CYTOGENETIC EFFECTS OF IRRADIATION WITH UV AT 6 ROMANIAN CULTIVARS OF *PHASEOLUS VULGARIS* L.

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Key words: chromosomal aberrations, UV radiations, bean, *Phaseolus vulgaris* L., cultivar.

Abstract: In the actual conditions of increased UV radiation level on Earth surface, the analysis of chromosomal aberrations which may appear during cell division in root apex is important for the estimation of mutagenic effect of UV. The study proves that the cytogenetic effect of UV-B on 6 Romanian bean cultivar (Vera, Ami, Ardeleana, Avans, Star, and Diva), exposed to UV-B/white light for 0,5h; 1,5h; or 3h is very similar, the DNA lesions leading to the same types of chromosomal aberrations, and with aberrations frequency correlated with the mitotic index. For all cultivars, the percent of mutations occurrence are similar to the natural induced ones, proving the low mutagenic effect of UV.

INTRODUCTION

Ozone selectively filters out the shorter UV wavelengths, so the most important consequence of the ozone depletion is an increase in the amount of UV-B (280-315nm) radiation reaching the earth's surface (Björn *et al.*, 1999a). The shift of spectral UV composition towards shorter wavelengths has on higher plants damaging effects, including DNA damage (Buchholz *et al.*, 1995). UV-B is efficiently absorbed by plants and can cause damage to nucleic acids, proteins, lipids, plant pigments, also direct, as well as mediated by reactive – oxygene-species (Bornman *et al.,* 1997).

Chromosomal aberrations occured 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 chromosomal aberrations induced by UV-B radiation at six romanian cultivars of *Phaseolus vulgaris* L. In *Phaseolus vulgaris* L, UV-B caused reduction in the levels of carotenoid and of chlorophyll *a, b* (Strid and Pora, 1992).

The choice of *Phaseolus vulgaris* as biological material for investigations, can be explained by the importance of seeds and legumes in humans nutrition, being considered since centuries the poor peoples "meat" because of the increased level of high quality proteins, and the high energetically level, and due to the importance in soil amelioration, being used as very good previous culture plant for many other species.

MATERIAL AND METHODS

Biological material: *Phaseolus vulgaris* seeds of 6 Romanian cultivars

Mutagenic agent: UV-B radiations. Light sources designated UV/white light, is a mixture of:

1. 2 L 40 W/73 (UV-A), Osram
2. 2 TL 40 W/18 (Blue Light), Philips
3. 1 TL 40 W/12 (UV-B), Philips.

Fluency rate of UV-B lamp is 3 W m^{-2}, total fluency rate is 8 W m^{-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 cut-off filters (Schott and Gen, Mainz, Germany) were used for the experiment: WG 305 with 11, 9 W m^{-2} fluency rates, 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). The quartz filter (Q) does not cut off any UV radiation, has 100% transmission for the full UV spectrum.

Working steps:

1. Seeds germination: 20 seeds for each experimental variant, from each cultivar were sawn in transparent plastic 9/9 cm boxes filled with vermiculite (Deutsche vermiculite Dämmstoff Gmbh Sprockhövel), in growth chamber, in dark, at 25° C.

2. UV-B/white light irradiation: 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 cut-off filters (WG 360, WG 320, WG 305, WG 275, 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.

3. Cytogenetic studies: after irradiation, roots were colored 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

The frequency of cells with chromosomial aberrations

If we consider the values induced by different wavelength UV (for **0,5h** irradiation time) in ana-telophase (A-T) of the 6 bean cultivars (Vera, Ami, 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 Ami and Diva cultivar, following Vera, Star, Ardeleana and Avans (Fig. 8).

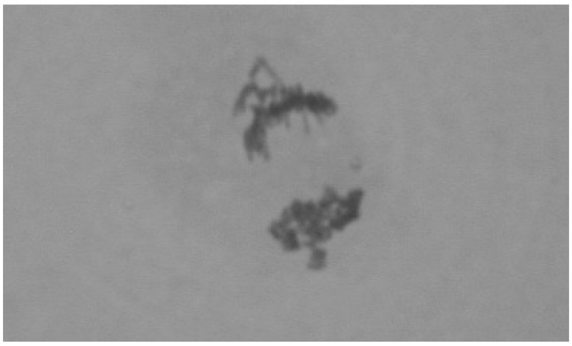


Fig. 1. A-T with expulsed chromosoms, Ardeleana, Q, 0,5h

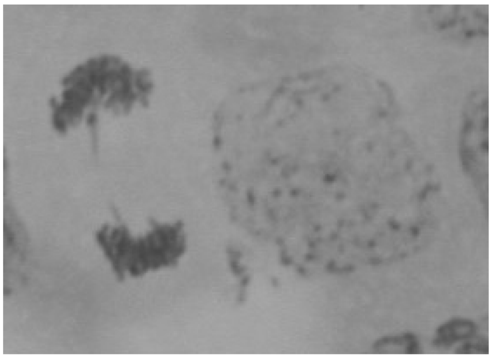


Fig. 2. A-T with broken bridge, Ardeleana, WG275, 0,5h

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 (Fig. 9).

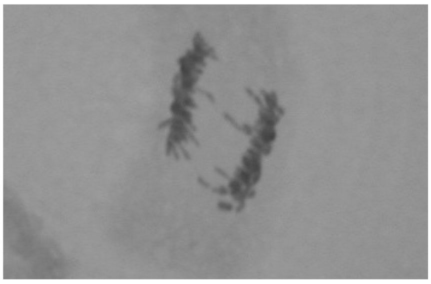


Fig. 3. A-T with broken bridges, retardate chromosome, Vera, Q, 1,5h

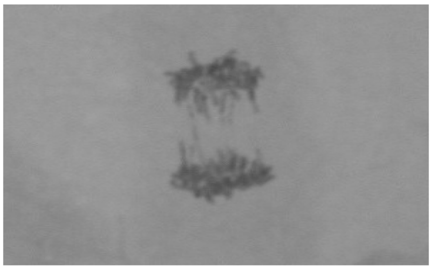


Fig. 4. A-T with broken bridges, expulsed, retardate chromosome, Star, WG32 01,5h

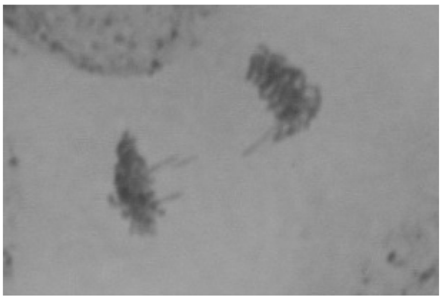


Fig. 5. A-T with broken bridge, retardate chromosome Avans, WG275, 1,5h

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 (Fig. 10).

The Ardeleana, Star, Diva, Ami and Vera cultivars reacted very similar to Avans. Aberrations types were in order of their occurring frequency: simple or multiple bridges, retardate chromosomes, expulsed chromosomes (Fig. 1), simple or multiple bridges combined with chromosomes or chromosomal fragments (Fig. 2, Fig. 3, Fig. 4) and in a very low percent some other aberrations types as more than one retarded chromosomes, expulsed genetic material, and multiple division poles. Ami cultivar presented also binucleated cells.

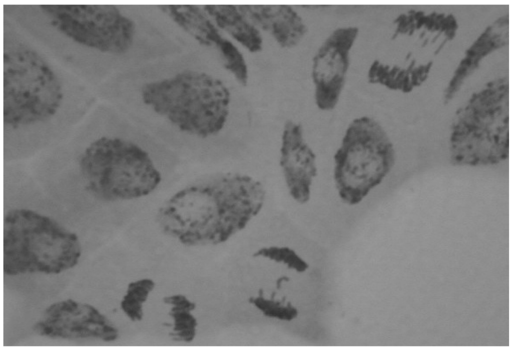


Fig. 5. A-T with multiple broken bridges, retardate chromosome Diva, Q 3h

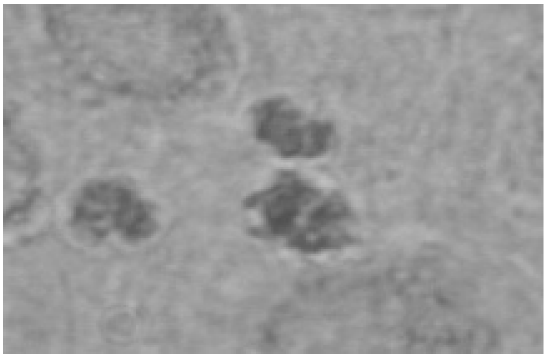
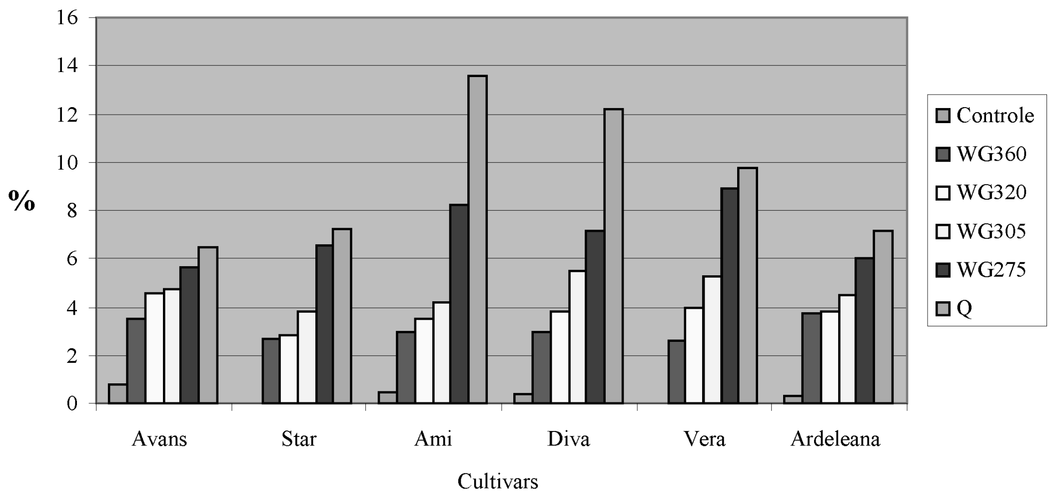


Fig. 6. Tripolare A-T, Avans, Q, 3h

The most frequent aberration types induced by UV, in the case of Avans cultivar, 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 (Fig. 5, Fig. 6).

Fig. 8. Frequency of aberrant A-T for different cultivars of *Phaseolus vulgaris* L., 0,5h irradiation time



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.

For all the 6 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-Ţigănaş, 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 and Terramura, 1989, Tosserams *et al*., 2001).

Fig. 9. Frequency of aberrant A-T for different cultivars of *Phaseolus vulgaris* L., 1,5h irradiation time

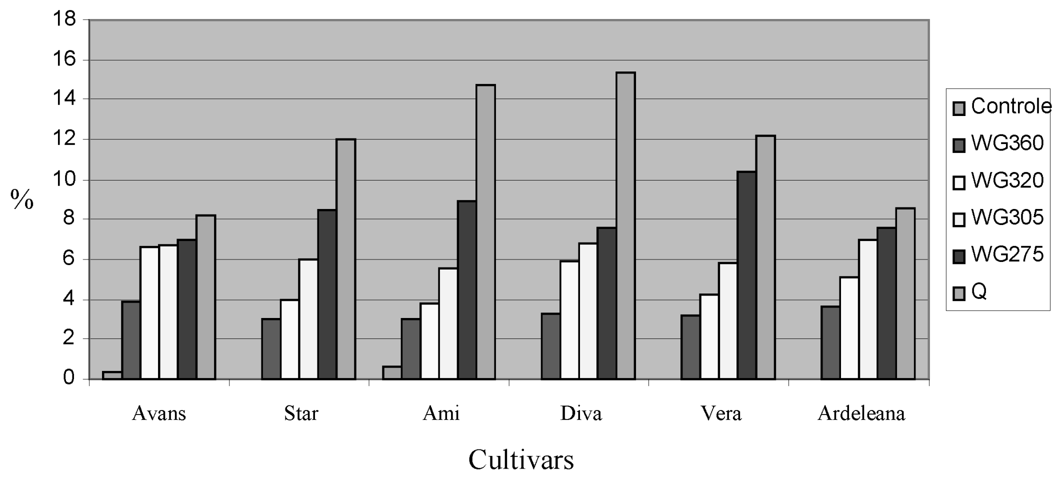
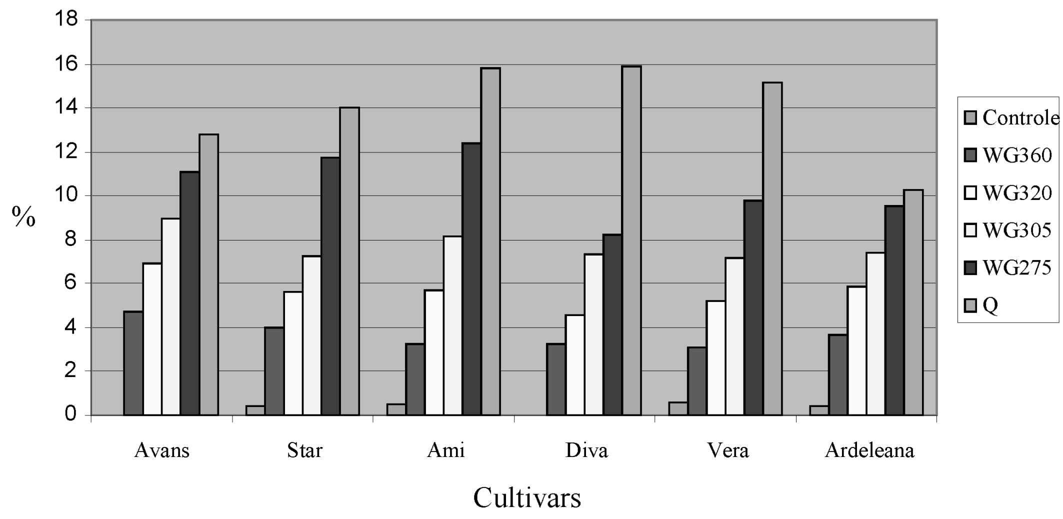


Fig. 10. Frequency of aberrant A-T for different cultivars of *Phaseolus vulgaris* L., 3h irradiation time



CONCLUSIONS

For all 6 investigated cultivars, the frequency of aberrations induced by UV, increase with the decrease of wavelength and with the increase of irradiation time, but the percent of mutations occurrence are similar to the natural induced ones, proving the low mutagenic effect of UV.
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.
All the 6 cultivars reacted very similar, regarding both aberrations type or aberrations frequency, as response to irradiations with different UV wavelength for different irradiation time, so no cultivar can be considered the most resistant.
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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