Studii și Cercetări Științifice. Biologie
Scientific Study & Research. Biology
Print ISSN 1224-919X Online ISSN 2457-5178

2025, 34 (2), 9-16

MODULATION OF THE MITOSIS PROCESS IN THE ROOT APEX CELLS OF *CARUM CARVI* L. AFTER CAFFEINE TREATMENT

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KEYWORDS

Carum carvi
Mitotic index
Chromosomal aberrations
Caffeine.

ABSTRACT

The present study, focused on investigating the effect induced by treatment with caffeine in different concentrations, applied for 6 and 12 hours respectively, highlights the response of cells from the root meristem of caraway (*Carum carvi* L.) at the level of mitosis process. To achieve the proposed objectives, the following indicators were evaluated: mitotic index, frequency of mitotic division phases, as well as the frequency of division aberrations. Thus, the application of caffeine treatment induced a decrease of division cells frequency, in general, proportional to the increase in concentration and the extension of the duration of seeds exposure to the tested alkaloid, so that at maximum dose and 12 hours of treatment, cell division was blocked. Furthermore, the genotoxic effect of caffeine is evidenced by the increase of chromosomal aberrations frequency, this parameter being almost 4 times higher than the Control variant.

INTRODUCTION

During 1820–1827 period, some substances isolated from green coffee beans, guarana and tea were called "caffeine" or "caffeine", "guaranine" and "theine", depending on the plant from which they were extracted. However, later (1838–1840), it was proven that they were the same compound, generally called caffeine (Arnaud, 1987; Arnaud, 2005). This substance has been identified as a natural constituent in about 100 plant species, being present in the seeds, nuts and leaves of various plants and, therefore, founded not only in coffee beans, tea leaves or guarana beans, but also in cocoa beans or cola (Batish et al., 2008; Faudone et al., 2021).

Caffeine is a common ingredient of beverages such as coffee, tea, and various soft drinks, and is used as a flavouring agent in foods and beverages and as an active component in a wide-ranging of pharmaceuticals and drugs (Abu-Hashem et al., 2024; Ashihara et al., 2001; Heckman et al., 2010). Chemically, caffeine (1,3,7-trimethylxanthine) is a trimethylxanthine having three methyl groups at positions 1, 3, and 7. It is a purine alkaloid belongs to methylxanthine alkaloids family, along with theophylline and theobromine (Ashihara et al., 2017; Ebrahimi et al., 2013; Kato et al., 2004; Monteiro et al., 2016).

Being a purine alkaloid, can be included into a DNA chain, instead of adenine, during the active synthesis of the DNA macromolecule, affecting both DNA structure and synthesis (S-phase), can abrogates G2/M cell-cycle checkpoint at high concentrations, allowing the damaged cells to proceed to the next phases of the cell cycle, being an efficient inhibitor of the DNA repair mechanism (Hatzi et al., 2015; Moura et al., 2021). Thus, caffeine is considered to be a weak mutagen by some authors (Kihlman et al., 1974; Kihlman et al., 1982), the mutagenic potential may improve some quantitative and qualitative characters of different plants (Cheng et al., 1990; Kumar et al., 2004; Vichi et al., 2024; Yousuf et al., 2023), and the allelopathy properties were also identified (Pham et al., 2019; Sugiyama et al., 2016; da Rosa et al., 2025; de Melo et al., 2018).

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https://doi.org/10.29081/scsb.2025.34.2.02

Furthermore, caffeine, alone or in combination with other agents, has been reported to have cytotoxic, genetic, carcinogenic, and teratogenic effects, altering DNA replication, can induce genetic mutations in bacteria and fungi, and can produce chromosomal aberrations in both plant and animal cells but the results are generally contradictory and confusing (Ransom, 1912; Haynes et al., 1984; Nehlig et al., 1994).
Taking into account the aforementioned, the objective of the present study is to analyse the effects induced by caffeine, as a potential mutagenic agent, on mitotic division in the root apex of *Carum carvi* L.

MATERIALS AND METHODS

Healthy dill seeds served as biological material. For treatment, four concentrations of caffeine (0.1%, 0.25%, 0.50% and 1%) were applied for 6 and 12 hours, at room temperature, in the dark. As a control, the seeds were maintained under the same conditions in distilled water. After treatment, the seeds were washed thoroughly (three times) and placed in Petri dishes on wet filter paper, in an incubator set at 22°C. At 7-10 mm root length, the material was fixed in ethanol and acetic acid mixture (3:1) for 12 hours. Until processing, the rootlets were placed in 70% ethanol and kept in a refrigerator (+4°C). Microscopic analyses were preceded by hydrolysis of the seedlings for 5 minutes in HCl solution prepared in distilled water (1:1). Carbol fuchsin solution was used to stain the meristem tips for 24 hours (Singh, 2018). Based on the squash method, five microscope slides/variant were prepared and ten microscopic fields were analysed using a Nikon Eclipse 600 microscope. The representative microphotograph, were taken at a 100x immersion objective with a Nikon Cool Pix 950 digital camera.

Cytogenetics parameters calculation

The percentage of cells in mitosis was calculated using next formula:

$$\text{Mitotic index (\%)} = \text{total cells in mitosis/total analysed cells} \times 100 \quad (1)$$

The frequency of the mitotic phases was calculated as ratio between number of the cells in specific phase (prophase, metaphase and anaphase) and total analysed cells x 100, as following equation:

$$\text{Division phases (\%)} = \text{Stage of mitosis/ total cells in mitosis} \times 100 \quad (2)$$

The frequency of chromosomal aberrations was assessed as percent between type of aberration and total cells in division x 100, according the formula:

$$\text{Type of aberration (\%)} = \text{number of the cells with particular aberration/total cells in mitosis} \times 100 \quad (3)$$

At least 7000 cells/variant were counted, including the control.

RESULTS AND DISCUSSION

Mitotic index

The response of the *Carum carvi* L. radicular cells after caffeine application depends both concentration and the duration of treatment. As shown in Figure 1, after 6 hours of the cumin seeds caffeine exposure, the mitotic index decreased proportionally with the increase of alkaloid concentration, ranging from 9.08% (Control) to 7.36% (1% caffeine). Extend the duration of treatment up to 12 hours exerted a slight mitogenic effect at the minimum concentration, the mitotic index increasing from 8.56% (Control) to 9.43% (0.1% caffeine). On the other hand, at this longer treatment period, the decrease of cells division frequency reached of 5.82% at the 0.5% caffeine variant, the reduced of this parameter being over 1.5 times than Control. Moreover, the maximum concentration of the alkaloid (1%) conducted to the total blocking of mitotic activity in cumin root cells. Our results are in accordance with some studies that mention the increasing of the mitotic index in *Triticum aestivum* L. (Tudose et al., 1972-1973) and *Helianthus annuus* varieties (Vlad Rusen et al., 2007) after caffeine application in low concentrations. The short-term treatment (3 h) has induced an intensification of mitotic process in root meristems of *Anethum graveolens* and *Phaseolus vulgaris* L. (Truță et al., 2011) after 0.25 and 0.5% caffeine exposure. Immersion of the seeds for 12 h in 0.01% and 0.05% caffeine stimulated the mitotic process in hemp (Truță et al., 2018). However, 24 h of treatment have strong inhibitory effect on cell division and growth of *Triticum aestivum* L. (Iosob et al., 2018), and grown of two *Capsicum annuum* varieties in the caffeine for six days was associated with suppression of cell division even at 0.25% caffeine (Roșu et al., 2006). These data highlight the importance of the treatment

conditions, both in terms of duration, concentration and the moment in which caffeine is used (before or after seeds germination), as well as depending on the species/variety of plants tested.

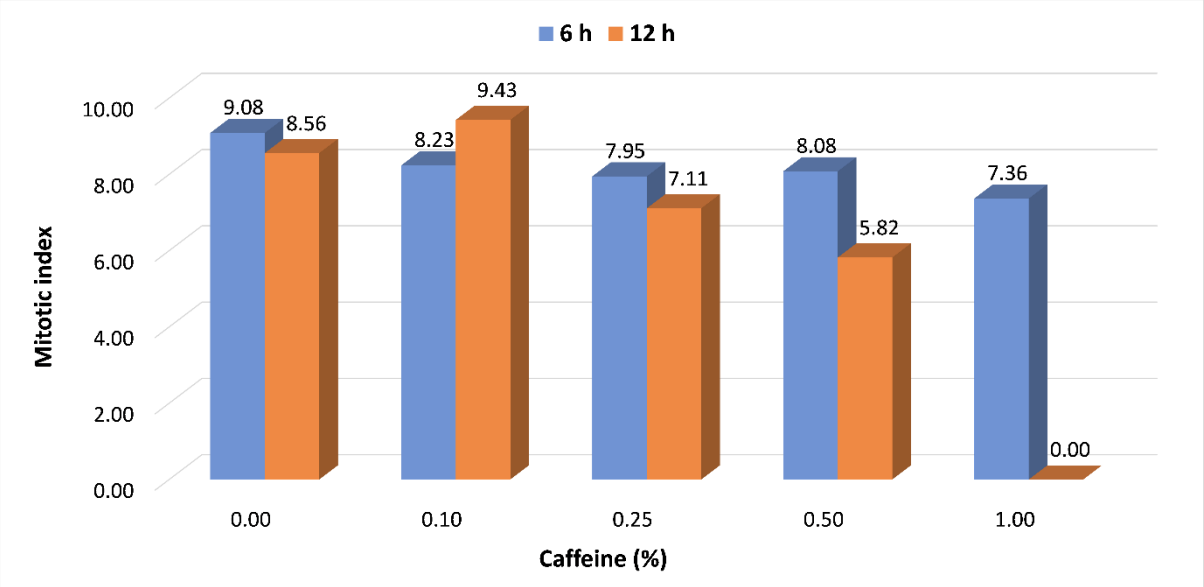

Figure 1. Mitotic index in *Carum carvi* L. root meristem cells after caffeine treatment

Mitotic division phases

As can be seen in Figure 2, for all variants, the distribution of the four mitotic phases is similar, in the sense that the highest proportion is held by cells in prophase, followed by those in metaphase, telophase and anaphase. The 6-hours of treatment was accompanied with decrease of prophases frequency, excepting 0.1% caffeine application. Regarding metaphases, no significant changes have been occurred.

In the case of 12-hour caffeine treatment, important variations were identified in prophases frequency and slight fluctuation of cells in metaphase, anaphase and telophase. Thus, the frequency of prophases decreases from 5.68% (Control) to 2.85 (0.50% caffeine), and increases to 6.51% upon 0.1% caffeine exposure (Figure 2). A negligible increase in metaphases percent was observed after 0.5% caffeine application, reaching of 1.69%, compared to 1.54% in Control variant.

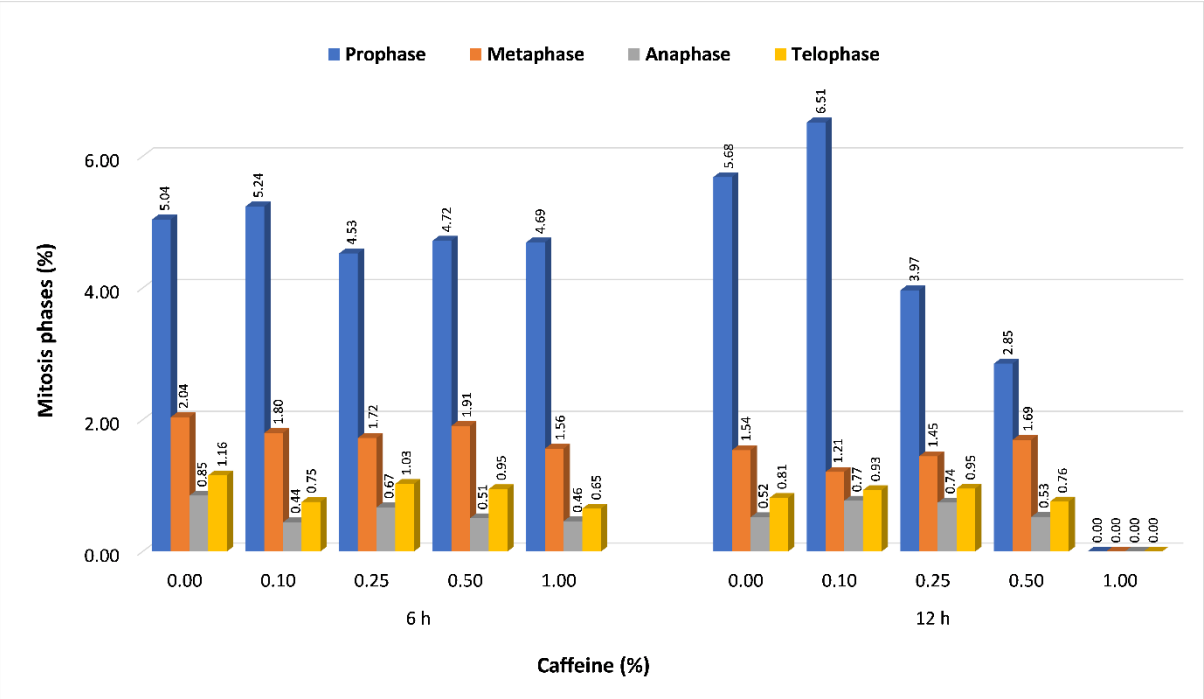

Figure 2. Mitotic division phases in *Carum carvi* L. root meristem cells after caffeine treatment

Frequency and type of mitotic division aberrations

Treating of cumin seeds with caffeine for 6 h increased the frequency of division aberrations in root cells with values between 0.49% (Control) and 1.95% (1% caffeine), the rate intensification of this indicator being 3.98 times higher than untreated Control. The mitoclastic effect of caffeine after 12 hours of treatment is evidenced by the increase of aberrations frequency, proportional to the increase in alkaloid concentration up to the 0.5 variant, having into account that the 1% caffeine exposure induced total suppression of cell division (Figure 3).

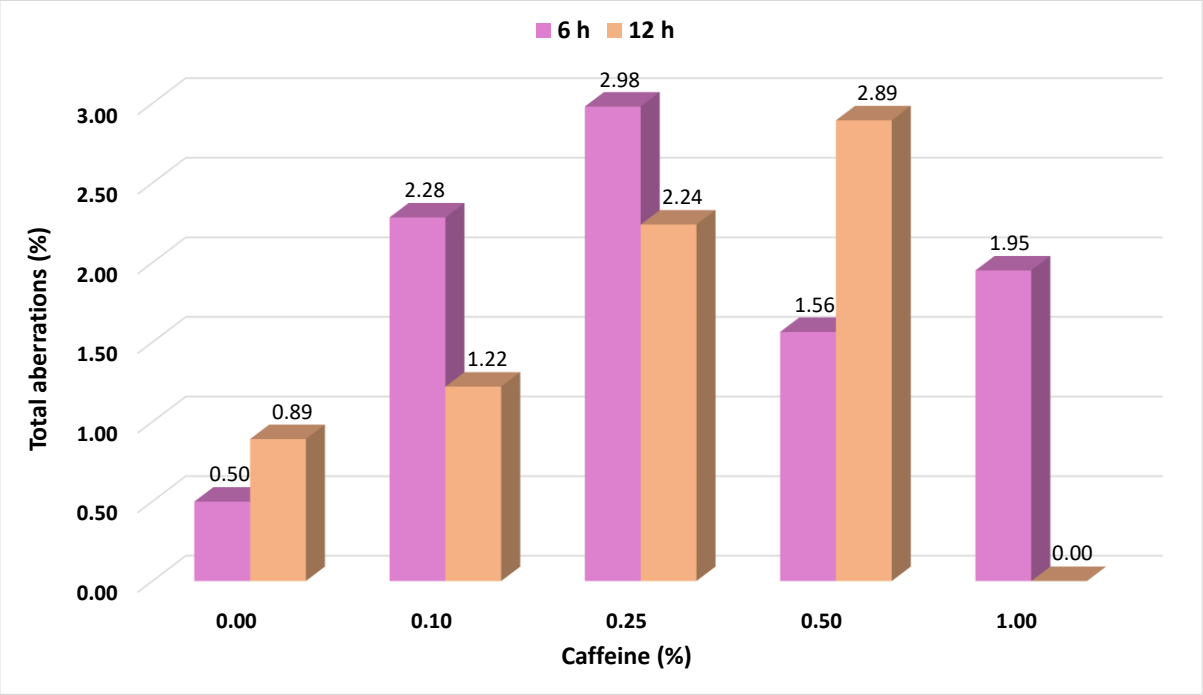

Figure 3. Aberration frequency in *Carum carvi* L. root meristem cells after caffeine treatment

Both after 6-hour and 12-hour treatment, the following types of aberrations were reported: bridges (single and multiple), ana-telophases with lagging or vagrant chromosomes, multipolar ana-telophases, metaphases with vagrant chromosomes, as well as C-metaphases (Tables 1-2, Figures 4-5).

Table 1. The main aberration types in *Carum carvi* L. root meristems cells, after 6 h caffeine treatment

Caffeine (%) - 6 h	Total aberrations (%)	A-T aberrations (%)						Metaphase aberrations (%)	
		Single bridge	Multiple bridges	Lagging chrs.	Vagrant chrs.	Multipolar A-T	Multipolar A-T and multiple bridges	Vagrant chrs.	C-metaphase
0.00	0.50	0.25	0.00	0.00	0.00	0.00	0.25	0.00	0.00
0.10	2.28	0.33	0.65	0.16	0.33	0.33	0.49	0.00	0.00
0.25	2.98	0.66	0.50	0.00	0.00	0.50	0.33	0.66	0.33
0.50	1.56	0.13	0.26	0.13	0.26	0.26	0.00	0.39	0.13
1.00	1.95	0.26	0.26	0.00	0.00	0.39	0.26	0.52	0.26

Table 2. The main aberration types in *Carum carvi* L. root meristems cells, after 12 h caffeine treatment

Caffeine (%) - 12 h	Total aberrations (%)	A-T aberrations (%)						Metaphase aberrations (%)	
		Single bridge	Multiple bridges	Lagging chrs.	Vagrant chrs.	Multipolar A-T	Multipolar A-T and multiple bridges	Vagrant chrs.	C-metaphase
0.00	0.89	0.15	0.00	0.00	0.30	0.00	0.00	0.00	0.00
0.10	1.22	0.37	0.12	0.00	0.12	0.24	0.12	0.00	0.24
0.25	2.24	0.75	0.19	0.19	0.00	0.56	0.19	0.19	0.37

0.50	2.89	0.18	0.72	0.00	0.00	0.36	0.54	0.36	0.72
1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

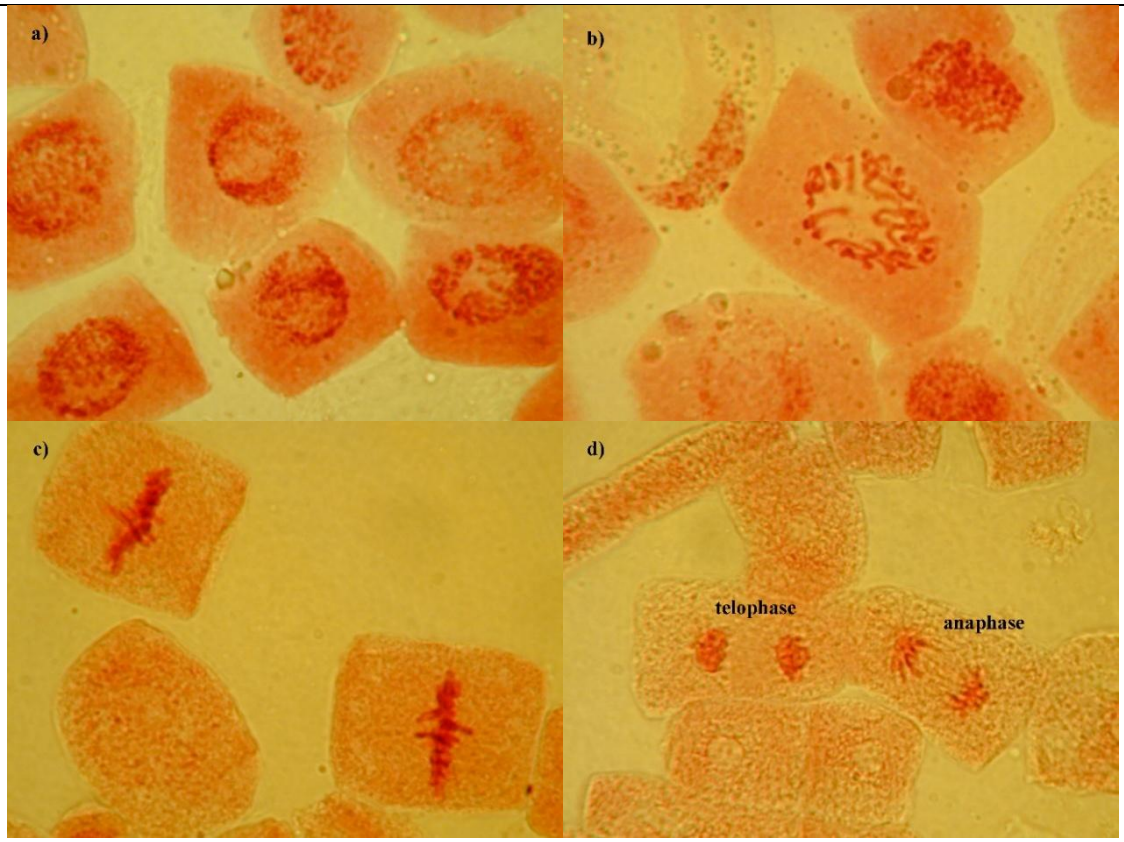

Figure 4. Normal mitotic phases in *Carum carvi* L. root meristem: a) – interphase; b – prophase; c – metaphase; d – anaphase and telophase)

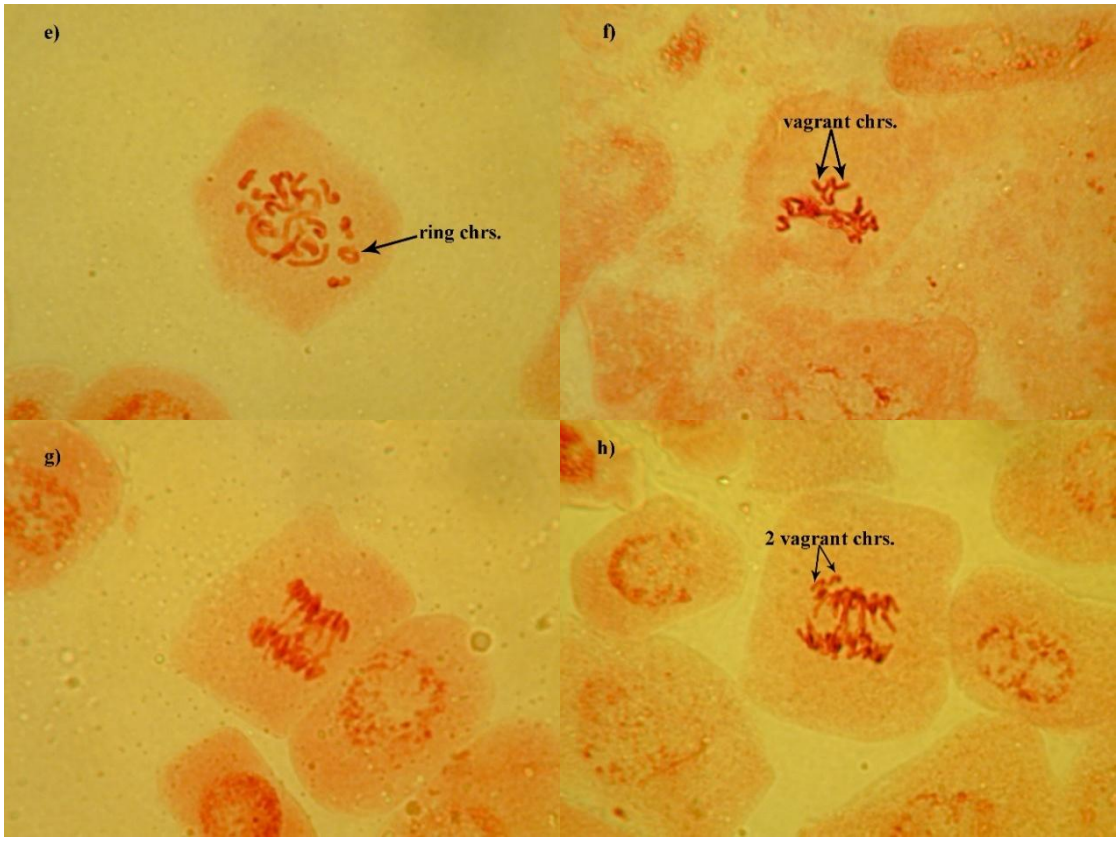

Figure 5. Aberrant mitotic phases in *Carum carvi* L. root meristems after caffeine application: e – prometaphase with vagrant and ring chromosomes; f – metaphase with vagrant chromosomes; g – multiple bridges in A-T; h – multiple bridges and vagrant chromosomes

The increased frequency of ana-telophases with aberrations, as a result of the use of caffeine, has been reported in numerous cultivated species, including: *Vicia faba* L. (Tudose et al., 1979), *Secale cereale* L. (Tudose et al., 1978a), *Fagopiron esculentum* Mönch. (Tudose et al., 1978b), *Helianthus annuus* L. (Vlad Rusen et al., 2007), *Capsicum annuum* L. (Roșu et al., 2006), *Phaseolus vulgaris* L. and *Raphanus sativus* L. (Truță et al., 2011), or *Cannabis sativa* (Truță et al., 2018).

Several studies show the effect of caffeine on the cytokinesis process in plants. Thus, early studies of Giménez-Martín et al. (Gimenez-Martin et al., 1965) present the partial or total cytokinesis inhibition in *Allium cepa* L. root meristems treated with 0.05, 0.1, 0.2, and 0.5% caffeine, leading to 4% of binucleate cells in root meristematic population. In addition, the phenomenon was associated with remarkable mitotic aberrations formation at 0.2 and 0.5% solutions treatment, represented by 1.5% chromosome bridges of the total anaphases. Also, Kihlman et al. (1971), Navas et al. (1986) showed the binucleate cells induction in *Allium cepa* L. flat violet variety after 2.5 mM caffeine treatment, this process being more intense by application of increasing vanadate concentrations (0.1 and 10 mM), the highest effect being obtained at 1 mM vanadate. Ultrastructural studies (Hepler et al., 1990; Lahouti et al., 2007) present the degradation of cell plate formation after 5 mM caffeine application by blocking of cytokinesis, giving rise to binucleate cells. Some tests performed on stamen hair cells of *Tradescantia virginiana* showed that the caffeine allows the cell plate to appear and grow normally until about 80%, but then led to it to break down until no refractile structure remains (Bonsignore et al., 1985). Moreover, cytokinesis inhibition by caffeine in *Allium cepa* L. meristem cells has shown the antagonism between calcium and/or magnesium and caffeine, involving some aspect of membrane recognition and/or fusion, where calcium and magnesium are essential requirements (Becerra et al., 1978; Lopez-Saez et al., 1982). Furthermore, Maniu et al. (1998) present the clastogenic effect of caffeine by identification of aberrant ana-telophases and a relatively high frequency of micronuclei in wheat root cells. Recent studies (Kumar et al., 2022) highlight the importance of mutagenesis in inducing new genetic variations with the help of natural compounds, such as caffeine. Thus, comparable to our results, the authors present the dose-effect relationship of caffeine in decreasing of active mitotic index, as well as the accumulation of chromosomal aberrations, the response being differentiated in two models, namely, *Anethum graveolens* L. and *Foeniculum vulgare* Mill., the first species presenting a greater sensitivity than the second.

CONCLUSION

Caffeine treatment induced: (i) a decrease in the mitotic index, in a dose-dependent manner; (ii) the accumulation of cells with aberrations, proportional to the increase in concentration and the prolongation of the treatment duration; (iii) the range of aberrations was represented by bridges (single and multiple), lagging or vagrant chromosomes, multipolar ana-telophases, as well as C-metaphases.

ACKNOWLEDGEMENTS

The financial support was provided through the Nucleu Program as part of the 2022-2027 National Research, Development and Innovation Plan, funded by MCID, projects no. PN23020401 and PN23020402, contract no.7N/03.01.2023.

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