CORRELATIONS BETWEEN THE INDUCTION OF CYCLOBUTYL-PYRIMIDINE-DIMERS AND THE CYTOGENETIC EFFECTS OF UV RADIATIONS AT BEAN (*PHASEOLUS VULGARIS* L.)

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Abstract. *The study was focused on estimating the early effects of UV-B at molecular and chromosomal level and the specific responses of four bean cultivars from Romania. The molecular damage was estimated by analysing the cyclobutane - pyrimidine dimers (CPDs) formation and their photorepair, and the cytogenetic effects by the occurrence and frequency of chromosomal aberrations, which may appear during cell division in root apex. The study proves that for Ardeleana, Avans, Star, and Diva cultivars, UV-B had little influence on the amount of CPDs formation in hypocotyls hook, and the cytogenetic effect is also very similar, the DNA lesions leading to the same types of chromosomal aberrations, with aberrations frequency correlated with the mitotic index, presenting a low sensitivity to increases of UV-B radiation. The choice of Phaseolus vulgaris (LINNAEUS 1753) as biological material for investigations can be explained by the importance of seeds and legumes in humans' nutrition, because of the increased level of high quality proteins, and the high energetically level, and due to the importance in soil amelioration.*

Keywords: *dimmers, aberrations, bean, UV-B.*

Rezumat. Corelaţii între inducerea formării dimerilor ciclobutil pirimidinici şi efectele citogenetice ale radiaţiilor UV la fasole (*Phaseolus vulgaris* L.). *Studiul s-a axat pe estimarea efectelor radiaţiilor UV-B la nivel molecular şi cromosomial şi a răspunsurilor specifice a patru soiuri româneşti de fasole. Leziunile apărute la nivel molecular au fost evaluate în urma analizei formării şi respectiv fotoreparării prin clivare a dimerilor ciclobutan pirimidinici (CPDs), iar efectele citogenetice au fost estimate în urma analizei frecvenţei de apariţie a aberaţiilor cromosomiale, la nivelul apexului radicular. Studiul dovedeşte că în cazul soiurilor: Ardeleana, Avans, Star şi Diva, radiaţia UV-B are o eficienţă scăzută în inducerea formării dimerilor de timină la nivelul cârjei hipocotilare, leziunile apărute la nivel ADN determinând apariţia aceloraşi tipuri de aberaţii cromosomiale indiferent de soi, având frecvenţa corelată cu indicele mitotic, dovedind o sensibilitate redusă la intensificarea iradierii cu UV-B. Alegerea fasolei Phaseolus vulgaris (LINNAEUS 1753), drept material biologic folosit în investigaţii, poate fi explicată prin importanţa folosirii seminţelor şi păstăilor în nutriţia omului, datorită conţinutului ridicat în proteine de calitate, a nivelului energetic ridicat cât şi a importanţei folosirii speciei, drept plantă precursoare în ameliorarea solului.*

Cuvinte cheie: *dimeri, aberaţii, fasole, UV-B.*

INTRODUCTION

In the last decades, the scientific world research focused, because of stratospheric ozone lay depletion, on the effects of UV irradiations (particularly UV-B) on organisms' survival, on the induction of changes at the level of biochemical and genetic processes, and on the variability of characters.

The most important consequence of the stratospheric ozone depletion is an increase in the amount of UV-B (280-315nm) radiation reaching the earth's surface, because ozone selectively filters out the shorter UV wavelengths, (BJÖRN et al., 1999a). The shift of spectral UV composition towards shorter wavelengths has on higher plants damaging effects, including DNA damage, commonly represented by formation of cytotoxic cyclobutane pyrimidine dimmers (CPDs), which can be reversed by splitting of CPDs by subsequent exposure to UV-A – blue light radiation (360-420nm), phenomenon termed photoreactivation (photorepair), via DNA photolyase (PRE) (BUCHHOLZ et al., 1995). DNA damage and repair, illustrated by kinetics of CPDs formation and repair as well as chromosomal aberrations occurred during cell division as response to mutagenic agents has been investigated in several plant species, but information of behavior shown by different cultivars of the same species can be important for the study of individual variability in a population. In the present study, we examined the formation and photorepair of DNA damage and the chromosomal aberrations induced by UV-B radiation in four Romanian cultivars of *Phaseolus vulgaris* L. (LINNAEUS 1753).

MATERIAL AND METHODS

Plant Material and Light Treatments

Biological material: *Phaseolus vulgaris* L. seeds of 4 Romanian cultivars (Diva, Ardeleana, Avans, Star), obtained from S.C.D.A. Podu-Iloaiei Iaşi Romania.

Mutagenic agent: UV-B radiations.

Light Sources for induction of cytotoxic cyclobutane pyrimidine dimmers (CPDs):

Short-wavelength UV radiation was obtained from a Philips TL 40-W/12 fluorescent tube (λ_{max} 310 nm, fluence rate 4.81 Wm^{-2} for 20 cm irradiation height). This light source was used unfiltered (covered with quartz) or filtered through 3mm Schott cutoff filters WG 360 (8, 3 W m^{-2}). The filters cut UV-B radiation with certain wavelength (50% transmission for the given wavelength).

Light Sources for photorepair, via DNA photolyase:

UV-A was obtained from Osram L36-W/73 tubes (3.3 Wm-^{2}), fluence rate 19.6 Wm^{-2} for 20 cm irradiation height.

Irradiation time: 30 minutes for UV-B and 60 minutes for UV-A.

Light sources used for cytogenetic studies designated UV/white light, is a mixture of:

2 L 40 W/73 (UV-A), Osram

2 TL 40 W/18 (Blue Light), Philips

1 TL 40 W/12 (UV-B), Philips.

Fluence rate of UV-B lamp is 3 Wm^{-2} total fluence rate is 8 Wm^{-2}.

The lamps were positioned in the next order: 1, 2, 3, 2, 1.

The Osram L 40 W/73 (UV-A source) includes sufficient UV-B radiation to activate UV-B photoreceptors (BEGGS & WELLMANN, 1985).

Relevant WG type cutoff filters (Schott and gen, Mainz, Germany) were used for the experiment: WG 305 with 11, 9 Wm^{-2} fluence rate, WG 320 (11 Wm^{-2}) and WG 360 (8, 3 Wm^{-2}). The last one was used for UV-B control. The filters cut UV-B radiation with certain wavelength (50% transmission for the given wavelength).

Working steps:

For each experimental variant, 20 seeds of four bean (*Phaseolus vulgaris* L.) cultivars were selected and sown in 300ml humid Vermiculite (Deutsche vermiculite Dämmstoff Gmbh Sprockhövel), in 9/9 cm plastic box. Seedlings were germinated and grown at 25°C for 4 days with controlled conditions of humidity and light/darkness alternation, in a phytochamber.

For testing UV irradiation effects, including DNA damage, represented by formation of cyclobutane pyrimidine dimmers (CPDs), the hypocotyls were cut longitudinally in halves, placed with the cut face on wet filter paper, and covered with a 3 mm quartz plate penetrated by UV or transmission WG360 cutoff filter (50% transmission at the given wavelengths, cutting completely UV-B, representing Control variant for each cultivar).

The hypocotyls halves were then, depending on the experimental variant, frozen in liquid nitrogen for estimating CPDs formation, or subsequent irradiated with UV-A for photorepair.

Six hypocotyls halves for each cultivar were ground for 3 minutes using sand 0.5 ml of CTAB buffer. DNA extraction was carried out essentially as described by TAKEUCHI et al. (1996) using a CTAB-based procedure. DNA damage was assayed by determination of cyclobutane pyrimidine dimers (CPDs) using a method adapted from MORI et al., 1991. DNA samples were denaturized, immobilized on an ELISA (Enzyme Linked Immunosorbent Assay) plate, and CPDs detected using the primary TDM-2 monoclonal antibody and the secondary HRP antibody. The method is based on the detection of the primary bound antibody by the secondary antibody (Sigma, St. Louis) and measuring the absorbance at 490 nm. CPD photorepair was assayed measuring the rate of disappearance of CPDs from DNA by activation of the DNA photolyase after exposing the samples to UV-A.

The rates of the CPDs photorepair in hypocotyls, were calculated as follows:

$$\% \text{ Rep.} = \frac{\sum Q - \sum R}{\sum Q - \sum K} \times 100$$ where % Rep represent the percent of restored dimmers, Q-the UV-B treatment variant, R-the UV-B+UV-A treatment variant and K the Control variant.

For cytogenetic studies: for each cultivar, there were 6 experimental variants (5 treatment variants and 1 control). For treatment variants, 72h old seedlings (15 for each variant) were irradiated in boxes covered with cutoff filters (WG360, WG320, WG305, WG275, Q) for 0,5h; 1,5h; or 3h with the light source described above. For control variant, 15 seedlings (72h old) were kept in dark at 25° C. After irradiation, roots were coloured by Feulgen method and microscope slides were prepared using root tips of 0.5-1 cm, following Squash techniques for cytogenetic studies (CÎMPEANU et al. 2002).

RESULTS AND DISCUSSIONS

After extraction, the DNA containing samples were read at the spectrophotometer at λ260nm respectively λ280nm, to check out if the proportion between the two obtained values are in the range of 1.8-2.0, which is an essential condition before following the next steps. Results were very similar for all the four cultivars, for all experimental variants, as shown in the Table 1.

Table 1. Ratio between readings at λ260nm λ280nm.
Tabel 1. Raportul între absorbțiile la λ260nm şi λ280nm.

Cultivar	Variants		
	Samples irradiated through WG 360 filter (C)	Sample irradiated through Q filter (Q)	Samples irradiated with UV-B followed by UV-A irradiation (R)
Diva	1,93	1,96	1,94
Ardeleana	1,91	1,95	1,95
Avans	1,96	1,95	1,86
Star	1,91	1,95	1,92

Regarding the action of the UV-B irradiation on the induction of CPDs, the results showed that the level of UV-B had little influence on the amount of CPDs formed in hypocotyls in the case of the four Romanian bean cultivars. The unexpected identification of dimmers also in Control samples, can be explained by the unspecific binding of thymine monomers or other azotate base to DNA, or the induction by UV-A in the case of a high energetic flux (Fig.1). UV-B radiation was probably effectively filtered by the UV-B absorbing compounds or CPDs were effectively photorepaired by the action of the DNA photolyase. Estimations of the rate of the CPDs photorepair in hypocotyls, indicate that, after 1h under photorepair conditions (UV-A irradiation) depending on cultivar, between 7.2-16.85 % of the CPDs disappeared (Table 2). This experiment indicate that the investigated bean cultivars presents a low sensitivity to increases of UV-B radiation, the photorepair, even if not effective enough to remove all CPDs, helps in avoiding the increase of DNA damage by this radiation.

It was also observed that for each cultivar, DNA quantity increased due to UV-B irradiation compared with the Control.

For each tested cultivar, on the ELISA plate, there were placed 200ng DNA per cell, in three repetitions: one negative control variant (represented by TE buffer), the Control variant (C) represented by the WG360 filtered UV irradiated samples, the DNA lesion (Q) variant represented by UV-B irradiated samples, and the photoreparation (R) variant, represented by samples irradiated with UV-B followed by UV-A irradiation.

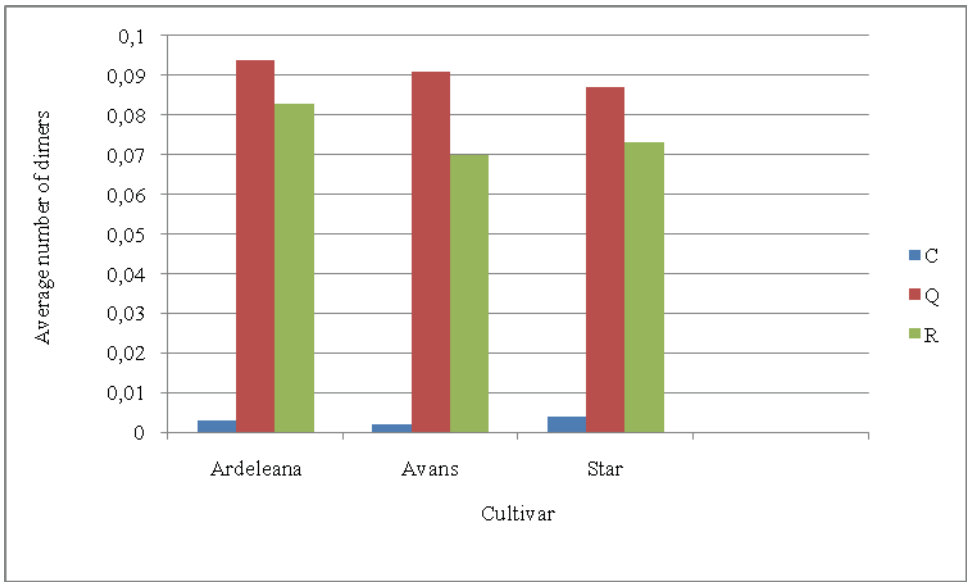

Figure 1. Average number of CPDs induced by UV irradiation at *Phaseolus vulgaris* L.
Figura 1. Numărul mediu de dimeri formați în urma iradierii cu UV la *Phaseolus vulgaris* L.

After reading the **ELISA** plate at DYNEX.MRX at λ490nm, for all investigated cultivars it could be noticed an increase of dimmers number following UV-B irradiation and a decrease after subsequent UV-A irradiation, because of photoreparation, as shown in Fig.1. It can be observed that the percent of restored dimmers is 6.95% for *Diva,* 11.71% for *Ardeleana,* 23.08% for *Avans* and 16.10% for *Star* (Table 2).

Table 2. The percent of restored dimmers for each investigated cultivar.
Tabel 2. Procentul dimerilor clivați în urma fotoreparării, pentru fiecare dintre soiurile investigate.

Cultivar			
Diva	Ardeleana	Avans	Star
6.95%	11.71%	23.08%	16.10%

Knowing the average number of dimmers formed in the case of DNA lesions variant (Q: UV-B irradiated samples), and the average number of dimmers which were not cleaved during photoreparation R, it was calculated the average number of dimmers which were cleaved during photoreparation (Table 3).

Table 3. Average number of dimmers which were cleaved for each investigated cultivar.
Tabel 3. Numărul mediu de dimeri clivați, pentru fiecare soi investigat.

Cultivar			
Diva	Ardeleana	Avans	Star
0.01	0.011	0.021	0.014

Comparing the photoreparation efficiency for the 4 bean cultivars, it can be observed that from this point of view, the most resistant to the damaging UV-B action is Avans, followed by Star, Ardeleana, Diva (Fig. 2). Diva is the most sensitive, because both: highest number of formed dimmers and lowest percent of restored dimmers.

A possible way of explanation can be the distribution of dimmers leading to differences in photoliase accessibility (which is the cleavage enzyme).

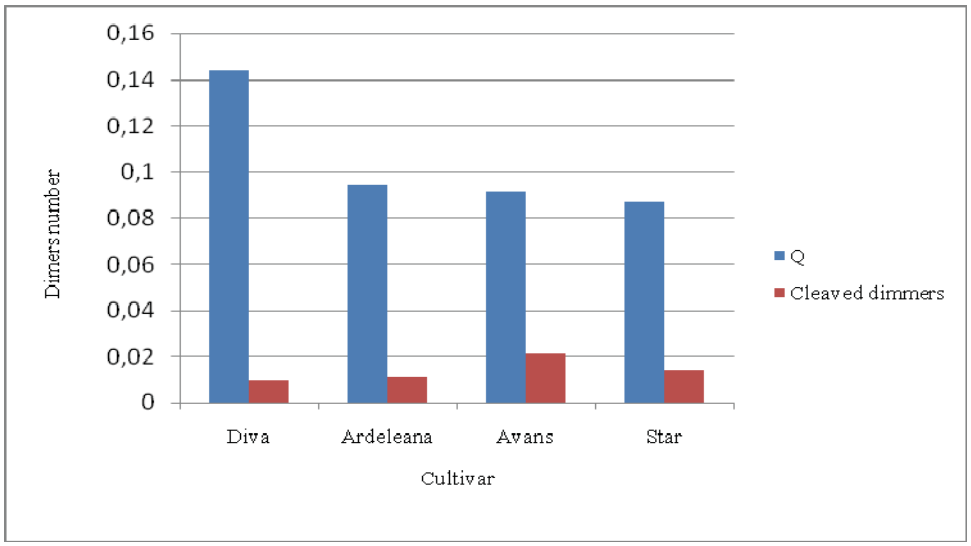

Figure 2. The photoreparation efficiency for the 4 bean cultivars.
Figura 2. Eficiența fotoreparației în cazul celor 4 soiuri de fasole.

The frequency of cells with chromosomal aberrations

If we consider the values induced by different wavelength UV (for **0.5h** irradiation time) in ana-telophase (A-T) of the 4 bean cultivars (Ardeleana, Avans, Star, Diva), it could be noticed that the frequency of cells with chromosomal aberrations increase with the decrease of radiation wavelength (meaning UV-A, to UV-B and finally UV-C kind of radiation). The maximal number and types of aberrations were found in the case of full spectrum UV (filter Q), especially for Diva cultivar, following Star, Ardeleana and Avans.

For **1.5h** irradiation time, results were similar regarding aberrations frequency connected with cultivar type: the frequency of cells with chromosomal aberrations increase with the decrease of radiation wavelength, the maximal number and types of aberrations were found in the case of full spectrum UV.

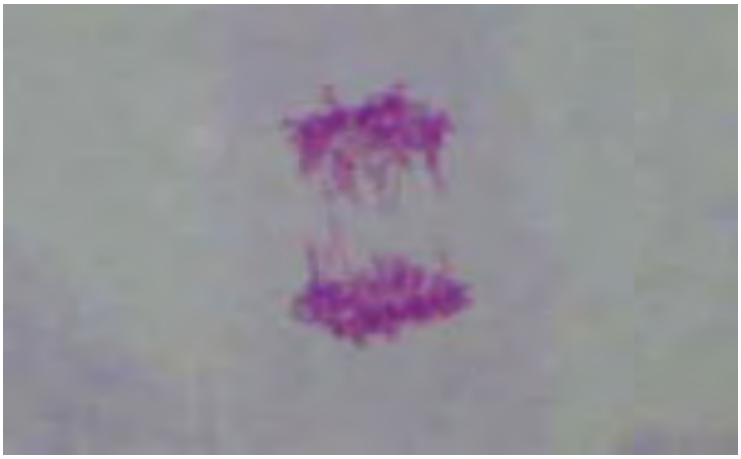

Figure 3. A-T with broken bridges, expulsed, retardate chromosome, Star, WG320, 1.5h.
Figura 3. A-T cu punți rupte, retardatari şi un expulzat, Star, WG320, 1,5h.

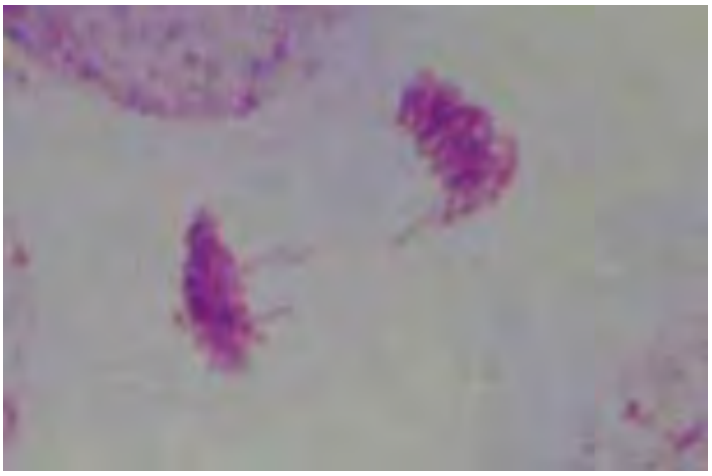

Figure 4. A-T with broken bridge, retardate chromosome Avans, WG275, 1,5h.
Figura 4. A-T cu punțe ruptă şi retardatari, Avans, WG275, 1,5h.

For **3h** irradiation time, it could be observed the increase of aberration frequency for all irradiations variants (WG 360, WG 320, WG 305, WG 275, Q), meaning UV-A, UV-B, UV-C and full spectrum of UV, proving the importance of treatment period next to UV harmfulness (increasing with the decrease of wavelength) in disturbing cell division.

The Ardeleana, Star, Avans and Diva cultivars reacted very similar. Aberrations types were in order of their occurring frequency: simple or multiple bridges, retardate chromosomes, expulsed chromosomes, simple or multiple bridges combined with chromosomes or chromosomal fragments (Figs. 3-5) and in a very low percent some other aberrations types as more than one retarded chromosomes, expulsed genetic material, and multiple division poles.

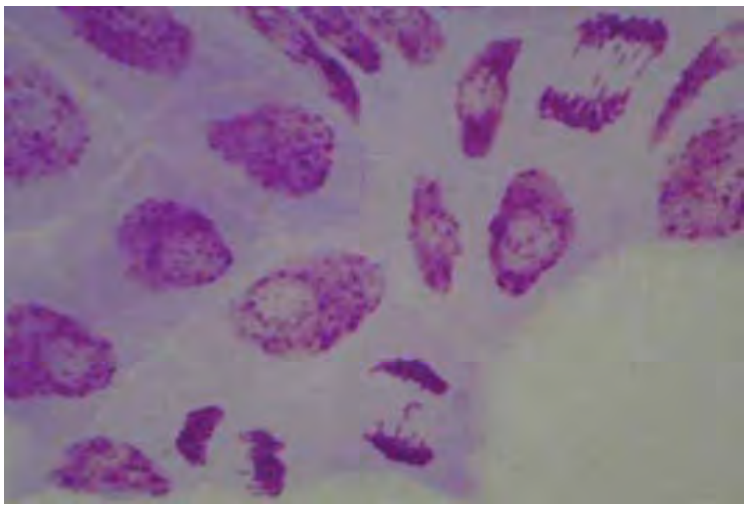

Figure 5. A-T with multiple broken bridges, retardate chromosome Diva, Q 3h.
Figura 5. A-T cu punți rupte, retardatari Diva, Q 3h.

The most frequent aberration types induced by UV, are multiple broken bridges, but could be observed also combinations between bridges and retardate or expulsed chromosomes, one or more retardate chromosomes, unequal and also tripolar ana-telophase (Figs. 4-5).

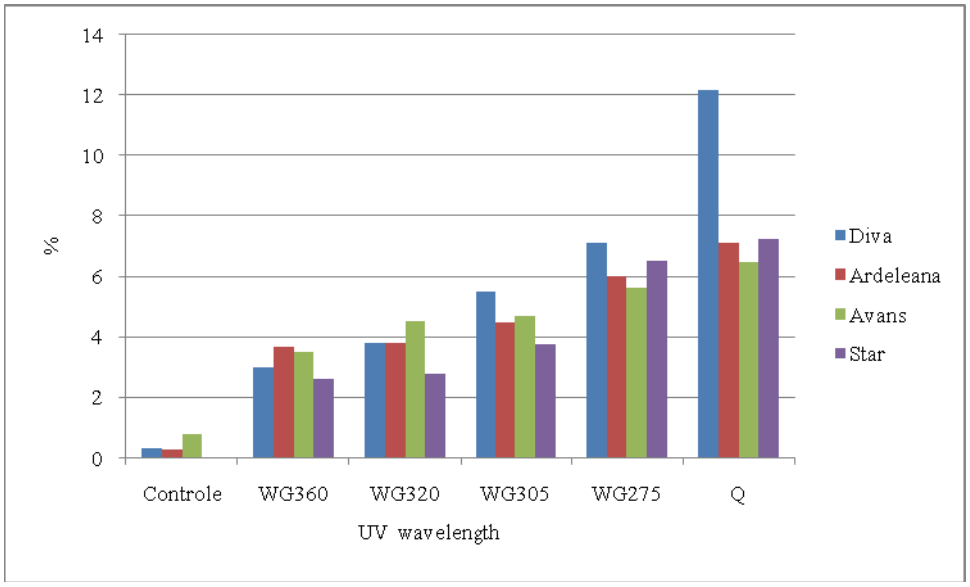

Figure 6. Frequency of aberrant A-T different cultivars of bean, depending on UV wavelength, 0.5h irradiation time.
Figura 6. Frecvența A-T aberante, pentru diferitele soiuri de fasole, in funcție de lungimea de undă a radiației UV, durata iradierii 0,5h.

After analyzing aberrations type, it can be noticed that in the case of all investigated cultivars, UV irradiation induced lesions at DNA level but also affected division spindle. The induction of lesions at DNA level was proven also by the increase of CPD dimmers number following UV-B irradiation.

For all the 4 cultivars, the results regarding the aberrations frequency can be correlated with the mitotic index. The aberrations frequency value increases and the mitotic index decrease (as a plant protection mechanism) correlated with the decrease of UV wavelength and increase of irradiation time (BĂRA & GRAMA-ȚIGĂNAȘ, 2005). The mitotic index decrease, proves an inhibition of cells division, shown that a supra UV-B dose could cause reduction in plant growth and in biomass production, similar to some other studies (SULLIVAN & TERRAMURA, 1989, TOSSERAMS et al., 2001).

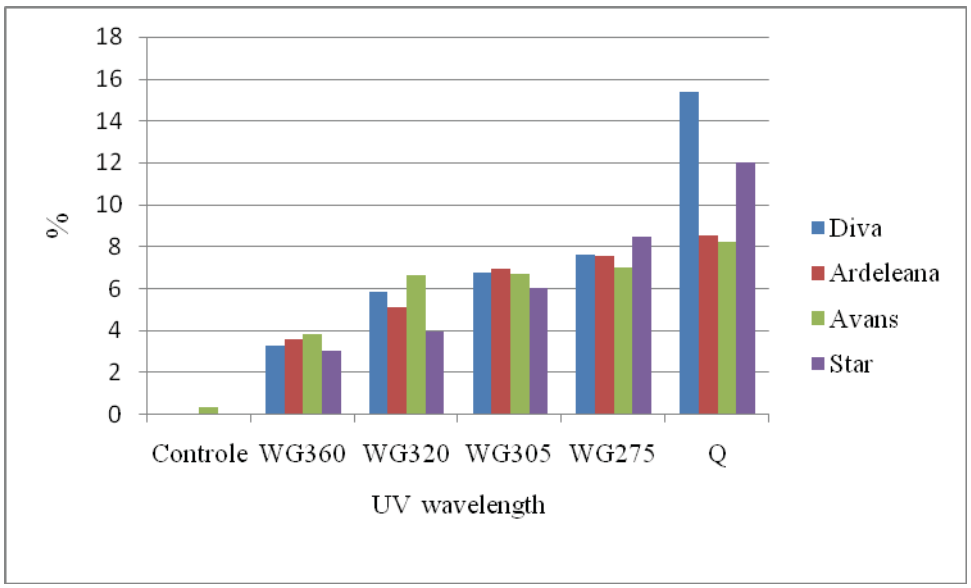

Figure 7. Frequency of aberrant A-T for different cultivars of bean depending on UV wavelength, for 1.5h irradiation time.
Figura 7. Frecvența A-T aberante, pentru diferitele soiuri de fasole, in funcție de lungimea de undă a radiației UV, durata iradierii 1,5h.

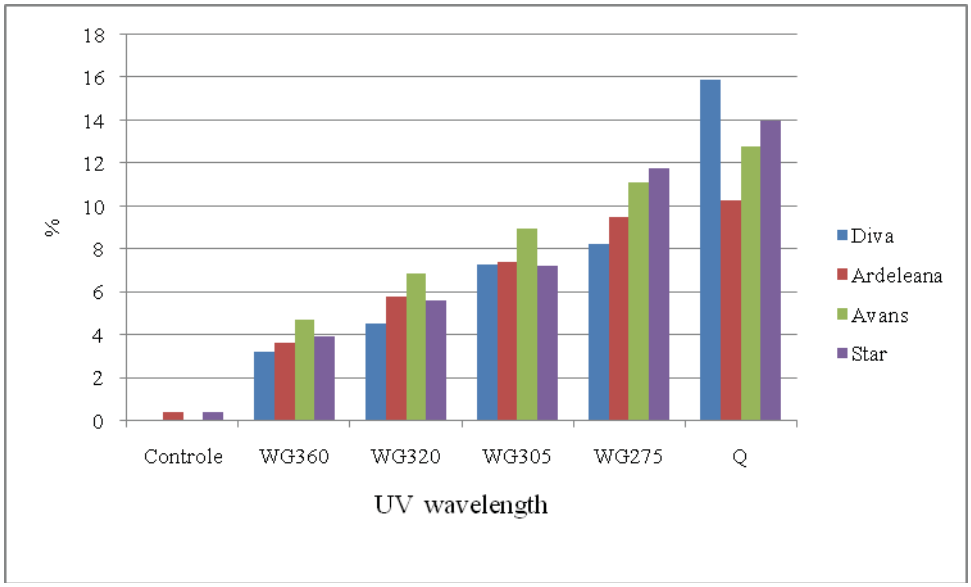

Figure 8. Frequency of aberrant A-T for different cultivars of bean, depending on UV wavelength, for 3h irradiation time.
Figura 8. Frecvenţa A-T aberante, pentru diferitele soiuri de fasole, in funcţie de lungimea de undă a radiaţiei UV, durata iradierii 3h.

It can be observed (Figs. 6-8) that for all irradiation time variants, the frequency of aberrant A-T, reaches the highest values for Diva cultivar, in the case of full spectrum UV (filter Q), in concordance with the DNA level damage, proved by the highest number of induced dimmers and lowest percent of restored dimmers. Persisting lesions occurred due to the dimmers which were not cleaved after photoreaparation, leaded to chromosomal aberrations, the frequency and types of which, were, as expected increased for Diva cultivar comparing with the other 3 cultivars (Avans, Star and Ardeleana, which reacted quite similar).

CONCLUSIONS

1. UV-B had little influence on the amount of CPDs formed in hypocotyls hook, on aberrations type or aberrations frequency, for the Romanian *Phaseolus vulgaris* L. investigated cultivars, all showing a low sensitivity.
2. The frequency of aberrant A-T reaches the highest values for Diva cultivar, in the case of full spectrum UV, in concordance with the DNA level damage, proved by the highest number of induced dimmers and lowest percent of restored dimmers.
3. For all investigated cultivars it could be noticed an increase of dimmers number following UV-B irradiation and a decrease after subsequent UV-A irradiation, because of photoreparation.
4. The photorepair, even if not effective enough to remove all CPDs, helps in avoiding the increase of DNA damage by this radiation.
5. Regarding kinetics of CPDs formation and cleavage at DNA level, for the investigated four bean cultivars, the most resistant is *Avans*, than *Star*, *Ardeleana* and *Diva*.
6. For all 4 investigated cultivars, the frequency of aberrations induced by UV increases with the decrease of wavelength and with the increase of irradiation time, but the percent of mutations occurrence is similar to the natural induced ones, proving the low mutagenic effect of UV.
7. After analyzing aberrations type, it can be noticed that in the case of all investigated cultivars, UV irradiation induced lesions at DNA level but also affected division spindle, occurring multipolar ana-telophase, retardate chromosomes.
8. The maximal number and types of aberrations were found in the case of full spectrum UV, correlated with the mithotic index decrease.

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