MITOSIS ANA-THELOPHASE CHROMOSOMAL ABERRATIONS INDUCED BY UV IRRADIATION UNDER THE ANTIOXIDATIVE PROTECTION OF VITAMIN C, BY *CALENDULA OFFICINALIS* L.

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Keywords: UV, radiation, chromosomal aberration, meristem apex.
Abstract: due to the stratospheric ozone layer depletion, the researches focused in the last decades on the study of solar radiations reaching Earth surface. The target of the study was to establish the biological response of *Calendula officinalis* L. to UV irradiation, under the anti-oxidative protection of vitamin C, (which helps next to UV induced anti-oxidative enzymes, in the protection against a large variety of products capable to induce free radicals formation).
Our investigations were focused on detecting chromosomal aberrations which occurs during cells in division in meristem root tips, under UV irradiation for 15 or 30 minutes, in the presence or absence of vitamin C, reported to the non-irradiated control samples. Regarding the mitotic index, it could be noticed an inhibition of cell division frequency under UV stress for all irradiated variants, not depending of presence or absence of vitamin C, positively correlated with the increase of irradiation period. Maximal chromosomal aberrations frequency, were induced by UV radiations in the root tips of seedlings germinated in the absence of vitamin C, decreasing in the presence of vitamin C, due to the antioxidant protective role of this. In the absence of UV irradiation, the chromosomal aberrations frequency was lower comparing with irradiated variants, for all seedlings, even if germinated in the presence or absence of vitamin C. Between chromosomal aberrations were detected: bridges, expulsed and retardate chromosomes, fragments.

INTRODUCTION

Due to the increased level of UV-B radiation (280-315 nm), reaching Earth surface as a consequence of stratospheric ozone layer depletion (ozone selectively filters out the shorter UV wavelengths), there might appear in plants as fixed organisms, either stress or acclimatization responses, consisting in molecular, physiological or morpho-anatomical changes.

A modern question is which irradiation effects on vegetal organisms are, concerning the influence of radiations at the level of biochemical and genetic processes, and on the variability of characters in the aim of culture plants amelioration.

Our study intends to determine the effects of UV-B irradiation on *Calendula officinalis* L., at cytogenetically level.

MATERIALS AND METHODS

Biological material: *Calendula officinalis* L. seeds.

Mutagenic agent: UV radiations. Light source: TL 40 W/12 (max 310nm, fluency 19.6 Wm-2 30cm irradiation high).

Experimental variants:

1: “Control” (100 non-irradiated seeds germinated 72h in dark, on filter paper moisturized with 10 ml distilled water,

2: “Vitamin C” (100 non-irradiated seeds germinated 72h in dark, on filter paper moisturized with 10 ml vitamin C solution 2%)

3: “UV 15” (100 seeds germinated 72h in dark, on filter paper moisturized with 10 ml distilled water, irradiated with complete UV spectrum, for 15 minutes)

4: “UV 15 + Vitamin C” (100 seeds germinated 72h in dark, on filter paper moisturized with 10 ml vitamin C solution 2% irradiated with complete UV spectrum, for 15 minutes)

5: “UV 30” (100 seeds germinated 72h in dark, on filter paper moisturized with 10 ml distilled water, irradiated with complete UV spectrum, for 30 minutes)

6: “UV 30 + Vitamin C” (100 seeds germinated 72h in dark, on filter paper moisturized with 10 ml vitamin C solution 2% irradiated with complete UV spectrum, for 30 minutes)

Working steps: Roots of 30 seeds from each experimental variant were colored by Feulgen method and microscope slides were prepared following Squash techniques for cytogenetically studies [Cimpeanu et al. 2002].

RESULTS AND DISCUSSIONS

Regarding the percent of cells in division reported to all analyzed cells (mitotic index), it could be observed (**Tab. 1, Fig. 1., Fig. 2.**) an inhibition due to UV irradiation stress (experimental variants 3 and 5) and an increase induced by vitamin C presence (variant 2). In the same time, the irradiation in presence of Vitamin C was less inhibiting (4 and 6). The inhibition of cell division under UV, can be explained as being an protective mechanism of plant against lesions at molecular level. During cell division, DNA is more exposed to potential mutagenic agents, increasing damage risk.

The distribution of dividing cells on division phases is similar for all non-irradiated experimental variants (**Tab. 1**), not depending of presence of Vitamin C, and also for the case of 15 minutes UV irradiation under vitamin C protection. It can be noticed that the most frequent found were prophases, than metaphases, telophases and anaphases.

Changes in the division phases repartition are the same for short time irradiation without vitamin C protection and for 30 minutes UV irradiation time in the presence of vitamin C: prophases, than metaphases, anaphases and telophases.

Regarding the maximal chromosomal aberrations frequency, it could be noticed (**Tab. 2, Fig. 3, Fig. 4**) that it was induced by UV radiations in the root tips of seedlings germinated in the absence of vitamin C, decreasing in the presence of vitamin C, due to the antioxidant protective role of this. In the absence of UV irradiation, the chromosomal aberrations frequency was lower comparing with irradiated variants, for all seedlings, even if germinated in the presence or absence of vitamin C. The most common aberrations type, for all experimental variants, was the retardate chromosome, simple or multiple bridges, sometimes broken bridges**.**

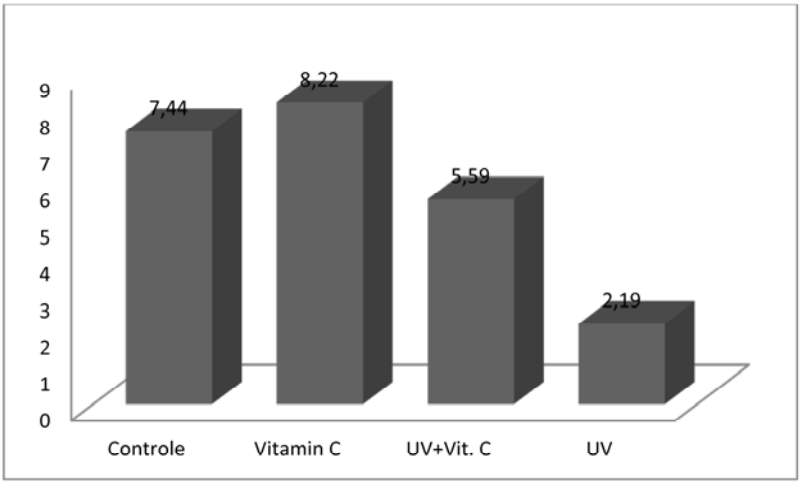

Fig.1. Mitotic index in radicular apex of *Calendula officinalis* L. for different experimental variants, irradiation time 15 minutes

Table 1. The Mitotic Index andthedistribution of cells in division on phases in radicular apex of *Calendulaofficinalis* Ldepending on experimental variant

IRRADIATION TIME	EXPERIMENTAL VARIANT	Total cells	Total cells in interphase		Total cells in division		MITOTIC INDEX	Distribution on cell division phases							
								Prophase		Metaphase		Anaphase		Telophase	
		nr.	nr.	%	nr.	%		nr.	%	nr.	%	nr.	%	nr.	%
15 minutes	Controle	5035	4660	92.56	375	7.44	**7.44**	209	55.73	95	25.33	24	6.4	47	12.53
	Vitamin C	4943	4536	91.78	407	8.22	**8.22**	181	44.47	141	34.64	32	7.86	53	13.02
	UV+Vit. C	4155	3923	94.41	232	5.59	**5.59**	110	47.41	66	28.44	23	9.91	33	14.22
	UV	2590	2533	97.81	57	2.19	**2.19**	24	42.10	12	21.05	11	19.29	10	17.54
30 minutes	UV+Vit. C	2439	2389	97.95	50	2.05	**2.05**	21	42	18	36	7	14	4	8
	UV	2501	2488	99.48	13	0.52	**0.52**	4	30.76	2	15.38	1	7.69	6	46.15

Table 2. The frequency of Ana-Telophases (A-T) in meristematic apex of *Calendulaofficinalis* L. dependingon experimental variant

IRRADIATION TIME	EXPERIMENTAL VARIANT	Total A-T	Total normal A-T		Total aberant A-T	
		nr.	nr.	%	nr.	%
15 minutes	Control	71	69	97.19	2	2.81
	Vitamin C	85	76	89.42	9	10.58
	UV+Vitamin C	56	41	73.21	15	26.78
	UV	21	4	19.04	17	80.95
30 minutes	UV+Vitamin C	11	6	54.54	5	45.46
	UV	7	2	28.57	5	71.42

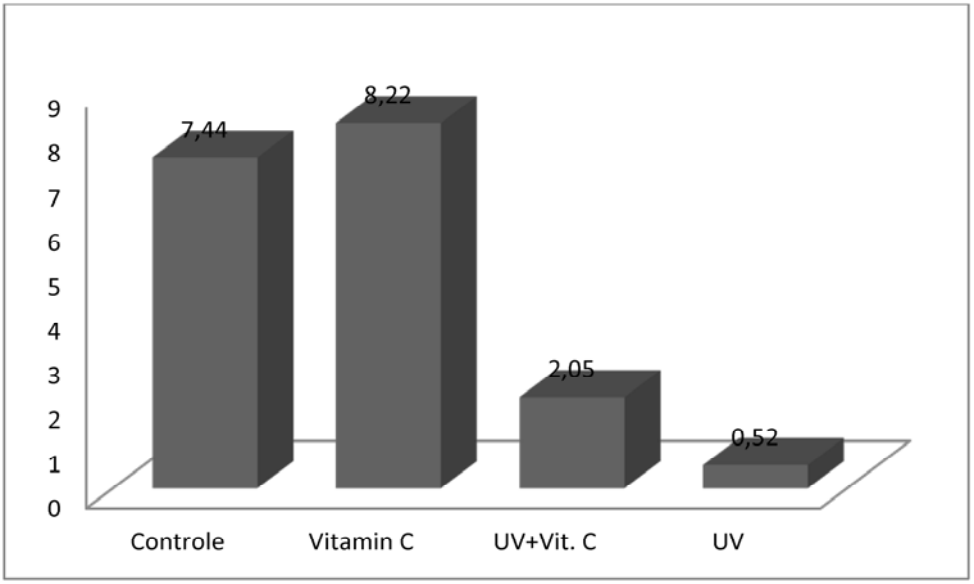

Fig.2. Mitotic index in radicular apex of *Calendula officinalis* L. for different experimental variants, irradiation time 30 minutes

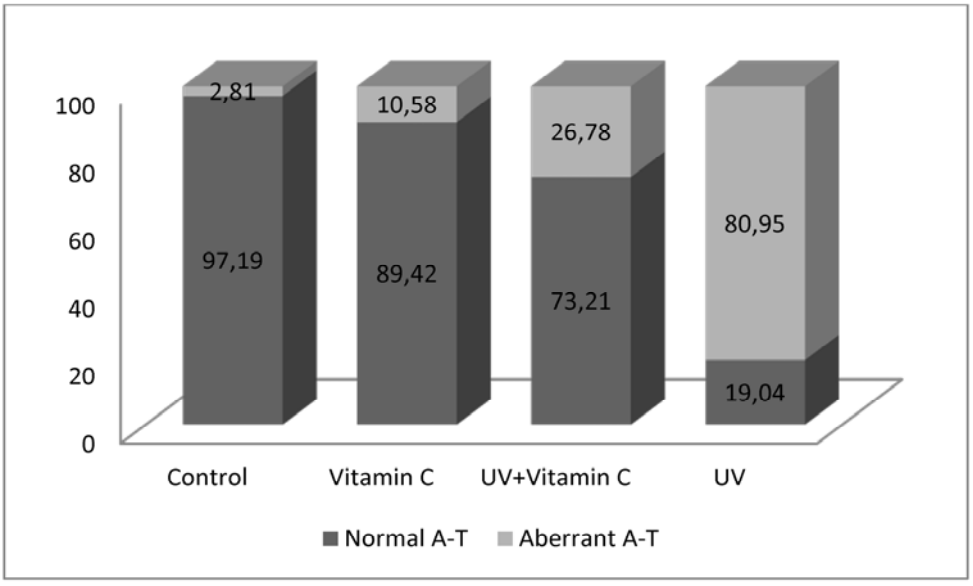

Fig.3. Comparison between the percentage of normal and aberrant A-T, in radicular apex of *Calendula officinalis* L irradiation time15 minutes

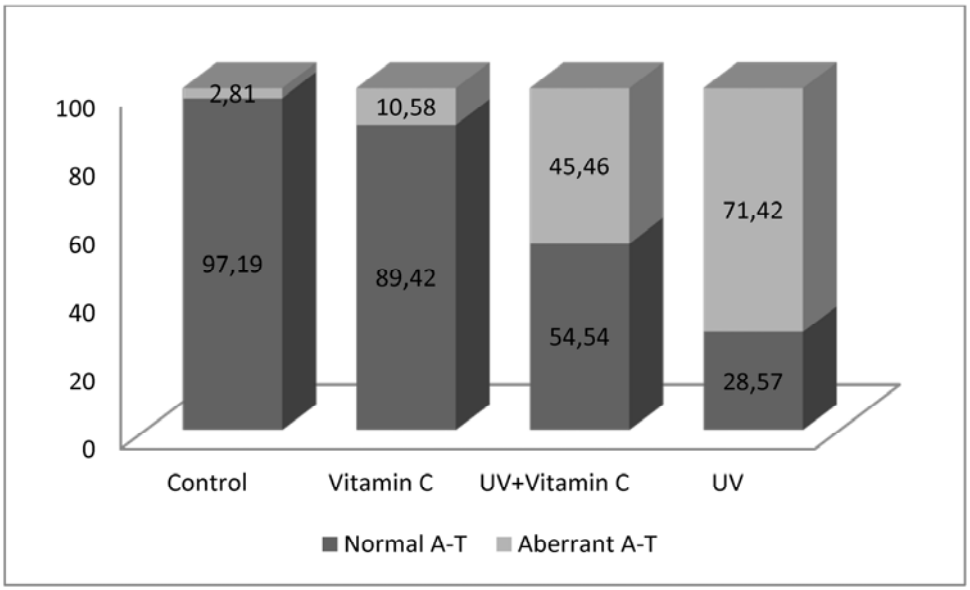

Fig.4. Comparison between the percentage of normal and aberrant A-T, in radicular apex of *Calendula officinalis* L irradiation time30 minutes

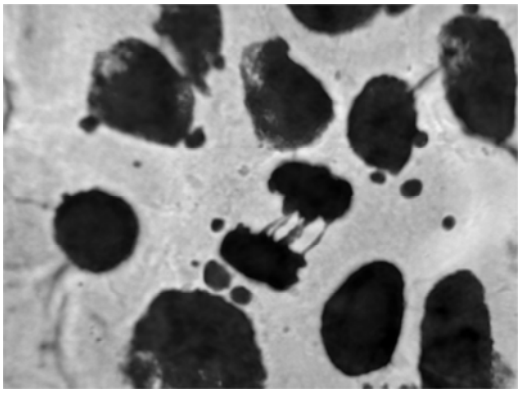

Fig.5. Telophase with multiple bridges

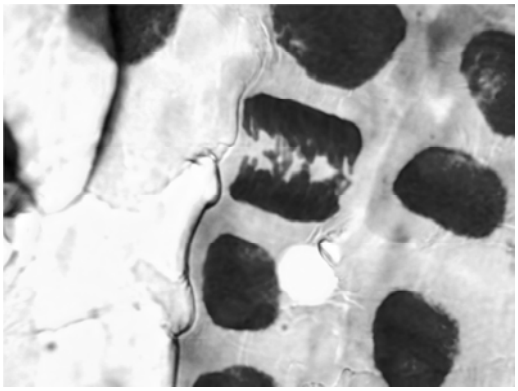

Fig.6. A-T with multiple bridges

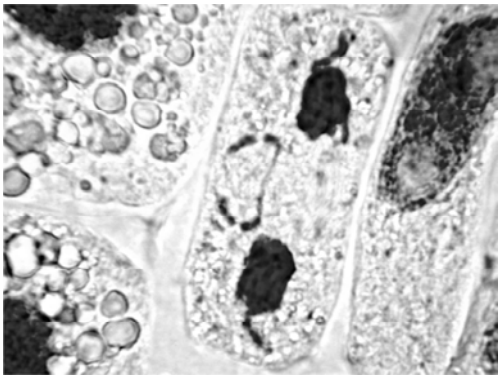

Fig.7. Aberrant telophase, with expulsed genetic material

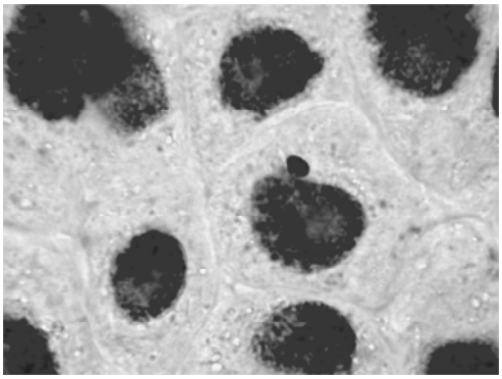

Fig.8. Micronucleus

CONCLUSIONS

UV irradiation induced a decrease of mitotic index, because the inhibition of DNA synthesis, with protective role.

Regarding cell division phases, for all experimental variants, most frequent occur prophases, than metaphases, anaphases and telophases.

The A-T aberrations frequency is highest in the case of UV irradiated seedlings germinated in water, and decrease for irradiated seedlings germinated in Vitamin C solution, suggesting the antioxidant protection.

The higher A-T aberrations frequency in the case of 15 minutes exposure to UV comparing with 30 minutes exposure can be correlated with the very low mitotic index for longer irradiation time, suggesting a disruptive action of the radiation on cell division.

UV induced DNA lesions and destroyed division spindle, leading to chromosomal aberrations types like bridges, retardate and expulsed chromosomes, chromosomal fragments, or combinations between this, during A-T mitosis in root tips of *Calendula officinalis* L.

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