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Study regarding the cytotoxic potential of cadmium and zinc in meristematic tissues of basil (*Ocimum basilicum* L.)

Irina Petrescu[1], Ioan Sarac[1], Elena Bonciu[2], Emilian Madosa[1], Catalin Aurelian Rosculete[2,*], Monica Butnariu[1]

[1] *Banat's University of Agricultural Science and Veterinary Medicine "Regele Mihai I al României" Timisoara, Romania*
[2] *University of Craiova, Faculty of Agronomy, Craiova, Romania*
*Corresponding author. E-mail: catalin_rosculete@yahoo.com

Abstract. The cytogenetic study on the meristematic tissues of basil (*Ocimum basilicum* L.) aimed to evaluate some cytotoxic effects induced by two heavy metals (cadmium - Cd and zinc - Zn) applied in three different concentrations: 10, 50 and 100 ppm. Cytogenetic tests reveal a decrease of the mitotic index and the occurrence of various chromosomal aberrations following heavy metal treatments. The cell division was significantly affected, especially in the case of Cd treatment, which showed the highest degree of toxicity in all variants compared to control variant. Instead, Zn has a lower degree of toxicity but only at concentrations of 50 ppm and 100 ppm. Types of chromosomal aberrations were relatively varied, being randomly distributed and concentration dependent, for both Cd and Zn. Were observed cells with large nucleus and disorganized-looking; interphases with pyknotic nucleus; cells with laggard chromosomes, pyknotic and sticky chromosomes, as well as cells with telophase bridge. The results reveal that Cd (at all tested concentrations) and Zn in concentrations higher than 10 ppm exhibit significant cytotoxic potential to *Ocimum basilicum* L. as a result of the effects reported in cell divisions of the meristematic tissues. We can also appreciate that the *Ocimum basilicum* L. species could be used as a test plant to determine the degree of soil pollution with heavy metals.

Keywords. Basil, cadmium, chromosomal aberrations, mitodepresive, zinc.

INTRODUCTION

Basil (*Ocimum basilicum* L.) is an herbaceous, annual and aromatic plant belonging to the *Lamiaceae* family. Basil is currently grown in many other parts of the world. Due to medical and culinary properties, as well as the spiritual and symbolic connotations that have been in the culture of the Romanian people since ancient times, basil is one of the most appreciated aromatic plants in Romania, along with rosemary, mint and sage. The basil is used as a seasonal functional food, being used both in tea and fresh salads, due to its health benefits. Functional foods have an important contribution to improving the quality of life (Butnariu and Caunii, 2013).

Basil is a plant used not only in the food industry but also in the pharmaceutical and perfume industry. For example, from a pharmaceutical point of view, the basil may be used as a nutritional supplement or therapeutic drug to protect against aspirin-induced gastric ulcers, a common problem resulting from the use of aspirin (Abd El-Ghffar *et al*., 2018).

Heavy metals are identifiable components in the environment, occurring in significant concentrations and under natural conditions. In the 21st century, the metaliferic loading of air, water, soils, and consequently of plants, animals and the human body became an urgent concern for nature pollution.

The aim of this study was to determine how the basil (*Ocimum basilicum* L.) responded to increasing Cd and Zn concentrations in terms of changes in cellular activity and especially in chromosomes structure. The vegetal meristematic tissues that are used for testing the effects of chemicals on chromosomes should be easy to obtain and less expensive and from this point of view, the basil can be suitable.

MATERIALS AND METHODS

Plant material

Dry seeds of *Ocimum basilicum* L. belong to the Genovese variety was placed in glass Petri dishes on filter paper. Three treatment variants with 4 replicates were performed for each of the heavy metals experienced (Cd and Zn). Solutions for the treatment of seeds have been obtained by dissolving the respective amounts of heavy metals in distilled water. Equal volumes of the different concentrations of cadmium nitrate $Cd(NO3)_2$ and zinc nitrate $Zn(NO3)_2$ solutions (10, 50 and 100 ppm), respectively were administered while the control was treated with distilled water. These concentrations have been established taking into account that basil is an aromatic and medicinal herbaceous plant that reacts easily to any stressful environmental factor, being easily contaminated with heavy metals during growth. The seeds (in the amount of 100 seeds per every variant) were germinated in climatic chamber (model Binder KBF 720, Binder manufacturer, USA), at 22°C. After 72 hours, the basil roots that grew to a length of 1-1.5 cm were cut and processed for microscopic preparation.

Microscopic preparations

The biological material were fixed with a mixture of absolute ethyl alcohol and glacial acetic acid in a volume ratio of 3:1 for 24 hours at 6°C in the refrigerator, followed by hydrolysis with 1 N hydrochloric acid for 5 minutes at room temperature. The stage of the meristematic roots staining was performed using the Feulgen-Rossenbeck method (Baik *et al.* 2017; Rosculete CA *et al.* 2019). Colouring was achieved in a basic fuchsine solution, in concentration of 10%. The microscopic slides were prepared using the squash technique (Asita Okorie *et al.* 2017).

Five slides for each variant were analysed for calculating the mitotic index and the chromosomal aberration frequency. The same slides used to calculate the mitotic index were studied to identify the chromosomal aberration. All slides were examined using a Kruss microscope with digital camera (Kruss manufacturer Hamburg, Germany).

Statistical analyses

Statistical analysis was done using MS Excel 2007. The data obtained were analysed to determine the effects of Cd and Zn treatments on the mitotic activity to *Ocimum basilicum* L. The mean and standard error (SE) were calculated for the mitotic index (MI) and differences between treatment means were compared using the LSD-test at probability level of 0.05% (Botu and Botu, 1997) after ANOVA analysis.

The mitotic index (MI) was calculated according to Balog (1982):

$$MI\ (\%) = \frac{\text{Total number of cells in division}}{\text{Total number of analysed cells}} \times 100$$

The index of the chromosomal aberrations (CA) and the percentage of germination (G) were also calculated:

$$CA\ (\%) = \frac{\text{Total number of aberrant cells}}{\text{Total number of cells in division}} \times 100$$

$$G\ (\%) = \frac{\text{Germinated seed}}{\text{Total seed}} \times 100$$

RESULTS

The heavy metals have differently influenced seed germination and root length to *Ocimum basilicum* L. as can be seen in Table 1. The inhibitory effect on germination is evident to the highest concentration of heavy

Table 1. Influence of cadmium and zinc on the seeds germination and root length to *Ocimum basilicum* L.

Variants	Germination (%)	Root length (X ± SE) (cm)
V1 (Control)	93.33	2.26±0.37
V2/Cd/10 ppm	80.00	1.16±0.19
V3/Cd/50 ppm	56.66	0.26±0.04
V4/Cd/100 ppm	23.31	0.21±0.04
V2/Zn/10 ppm	93.33	1.28±0.13
V3/Zn/50 ppm	74.11	1.18±0.19
V4/Zn/100 ppm	70.00	0.65±0.09

metals (V4/100 ppm) at which the germination percentage was 23.33% for Cd and 70.00% for Zn. Also the concentration of 50 ppm, both to Cd and Zn, inhibited the germination of basil seeds in the proportion of 56.66% (Cd) and 74.11% respectively (Zn).

The highest germination percentage was recorded to V2/Zn/10 ppm variant (93.33%, percentage equal to that of the untreated control).

As for the increase in length of the roots, the highest values were recorded in the variants with the lowest concentrations of heavy metals: V2/Zn/10 ppm (1.28 ± 0.13 cm) respectively V2/Cd/10 ppm (1.16 ± 0.19 cm). The most powerful inhibitory effect was found in the V4/Cd/100 ppm variant, where the average length of the roots was 0.21 ± 0.04 cm, compared to the control (2.26 ± 0.37).

Table 2 presents the results of the effects of Cd and Zn on the mitotic index and the cell division phases to *Ocimum basilicum* L. A significant reduction (p=0.05) of the mitotic index compared to the control was observed in all treatment variants.

Mitotic index value decreased with the increase concentration of heavy metal solutions. Thus, the intensity of mitotic activity was decreasing in order of treatment with Cd to Zn treatment. The higher mitodepresive effect was found in the treatment of Cd at the concentration of 100 ppm, when MI was 9.23%, i.e. 79.8% lower mitotic activity compared to control variant. However, in all variants treated with Cd, there was a significant decrease in the mitotic index compared to the control (10.26% - V3/50 ppm and 12.64% - V2/10 ppm).

In case of the Zn-treated variants, the decrease in the mitotic index was also correlated with the increase in the concentration of heavy metal, but the strongest mitodepresive effect compared to the control was found only at the concentration of 100 ppm (V4 - 17.29% and 50 ppm (V3 - 29.14%). In low concentrations (10 ppm), Zn did not negatively influence the values of the mitotic index as compared to control variant.

From point of view of the cell distribution on mitotic phases, the highest percentage was registered by prophase, followed by telophase, metaphase and anaphases in all the analysed variants, including the control.

Frequency of cells in prophase ranged from 73.47-85.61% for Cd-treated variants and 75.54-84.11% for Zn-treated variants. The frequency of cells in metaphase ranged from 7.33% (V4/Zn/100 ppm) to 10.29% (V2/Cd/10 ppm). On the other hand, the frequency of cells in anaphase stage ranged from 1.58% (V4/Cd/100 ppm) to 4.82% (V3/Zn/50 ppm). The smallest values of the mitotic index of telophase compared to control were recorded at the highest concentrations of heavy metals: 3.70% (V4/Cd/100 ppm) and 6.54% (V4/Zn/100 ppm) respectively.

Heavy metals tested induced a high number of mitotic aberrations when compared with control. The increase of mitotic aberrations was dependent on the increasing treatment concentrations (Table 3). The types of chromosomal aberrations identified in meristematic

Table 2. Mitotic index (%) and the cell division phases (%) to *Ocimum basilicum* L. treated with different concentrations of cadmium and zinc nitrate.

Variants	TCN	MI ± SE %	MI_P %	MI_M %	MI_A %	MI_T %
V1 (Control)	500	45.82±0.68	75.35	6.35	1.93	16.37
V2/Cd/10 ppm	500	12.64±0.42*	73.47	10.29	1.86	14.38
V3/Cd/50 ppm	500	10.26±0.35*	78.36	9.74	1.64	10.26
V4/Cd/100 ppm	500	9.23±0.34*	85.61	9.11	1.58	3.70
V2/Zn/10 ppm	500	43.61±0.63	76.28	7.46	2.01	14.25
V3/Zn/50 ppm	500	29.14±0.60*	75.54	9.22	4.82	10.42
V4/Zn/100 ppm	500	17.29±0.58*	84.11	7.33	2.02	6.54

TCN = Total cells number; MI = Mitotic index; MI_P = Mitotic index of Prophase; MI_M = Mitotic index of Metaphase; MI_A = Mitotic index of Anaphase; MI_T = Mitotic index of Telophase; SE = Standard error; * Significant at level 5% (p=0.05).

Table 3. Type and percentage of mitotic aberrations induced by cadmium and zinc on the meristematic roots to *Ocimum basilicum* L.

Variants	Mitotic aberrations (%)					Total aberrations (%)
	PN	PC	L	S	B	
V1 (Control)	0	0	0	1.05	0	1.05
V2/Cd/10 ppm	4.03	5.27	3.89	5.09	1.23	19.51*
V3/Cd/50 ppm	6.21	8.63	4.03	7.26	1.58	27.71*
V4/Cd/100 ppm	8.15	12.86	6.23	10.04	2.42	39.70*
V2/Zn/10 ppm	0	1.04	1.53	2.01	0	4.58
V3/Zn/50 ppm	3.05	1.01	2.03	2.34	0.89	9.32
V4/Zn/100 ppm	2.84	2.63	3.82	4.01	1.21	14.51

PN = Pyknotic Nucleus; PC = Pyknotic Chromosomes; L = Laggards; S = Stickiness; B = Bridges; * Significant at level 5% (p=0.05).

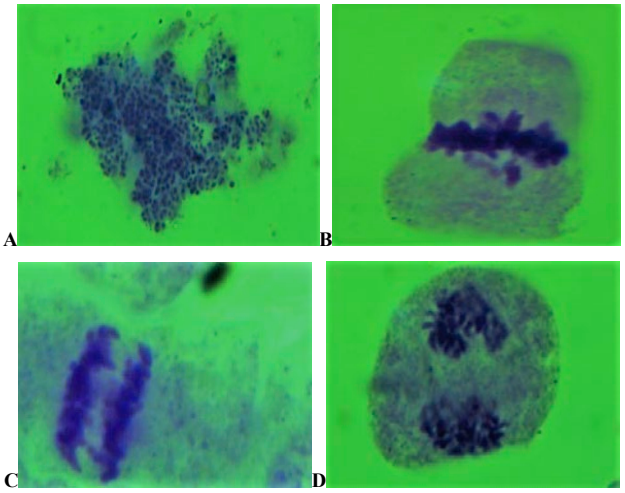

Figure 1. Some chromosomal aberrations identified in meristematic cells of *Ocimum basilicum* L. exposed to Cd and Zn: pyknosis (A); sticky metaphase whit laggards chromosomes (B); bridges (C); disturbed telophase whit pyknotic chromosomes (D).

cells of *Ocimum basilicum* L. were interphases with pyknotic nucleus; pyknotic and sticky chromosomes, cells with laggard chromosomes, as well as cells with telophase bridge.

The most common types of chromosomal aberrations were stickiness and pyknosis while the least frequent were bridges. Compared with the control variant, total chromosomal aberration rate recorded insignificant values for all variant exposed to Zn, from 4.58% (V2/Zn/10 ppm) to 14.51% (V4/Zn/100 ppm). On the other hand, in all variants exposed to Cd treatment total chromosomal aberration recorded significantly positive values from 19.51% (V2/Cd/10 ppm) to 39.70% (V4/Cd/100 ppm) respectively.

DISCUSSION

The contamination of soil and water by heavy metals is a major environmental problem. In this regard it presents an ecotoxicology risk for food chains because of strongly toxic properties of these elements for all human beings (Lassoued *et al.* 2014; Bonciu *et al.* 2018; Coroian *et al.* 2017; Puia *et al.* 2019). Understanding the phenomenon of bioaccumulation of heavy metals in living substance is of extremely complex. This contamination can have very long-term effects (Bilal *et al.* 2014; Lassoued *et al.* 2014; Bonciu *et al.* 2018).

Heavy metal pollution is one of the most serious problems of industrialization, who affects significantly soil and biodiversity and its impact continues to increase (Bae *et al.* 2016), due to these metals' non-biodegradability and high toxicity (Chul Kong, 2013). Generally, heavy metals are dangerous because they tend to bioaccumulate and can cause altered physiological and metabolic processes to plants or disturbing the metabolism of essential elements (Petrescu *et al.* 2015; Sarac *et al.* 2015; Wójcik and Tukiendorf 2014; Butnariu 2012; Mohanpuria *et al.* 2007; Dong *et al.* 2006).

In our experiment, the seed germination of *Ocimum basilicum* L. was heavily affected by the concentration of heavy metals, especially by Cd, which exhibited the highest degree of toxicity. Instead, zinc recorded a lower degree of toxicity and only at concentrations of 50 ppm and 100 ppm. The inhibition of seed germination with increasing concentration of Cd has been found also in other plants: *Vigna radiata* (Maheswari *et al.* 2017); *Triticum aestivum* (Guilherme *et al.* 2015); *Suaeda salsa* (Liu *et al.* 2012); *Spartiana alterniflora* (Mrozek and Funicelli, 1982), etc.

We can appreciate that the highest toxicity in basil seed germination as well as the increase in length of meristematic roots was induced by Cd at the concentration of 100 ppm. In other authors' opinion, at low concentrations Cd is not toxic to plants, but at higher concentrations it is toxic and preferentially accumulates in the meristematic and elongation root zones (Xu *et al.* 2009; Karcz and Kurtyka, 2007). The heavy metals can disturb the nucleolar cycle. Indirect immunofluorescence detects nucleolar material and their movement into the cytoplasm following heavy metal stress (Liu *et al.* 2016).

The length roots of *Ocimum basilicum* L. was influenced differently from one heavy metal to another and from one concentration to the other, the most power-

ful inhibitory effect being found at the highest Cd concentrations. Results suggest that Cd is highly toxic and can affect the metabolism of meristematic roots. Similar results have been reported by Gharebaghi *et al*. (2017) to two basil species (*Ocimum basilicum* L. and *Ocimum basilicum* var. *Purpurescens*).

Cytogenetic tests on *Ocimum basilicum* L. show a decrease of the mitotic index following heavy metal treatments. The mitodepresive effect of cadmium was obvious even at the lowest concentration (10 ppm). The other authors results showed that Cd causes irregularities in mitotic activity to *Pisum sativum* (Fusconi *et al*. 2007) and *Allium sativum* (Xu *et al*. 2009) and can induce increased frequency of the chromosomal aberrations to *Allium cepa* (1.5 times more than in control group), while mitotic index was significantly decreased (Evseeva *et al*. 2001). To *Allium cepa*, Cd affected the spindle and decreased anaphase and telophase stages while the metaphase stage was increased.

In the presence of certain external stimuli, the cellular progress can be blocked in one of the phases of the cell cycle or cell division, and their action is called mitoinhibition. Mitogens act to overcome intracellular braking mechanisms that block cell cycle progression, and their action is called mitostimulatory. Any deviation from the orderly and directed progression of the cell cycle, and respectively, of mitosis and cytokinesis, is reflected in a state of cytotoxicity and genotoxicity (Bonciu *et al*. 2018; Rosculete E *et al*. 2019) and some chromosomes variation (Bouziane *et al*. 2019).

Some research shows that the electron energy loss spectroscopy (EELS) and electron spectroscopic imaging (ESI) are good methods for identifying sites of localization of heavy metals at the sub-cellular level in cell organelles, cytoplasm or cell walls and clarifying the process involved in their uptake, transport and deposition or detoxification in plant cells (Liu and Kottke 2003, 2004).

The results of previous investigations indicate that heavy metals including Cd and Zn at excessive concentration can disturb cell division process and induce CA comprising c-mitosis and lagging chromosomes, anaphase bridges, and chromosome stickiness in the root tips of *A. cepa* (Liu *et al*. 1995). During mitosis, metal ions can interfere with the proper positioning of nucleolar organizing regions on chromosomes. Under metal stress, an obviously toxic phenomenon appears in nucleoli of root tips of *A. cepa* (Bonciu *et al*. 2018).

The results of this study highlight the strong cytotoxic effect of Cd to *Ocimum basilicum* L. even at low concentrations of 10 and 50 ppm. The most common types of chromosomal aberrations were stickiness; sticky chromosomes can lead, in opinion of some authors, to cell death (Singh, 2015; Karaismailoğlu, 2017). In most cases the percentages of abnormal mitotic phases were seen to increase with increasing concentration, this result being recorded in other studies also (Samanta and Bandyopadhyay, 2012; Verma *et al*. 2016; Şuţan *et al*. 2018).

The cytotoxicity effect of Zn occurred only at concentrations higher than 10 ppm. In low concentrations, Zn did not negatively influence the values of the mitotic index, the percentage of chromosome aberrations being insignificant.

Of the two heavy metals tested, Cd showed the highest degree of cytotoxicity and inhibits normal growth to *Ocimum basilicum* L. The results also suggest that basil may be used for bio-greening of soil, since it absorbs the heavy metals and synthesizes them in the cells. Besides, the decontamination of soils polluted with heavy metals through phytoremediation is one of the cheapest and simplest methods, and from this point of view, the cultivation of basil involves very low costs.

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