An attempt of discrimination between solar UVB and photoreactivating light: chromosomal aberration test in the cells of *Crepis capillaris*

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To discriminate between solar UVB and photoreactivating light, root tips of *Crepis capillaris* were placed into special chambers equipped with filters. Results of the two-year investigations are compared. The frequency of chromosomal aberrations increased stronger if the roots were exposed to a 'mixture' of UVB+UVA than UVB alone. Photoreactivation removed a significant part, but not all chromosomal aberrations. The causes of the phenomenon are discussed.

Key words: sunlight UVB, photoreactivation, chromosomal aberrations, *Crepis capillaris*

INTRODUCTION

The recent increase of atmospheric chlorofluorocarbons, methane and nitrous oxides leads to a significant reduction in the global stratospheric ozone [1]. The Antarctic 'ozone hole' has become larger and the depletion of ozone more severe [2]. A general erosion of ozone at the lower latitudes has also been well established [3]. As a consequence of the depletion of stratospheric ozone, the solar ultraviolet B (280–320) radiation reaching the Earth's surface increases. Enhanced UVB at the Earth's surface may have potentially damaging effects on human, plants and animals in terrestrial and aquatic ecosystems [4].

The UVB dose depends not only on the geographic, but also on the atmospheric conditions of a concrete region at the same geographic latitude. So, it is actual to determine biological effects of solar UVB on concrete regions. As for the Lithuanian region, information about biological effects of solar UVB is restricted. Especially little is known about solar UVB effect on plants. Plants require sunlight to grow. So, they are exposed for a long time to deleterous UV radiation of the sunlight. However, the most throroughly investigated effect of UVB on plants concerns its physiological effects, especially on the structure and function (reduced photosynthesis causing a decrease in growth rate; anatomical and plastid structure changes; reduced root/shoot ratio; smaller, thicker leaves), increased pigmentation (mainly flavonoids that absorb radiation in the UVB wave-length, offering protection to the underlying tissue) [5–9].

However, only a few works have been published about the action of UVB on the plant genetical apparatus, down-regulation of the mRNA level of chloroplast proteins [10], induction of the cyclobutane pyrimidine dimers and their photoreactivation [11–13]. That situation may be explained by photorepair.

Simultaneously to the damaging action of UVB, the longer wave-length radiation of the sunlight (400–700) necessary for photosynthesis (and called PAR – Photosynthetically Active Radiation) can minimize the damage induced by UVB. This protection occurs at the physiological, biochemical and molecular levels. The thicker leaves and increased level of flavonoids are attributed to the photorepair phenomenon [10]. Photorepair of UV-induced DNA damage by the blue light is called photoreactivation. Two types of DNA lesions – cyclobutane pyrimidine dimers (CPDs) and (6–4) photoproducts – are involved in photoreactivation, and for both types of DNA lesions the special enzymes, CPD–photolyase and (6–4) photolyase, are activated by the blue light. Photoreactivation is a very specific phenomenon for the CPD and (6–4) photoproducts, and only for the UV action. The third signal enzyme system exists in plants. It is the signal transduction system, and the blue light photoreceptor is very similar to the microbial CPD photolyase [14].

The simultaneous effects of DNA damage and of photoreactivation complicate significantly the qu-

antative evaluation of the real danger of solar UV for the plants. So, it is necessary to cut off the UVB region of the sunlight from the photoreactivating wave-length of solar radiation.

At the present work, attempts to solve that problem were made by exposing root tips of *Crepis capillaris* to the narrow region of the sunlight wavelengths in special chambers with filters. Chromosomal aberrations were investigated in the meristematic cells for evaluation of the damaging effect of solar UVB on the genetic apparatus of the plants. A photorepairable part of chromosomal aberrations induced by solar UVB was also determined.

MATERIALS AND METHODS

Heterogeneous cell population of *Crepis capillaris* (L.) Wallr. root tips was used to study the influence of solar UVB on the frequency of chromosomal aberrations. The meristematic cells of the root tips were analysed.

Irradiation by solar UVB. Seeds of *C. capillaris* were germinated in a thermostate at 25°C in the dark. The roots of 2-3 mm long were placed into Petri dishes and put into special chambers equipped with filters that passed only UVB, UVB+UVA, or photoreactivating (PR) part of the sunlight spectrum. Results of two-year investigations were compared. In 1999 the material was irradiated on 1 July. The exposition lasted 6.5 h, beginning from 10 a.m. In 2000, the roots of *C. capillaris* were irradiated on 15 May. The exposition lasted 4 h, beginning from 11.45 a.m. In both years the maximum sunny days were specially selected. The choice of these days was very difficult. Several faulty attempts were made in each year.

For comparison, as a control, part of roots were kept in the same chamber for the same time in the dark. In 2000, the conditions in comparison to 1999 were changed duration and time of exposition, the filters, photoreactivation and aeration in the chambers. The latter conditions were improved. This was necessary to do, because the conditions of ozone, induced by UV, may change at the time of exposition.

Photoreactivation conditions. In 1999 the material was photoreactivated after UVB irradiation. The roots were irradiated immediately after UVB treatment in one portion of the photoreactivating light.

In 2000 photoreactivation was carried out in portions: 10 min before UV treatment; 5 min after 1 h of treatment with UV; and 30 min at the end of UV treatment. Photoreactivation was made on the material which was treated by a 'mixture' of UVB+UVA. The experience of the 1999 showed us that UVB+UVA induced more chromosomal aberrations.

The filters used for UV irradiation and photoreactivation: in 1999: for UVB – Ж-20 and УФС-5, for UVB+UVA – ЖС-20 and УФС-8, for photoreactivation – ЖС-20 and УФС-6; in 2000: for UVB – ЖС-20, for UVB+UVA – УФС-2, for photoreactivation - УФС-5 and ЖС-23.

Chromosomal aberration test. All the operations with roots before and after irradiation were carried out in the red light. The root tips were treated with colchicine (100 mg/l) and fixed with an acetic acid and ethanol (1:3) mixture at 3; 6; 9; 12 hs after UV irradiation. The fixed root tips were stored in a 70% ethanol in the freezer until used. Chromosomal aberrations (CA) were studied on temporary preparations stained with acetocarmine. The metaphase cells were examined.

RESULTS

Sunlight exerts an effect on plants in wide range of wave-lengths. So, it is necessary to separate the real UV action from the action of the solar light in a wide spectrum of wave-length. This task was realized by the cultivation of *C. capillaris* roots in special chambers equipped with filters transmitting the narrow range of the sunlight wave-length.

Both in 1999 and 2000, two ranges of wave-length were tested: UVB and UVB+UVA (Tables 1 and 2).

Unexpectedly, a stronger effect on the induction of chromosomal aberrations had the 'mixture' UVB+UVA. In 1999 the level of chromosomal aberrations after the treatment with UVB+UVA increased more than ten times, in comparison to a spontaneous level of cells not treated with UV (Table 1). The effect of the UVB+UVA was about twice as strong as irradiation with UVB alone. Solar UVB increased the frequency of chromosomal aberrations 4.6 times (Table 1).

Since such a strong effect of UVB+UVA was unexpected, in 1999 the photoreactivation was carried out in roots irradiated with UVB. Photoreactivation decreased significantly the level of chromosomal aberrations in the irradiated cells. However, it was still higher than in untreated material. So, photoreactivation removed not all DNA lesions leading to chromosomal aberrations.

The same phenomenon was also observed in 2000, although photoreactivation was carried out on the roots irradiated with UVB+UVA (Table 2). Photoreactivation of part of chromosomal aberrations was undoubtedly and statistically significant ($t_d = 2.02$), but there was also part of chromosomal aberrations that had not yet been photoreactivated. Results of investigations in 2000 confirmed the fact that UVB+UVA is more effective than irradiation with UVB for induction of chromosomal aberrations

Table 1. **Induction of chromosomal aberrations (CA) and their photoreactivation in meristematic cells of *Crepis capillaris* root tips irradiated by solar UVB and UVB+UVA**

Results of 1999

Time after UV, h	Mitotic index	Number of metaphases	Metaphases with CA		Types of CA				
			number	% ± m	Chromosome		Chromatid		Undefined
					number	% ± m	number	% ± m	number
Without irradiation (control in the dark)									
3	–	406	1	0.246 ± 0.246	0	0	1	0.246 ± 0.246	0
6	32.1 ± 3.4	460	0	0	0	0	0	0	0
9	23.4 ± 1.7	366	0	0	0	0	0	0	0
12	29.8 ± 1.5	–	–	–	–	–	–	–	–
Total	28.4 ± 1.1	1232	1	0.081 ± 0.081	0	0	1	0.081 ± 0.081	0
Irradiation with UVB									
3	9.9 ± 0.8	308	1	0.325 ± 0.325	1	0.325 ± 0.325	0	0	0
6	6.4 ± 0.8	47	0	0	0	0	0	0	0
9	3.5 ± 0.7	174	1	0.575 ± 0.575	0	0	1	0.575 ± 0.575	0
12	4.4 ± 0.7	–	–	–	–	–	–	–	–
Total	6.1 ± 0.4	529	2	0.378 ± 0.267	1	0.189 ± 0.189	1	0.189 ± 0.189	0
Irradiation with UVB+UVA									
3	14.8 ± 1.3	261	3	1.149 ± 0.660	3	1.149 ± 0.660	0	0	0
6	19.9 ± 3.2	426	3	0.704 ± 0.405	1	0.235 ± 0.235	2	0.469 ± 0.331	0
9	19.2 ± 7.7	380	3	0.789 ± 0.454	3	0.789 ± 0.454	0	0	0
12	11.3 ± 3.2	–	–	–	–	–	–	–	–
Total	16.3 ± 1.1	1067	9	0.843 ± 0.280	7	0.656 ± 0.247	2	0.187 ± 0.132	0
After photoreactivation of UVB+UVA									
3	15.5 ± 1.8	349	1	0.287 ± 0.287	1	0.287 ± 0.287	0	0	0
6	13.1 ± 1.1	494	0	0	0	0	0	0	0
9	21.1 ± 3.4	130	1	0.769 ± 0.766	1	0.769 ± 0.766	0	0	0
12	–	–	–	–	–	–	–	–	–
Total	16.6 ± 0.9	973	2	0.206 ± 0.145	2	0.206 ± 0.145	0	0	0

Table 2. **Induction of chromosome aberrations (CA) and photoreactivation in meristematic cells of *Crepis capillaris* root tips irradiated with solar UVB and UVB+UVA**

Results of 2000

Time after UV, h	Number of metaphases	Metaphases with CA		Types of CA				
		number	% ± m	Chromosome		Chromatid		Undefined
				number	% ± m	number	% ± m	number
Without irradiation (control in the dark)								
6	1131	5	0.442 ± 0.147	1	0.088 ± 0.088	4	0.354 ± 0.177	0
9	371	1	0.270 ± 0.269	0	0	1	0.270 ± 0.269	0
Total	1502	6	0.399 ± 0.150	1	0.667 ± 0.210	5	0.332 ± 0.148	0
Irradiation with UVB								
6	469	4	0.853 ± 0.425	0	0	2	0.426 ± 0.314	2
9	842	5	0.549 ± 0.265	0	0	4	0.475 ± 0.237	1
Total	1311	9	0.686 ± 0.228	0	0	6	0.458 ± 0.186	3
Irradiation with UVB+UVA								
6	682	6	0.880 ± 0.358	0	0	5	0.733 ± 0.327	1
9	438	4	0.913 ± 0.454	1	0.228 ± 0.228	3	0.685 ± 0.394	0
Total	1120	10	0.893 ± 0.281	1	0.089 ± 0.089	8	0.714 ± 0.252	1
After photoreactivation of UVB+UVA								
6	523	3	0.574 ± 0.337	0	0	3	0.574 ± 0.337	0
9	483	2	0.414 ± 0.292	0	0	2	0.414 ± 0.292	0
Total	1006	5	0.497 ± 0.222	0	0	5	0.497 ± 0.222	0

(Table 2). Solar UVB+UVA induced both chromosome and chromatid types of aberrations. They were observed in both years. However, in 2000 only chromatid type of aberrations were induced after irradiation with UVB. Contrary to it, in 1999 the both types of chromosomal aberrations arose in the cells irradiated with UVB (Table 1).

A very effective test for evaluation of UV action on plant cells was the mitotic index. That index was determined only in 1999 (Table 1). The solar UVB undoubtedly (t_d = 2.02) decreased the mitotic index of the irradiated cells about four times. After irradiation by a 'mixture' UVB+UVA or after photoreactivation of the cells irradiated with UVB, the mitotic index increased about twice, but it did not reach the initial level noted for untreated control cells (Table 1).

The longer was the time after irradiation, the lower decrease of the mitotic index was observed. The root material fixed at 3 h and 12 h after irradiation was compared. The level of cell division decreased from 9.9 at 3 h to 3.5–4.4 at 9–12 h after treatment with UV, *i.e*. decreased 2.8–2.2 times.

In comparison to the untreated cells, inhibition of cell division with UV is especially drastic: the mitotic index decreased at the same fixations 6.7–6.8 times (Table 1).

DISCUSSION

The use of special chambers equipped with filters cutting off a definite region of the sunlight and transmitting only UVB, UVB+UVA or photoreactivating light allowed us to determine the real activity of solar UVB on induction of chromosomal aberrations in plant cells.

A significant decrease of the level of chromosomal aberration was noted after irradiation with photoreactivating light in photoreactivation experiments conducted both in 1999 and 2000 (Figure).

Since photoreactivation removes only pyrimidine dimers, by monomerisation, a reduction in the biological effect after photoreactivation means that the pyrimidine dimers are involved in that biological effect of UV irradiation [14]. So, if the level of chromosomal aberrations is decreased when photoreactivation is used, it means also that pyrimidine dimers are involved in the formation of chromosomal aberrations, including those in meristematic cells of *Crepis capillaris*. However, attention must be given to the following facts: (1) a stronger effect on induction of chromosomal aberrations was observed, if *C. capillaris* roots were irradiated with a 'mixture' of UVB+UVA; (2) a certain part of chromosomal aberrations and DNA lesions induced by UV is insuperable for photoreactivation enzymes.

The latter phenomenon was also confirmed by analysis of the mitotic index. UV irradiation decreased cell division very strongly. So, the mitotic index is a very sensitive test for determination of the biological action of solar UV, but photoreactivating light was unable to restore completely cell division. A significant inhibition of cell division was still observed after photoreactivation.

Maintenance of a certain part of chromosomal aberrations after photoreactivation has been also observed earlier in mammalian cells [15], and there are several explanations for that phenomenon:

(1) part of pyrimidine dimers realise into chromosomal aberrations before these DNA lesions may be removed with photoreactivation. This might happen in those cells which were in S-phase or even in

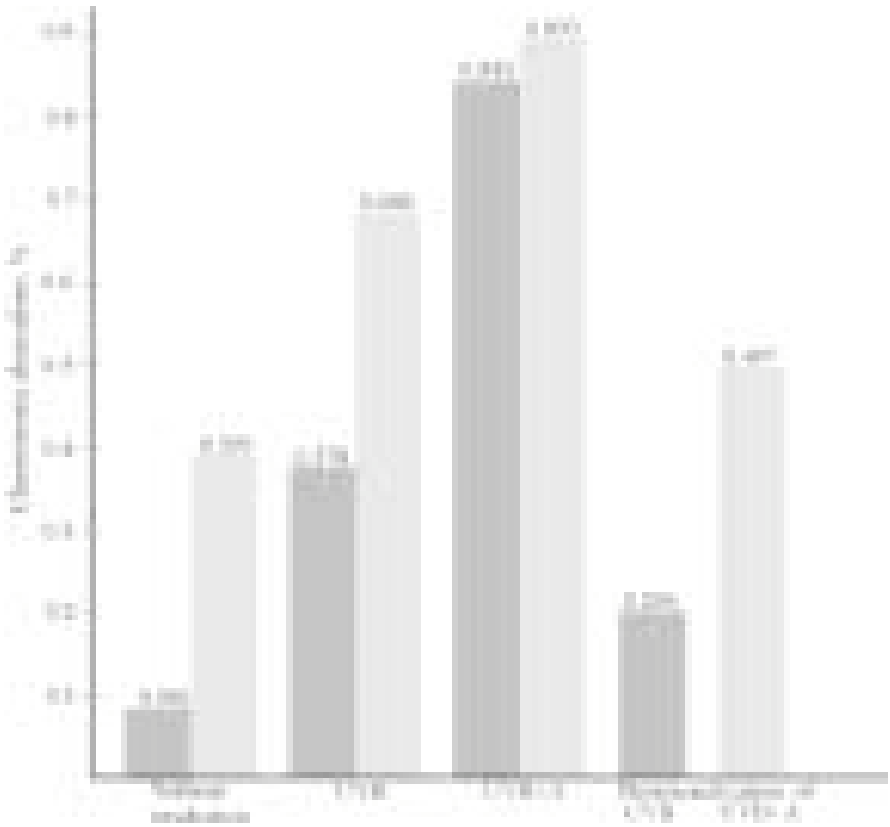

Figure. Comparison of the total frequency of chromosomal aberrations in the meristematic cells of *Crepis capillaris* root tips after irradiation by solar UVB, UVB+UVA and photoreactivation in 1999 and 2000

G2-phase of the cell cycle when exposed to the photoreactivating light.

That supposition is in agreement with the fact that only chromosome-type aberrations disappeared after photoreactivation in 2000 (Table 2). So, only one-strand chromosomal aberrations arising in G2-phase remained after that experiment. However, a certain part of chromatid-type aberrations also disappeared after photoreactivation (Table 1). In 1999, *vice versa,* the chromatid-type of aberrations disappeared, although the chromosome-type was also reduced (Table 2). This contradiction may be explained by the different materials used for photoreactivation in 1999 and in 2000. In 1999 was root tips were irradiated with UVB, while in 2000 a 'mixture' UVB+UVA was used for irradiation. Aeration and photoreactivation conditions in the chambers were also improved in 2000;

(2) although pyrimidine dimers are the main and most important group of DNA lesions induced by UV, other types of DNA lesions unrepairable with photoreactivation arise after UV irradiation [15–18]. Induction of DNA lesions is best of all investigated after irradiation with artificial UVC. After UVC irradiation of *Escherichia coli,* DNA lesions are found in the following frequency: dimers – TT – 38%, CU – 4.6–19%, TC – 19–22%, UT– 11.5%, cytozine hydrates – 6.9%, pyrimidine adducts – 4.6%; crosslinks DNA–protein – 0.01%, DNA breaks – 0.01% [18]. Only cyclobutane pyrimidine dimers were measured directly in plants, but not other types of UV radiation-induced DNA damage [11, 12, 13, 19–21].

It is suggested that the other DNA lesions that cannot remove photoreactivation cause a relatively small part of biological effects [15]. Although those types of DNA lesions was not studied in plants, the general principles of the UV action on plants are the same on other organisms. In addition, UV may induce DNA damage indirectly through the formation of singlet oxygen, free radicals or mutagenic derivatives of the endogenous organic substances [19, 22, 23];

(3) a pecularity of UV photobiology of plants is that the solar UV light and photoreactivating light act simultaneously, and photosynthetically active radiation (PAR) is necessary for the plant life. So, photosynthesis plays an important role in the protection against UVB damage [24]. The PAR action is excluded by using etiolated plant material. In the present work this was done with the aid of naturally etiolated roots;

(4) physiological conditions act also on the photoreactivation effect in plants. Ahmad et al. [25] showed that the level of expression of the photoliase gene *PHR1* in *Arabidopsis* can change at different leaf stages or age. So, the level of *PHR1* expression in the leaves of four-week-old mature plants was lower than in the leaves of etiolated seedlings exposed for 6 h to the white or UVA light. The role of physiological conditions in photoreactivation has been also confirmed by Hidema and Kumagai on rice (*Oryza sativa*) [11]. In *Arabidopsis* the photolyase activity is markedly temperature-sensitive [21].

Among important factors that may determine resistance of the plants to the solar UV are also pigments, presumptively the flavonoids. They serve as an optical screen absorbing UV while transmitting photosynthetically active sunlight radiation [26–28]. The less content of the plant pigments the stronger plant damage with UV [26–28]. In the roots, naturally, the content of pigments is less than in the leaves. This may explain the fact that even a short exposition (4–6 h) of the *Crepis* roots to UV gives a relatively strong effect on the level of chromosomal aberrations;

(6) DNA repeat sequences are involved in the formation of chromosomal aberrations. These repeats may share the property of promoting to different degrees the formation of the recombinogenic DNA structures which act as a focus for the expression of DNA damage [29].

All the factors and phenomena discussed above (1–6) may do their bit to the formation of the unphotorepairable chromosomal aberrations. It is proposed that these factors cause also a stronger effect of UVB+UVA 'mixture' on the induction of chromosomal aberrations than does irradiation with UVB alone. The level of such chromosomal aberrations was also quantitatively determined in photoreactivation experiments.

The present work confirmed the results of the previous works [13, 20, 30] that the main part of chromosomal aberrations induced in plant cells by UV, are caused by pyrimidine dimers. This part of aberrations is photorepairable. However, the rest part of chromosome aberrations caused by unphotorepairable DNA lesions is ecologically very important. They expressed the real danger of solar UV radiation on the plant genome, because in the ecological conditions a balance exists between DNA lesions induced with UV, and, on the other hand, photoreactivation. As mentioned above, the both phenomena – solar UV radiation and photoreactivation – act simultaneously. The question arises for the future investigations: which factors are able to disturb that balance?

When the plant material is exposed to the sunlight UV in special chambers, additional problems arise. Chromosomal aberrations and other lesions can be induced by ozone produced by UV radiation [24].

That's why so much attention is being paid to improve the conditions in special experimental chambers for the sunlight UV treatment [31]. The good aeration conditions in the chamber, provided in 2000 for UVB and UVB+UVA treatment, minimized or maybe fully excluded the effect of ozone.

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PASTANGOS ATSKIRTI SAULĖS UVB IR FOTOREAKTYVINANČIĄ ŠVIESĄ: CHROMOSOMŲ ABERACIJŲ TESTAS *CREPIS CAPILLARIS* LĄSTELĖSE

S a n t r a u k a

Saulės UVB poveikiui atskirti nuo fotoreaktyvinančios spektro dalies *Crepis capillaris* šaknelių viršūnėlės buvo eksponuojamos specialiose kamerose, naudojant filtrus. Dvejų metų rezultatai yra lyginami.

Chromosomų aberacijų aptikta daugiau, kai šaknelės apšvitintos ne UVB, o UVB+UVA. Fotoreaktyvacija pašalino nemažai chromosomų aberacijų, kurias indukavo UVB arba UVB+UVA, tačiau ne visas. Po fotoreaktyvacijos liekamųjų chromosomų aberacijų prigimtis svarstoma.