total number of analysed cells $\times 100$;

Cytological anomalies index (CA%) = total number of aberrant cells / total number of cells in division $\times 100$. Data were expressed as the mean $\pm$ standard deviation (SD). The differences between treatment means were compared using the LSD-test at a probability level of 0.05% subsequent to the ANOVA analysis.

RESULTS AND DISCUSSION

The cytotoxicity and genotoxicity potential of the polluted water to *A. majus* L. meristematic cells were estimated by observing cytological parameters, respective MI index (Table 1) and CA index (Table 2).

It was found that in the variant exposed to the action of the polluted water, in all three replicates, the mitotic index was significantly reduced from 28.42% (control) to 17.15% (V1R3); 15.06% (V1R2) and 14.95% (V1R1). These values suggest the strong mitodepressive effect of polluted water on meristematic cells of *A. majus* L. MI is considered to reliably identify the presence of cytotoxic pollutants in the environment.^[17, 25] In our experience, the decrease of MI was between 47.40% (V1R1), 47.01% (V1R2) respectively 39.66% (V1R3). Similar results were reported by Radić et al. (2010), who reported a decrease of the mitotic index in *Allium cepa* cells after treated with polluted water by more than 40% compared to the untreated control.^[25]

Table 1: The mitodepressive effect of polluted water to *A. majus* L.

Time (h)	Variants	MI $\pm$ SD (%)
72	Control	28.42 $\pm$ 0.82
	V1R1 (treated)	14.95 $\pm$ 0.61*
	V1R2 (treated)	15.06 $\pm$ 0.39*
	V1R3 (treated)	17.15 $\pm$ 0.46*

MI=mitotic index; SD=standard deviation. The results are expressed as the mean $\pm$ SEM; *significant at $p \leq 0.05$, as compared to the control variant (LSD-test at a probability level of 0.05% subsequent to the ANOVA analysis).

Table 2: The genotoxic effect of polluted water to *A. majus* L.

Time (h)	Variants	S	R	Cm	NA	CA $\pm$ SD (%)
72	Control (untreated)	0	0	1.02	0	1.02 $\pm$ 0.24
	V1R1 (treated)	4.02	1.27	2.14	3.01	10.44 $\pm$ 0.54
	V1R2 (treated)	3.61	2.10	4.28	6.18	16.71 $\pm$ 0.62
	V1R3 (treated)	8.75	3.54	2.96	5.82	21.07 $\pm$ 0.36

S=sticky chromosomes; R=Ring chromosomes; Cm=C-mitosis; NA=nuclear alteration; CA=cytological anomalies; SD=standard deviation

A variety of bioassays has been used to demonstrate the mutagenic activity of industrial effluents and surface waters was demonstrated by many bioassays.^[21, 22, 32]

The decrease of mitotic activity was accompanied with induction of a number of cytological anomalies

compared with the untreated control, namely: sticky chromosomes, ring chromosomes; C-mitosis and nuclear alteration (Figure 2).

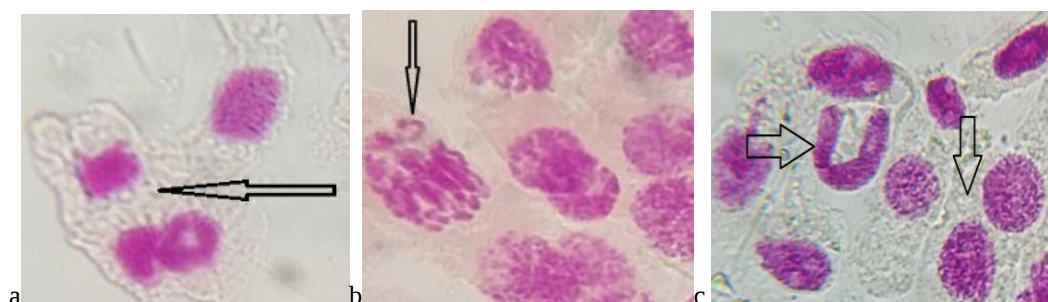


Figure 2. Some of cytogenetic anomalies identified in meristematic cells of *A. majus* L. under action of polluted water: sticky telophase (a); ring chromosome (b); nuclear alteration: nucleus with irregular shape and binucleated cell (c).

Chromosomal anomalies are considered concrete proofs of genotoxicity.^[3, 4, 5, 11, 26, 28] The cytological anomalies identified in the *A. majus* L. meristematic cells indicate the plant sensitivity to the genotoxicity phenomenon induced by exposed to polluted water. Other authors have reached similar conclusions, but in experiments with the *Allium cepa* under action of polluted water.^[9, 23, 25]

The most common cytological abnormalities were stickiness chromosomes: 3.61% (V1R2), 4.02% (V1R1) și 8.75% (V1R3), followed by nuclear alterations: 3.01% (V1R1), 6.18% (V1R2) și 5.82% (V1R3).

According to Fiskesjö (1997), stickiness is considered a common sign of toxic effects on chromosomes probably leading to cell death.^[12] Stickiness may be caused by genetic and environmental factors and several agents like glycidol^[24] or heavy metals.^[29] Carità and Marin-Morales (2008) suggests that cell damage may be transmitted to subsequent generations, possibly affecting the organism as a whole, as well as the local biota exposed to the effluent discharge.^[9]

In this study, the results indicate the presence of aberrant ring chromosomes in all three replicates, their frequency being 1.27% (V1R1), 2.10% (V1R2) and 3.54% respectively (V1R3). Ring chromosomes are not stable during cell division, often are eliminated during cell division^[20] and can form interlocking or fused rings.^[30] Ring chromosomes may form in cells following genetic damage by mutagens like radiation, but they may also arise spontaneously during development. Kumar (2018) reported the presence of ring chromosomes in *A. cepa* cells exposed to the action of some antibiotics.^[16] Ring chromosomes are formed when a chromosome was breaks and the ends are reuniting in a ring shape.

C-mitosis cytogenetic abnormalities were identified in meristematic cells of *A. majus* L. with a frequency of 1.02% (control), 2.14% (V1R1), 4.28% (V1R2) and 2.96% (V1R3). Similar results were reported by other authors, after testing the cytological response of the species *A. cepa* exposed to the action of polluted waters^[9, 23, 25] or the evaluation of cytotoxicity and genotoxicity of samples of drinking water, wastewater

and surface water using SOS/umuC assay with *Salmonella typhimurium* TA1535/pSK1002 and MTT assay with human hepatoma HepG2 cells.^[32]

Another type of cytogenetic abnormalities identified in meristematic cells of *A. majus* L. was nuclear alterations. The frequency of this type of cytological abnormality recorded values of 3.01% (V1R1), 6.18% (V1R2) and 5.82% (V1R3), whereas in the control variant no such anomalies were identified. At interphase, nuclear alterations were characterized by the presence of binucleated cells and nuclei with irregular shapes. Similar results were reported by Souguir et al. (2018) but in an experiment with *Vicia faba* seeds treated with Sodium Chloride.^[31]

CONCLUSION

The cytological anomalies identified in the *A. majus* L. meristematic cells indicate the plant sensitivity to the genotoxicity phenomenon induced by exposed to polluted water and suggests the potential of this species to be used as a test plant for assessing and monitoring water quality. However, this hypothesis may require additional testing.

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