Photoreactivation of the chromosomal damage induced by UVC in *Crepis capillaris* cells

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Root tip meristematic cells were used to study the influence of solar photoreactivating (PR) light on the frequency of chromosomal aberrations (CA) after treatment with short-wave ultraviolet light (UVC). PR light (sunlight, through a double glass filter, 320 nm – visible) at a dose 60000 J/m^2 used immediately after UVC irradiation decreased the frequency of CA induced in G_1 and G_2 phases. Photoreactivation was efficient at a temperature of 15–30 °C in the first cell cycle after UVC irradiation.

Key words: UVC, chromosomal aberrations, photoreactivation, *Crepis capillaris*

INTRODUCTION

As the protective ozone layer around the Earth absorbing ultraviolet radiation (UV) is being reduced, there is an increasing interest to the mechanisms of UV damage in the living organisms, its repair and prevention. Photosynthetic organisms need sunlight and are exposed to ultraviolet radiation present in sunlight. UVC rays below 280 nm are effectively absorbed by ozone in the stratosphere and are not present in sunlight at the Earth's surface. The UVC damage is not physiologically relevant to the plants growing in the sun, however, short-wave-length ultraviolet radiation from germicidal lamps has been studied more intensively, because DNA has a strong absorption maximum in the UVC range [1]. Although UVB is a minor component of sunlight, it has a disproportionately damaging effect on higher plants [2–5]. UVA region rays are unaffected by ozone layer reduction, its dose is not influenced by the factors that destroy the ozone layer. Thinning of the ozone layer also results in a shift of the spectral UV composition towards shorter wavelengths. In general, biological damage is exacerbated as the wavelength becomes shorter. Ultraviolet-sensitive targets include DNA, proteins and membranes, which must be protected for normal growth and development.

In the present work, the photoreactivation of UVC-induced damage on chromosomes of *Crepis capillaris* by the sunlight was evaluated in several aspects.

MATERIALS AND METHODS

One-year-old seeds of *Crepis capillaris* L. (Wallr.) were germinated at 25 °C in the dark. Roots 2–3 mm in long were irradiated by BUV-15 and BUV-30 lamps, dose 2000 J/m^2. The irradiation conditions ware as in [6]. After UV irradiation a part of seedlings was immediately irradiated with photoreactivating solar light (320 nm – visible light), dose 60000 J/m^2. The spectral range was cut off by a double glass plate filter. A part of seedlings was irradiated only with photoreactivating light, one more part remained without irradiation (control). Irradiated and control roots were treated with colchicine solution (100 mg/l) and germinated under the same conditions till fixation. The roots were fixed with ice acetic acid and ethanol (1:3) mixture: a) 7, 8.5, 10 and 12 h after UV irradiation to study the cells irradiated at G_1 phase of the cell cycle; b) 1, 2, 3 and 4 h after UV, to study the cells irradiated at G_2; c) to study the influence of temperature on UV-induced chromosomal damage, irradiated and control roots were germinated at 15°, 26° and 30 °C till fixation. The root tips were fixed 3, 6 and 10 h after UV irradiation. All the operations with roots before and after irradiation were carried out in red light.

RESULTS AND DISCUSSION

All types of ultraviolet radiation destroy different processes in plants. The most frequent UV-induced DNA damage – cyclobutane pyrimidine dimers (CPDs) and (6–4) photoproducts are mutagenic. The most important biological sequence of the damage is destruction of normal cell activity, namely: DNA replication, transcription, alterations in gene expres-

sion, stopping of protein synthesis [1, 2, 7]. The DNA damage induced by UV are removed with the help of excision repair, photoreactivation and post-replicative repair enzyme systems [8–10]. In case of their mistakes DNA breaks are possible, as a result chromosomal aberrations can appear, which can cause destruction of cells. *Crepis* root meristem cells (2n = 6) enabling to calculate chromosomal aberrations in some cell cycles by a C-mitosis method were used for investigation of CA in UVC-irradiated and photoreactivated cells.

Chromosomal aberrations were tested in an asynchronous population of root meristem cells. The duration of cell cycle of *Crepis* root meristem cells lasts on the average 10.4–12.4 h, G_1 phase 3.5 h, S – 5.3 h and G_2 + M/2 lasts 3.8 h [11]. Photoreactivation of UVC damage on G_1 chromosomes was evaluated 7–12 h after UV. The data show that UVC irradiation increased the frequency of CA about three times. Both types of CA (chromatid and chromosome) were noticed, but only the frequency of the chromatid-type CA increased after UV treatment. The level of chromosome type was about the same as without any treatment or as after irradiation only by photoreactivating light. After treatment with photoreactivating light the amount of CA decreased about twice and approximately reached the spontaneous level (Fig. 1).

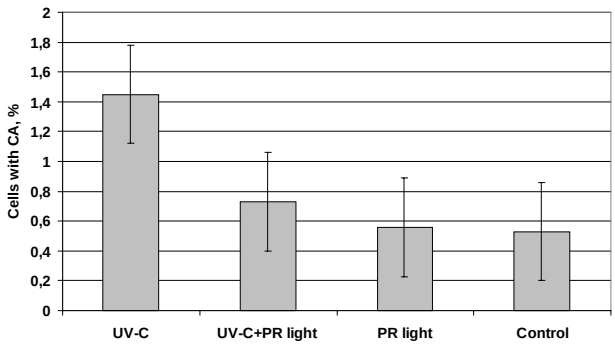

Fig. 1. Photoreactivation by the sunlight (320 nm-visible light) of chromosomal aberrations in *Crepis capillaris* cells at G_1 irradiated with UVC (total from 4 fixations)

Photoreactivation of UVC-induced damage on G_2 chromosomes was evaluated 1–4 h after UV irradiation. The first aberrations appeared in the third hour after UV, most cells with CA (2.94%) were found in the fourth hour after UV, *i.e*. in the early G_2 phase. In total, 1.04% cells had CA, most of them being chromatid-type aberrations. Photoreactivating light twofold decreased the number of CA. In cells illuminated only with PR light there were 0.21% and in control cells 0.29% of cells with CA (Fig. 2).

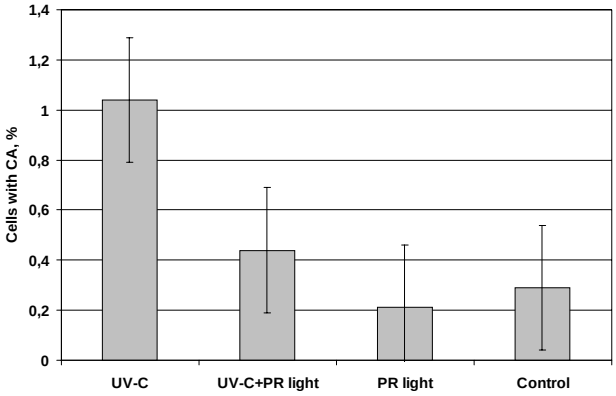

Fig. 2. Photoreactivation by the sunlight (320 nm-visible light) of chromosomal aberrations in *Crepis capillaris* cells at G_2 phase irradiated with UVC (total from four fixations)

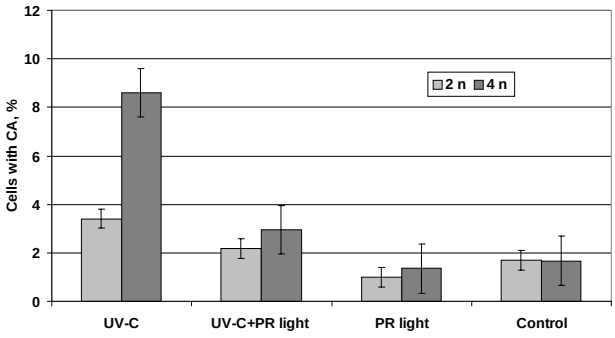

Fig. 3. Photoreactivation by the sunlight of chromosomal aberrations in two cell cycles of *Crepis capillris* after UV-C irradiation

Temperature is one of the most important components of the plant's environment. Temperature changes are features of the natural environment in which higher plants must survive and function. We have analyzed the influence of different physiologically usual temperatures on UVC-induced chromosomal damage and photoreactivation in *Crepis* root cells. CA were examined during the first cell cycle after UV. The greatest number of CA was found in UV-irradiated roots cultivated at 15 °C (1.43% cells with CA). After treatment with PR light at all investigated temperatures the CA level decreased significantly. PR light about seven-fold times decreased the frequency of CA at 30 °C and 4 times in cells cultivated at 15 °C (Table). In contrast to our results, it was established that *Arabidopsis* photolyase was temperature-sensitive, both *in vitro* and *in vivo* [12]. Our data show that photoreactivation was efficient in *Crepis* cells at t° of 15–30 °C. Photoreactivating solar light (60000 J/m^2, 320 nm – visible) decreased the amount of UVC-induced chromosome aberrations in this interval of temperatures to a spontaneous level.

Table. **Effect of post-temperature treatment upon the UVC induced and PR-solar- light photoreactivated chromosomal aberrations in *Crepis capillaris* cells**

Temperature, °C	Number of cells scored	Cells with CA	
		number	% ± m
UVC, 2000 J/m^2			
15	1889	27	1.43 ± 0.27
26	1293	9	0.69 ± 0.23
30	2139	22	1.03 ± 0.22
UVC, 2000 J/m^2 + PR light, 60000 J/m^2			
15	785	3	0.38 ± 0.22
26	1406	6	0.43 ± 0.17
30	1435	2	0.14 ± 0.09
Control (without irradiation)			
15	1463	8	0.55 ± 0.19
26	1391	5	0.36 ± 0.16
30	2180	6	0.27 ± 0.11

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UV-C INDUKUOTŲ CHROMOSOMŲ PAŽAIDŲ *CREPIS CAPILLARIS* LĄSTELĖSE ATSTATYMAS FOTOREAKTYVINANČIA SAULĖS ŠVIESA

S a n t r a u k a

Crepis capillaris šaknų meristemų ląstelėse tirtas fotoreaktyvinančios šviesos poveikis trumpabangiu ultravioletu (UVC) sukeltų chromosomų persitvarkymų dažniui. Fotoreaktyvinanti šviesa (Saulės šviesa, per dvigubą stiklo filtrą, 320 nm – matoma, 60000 J/m^2), panaudota iškart po UVC, mažino chromosomų persitvarkymų dažnį ląstelėse, švitintose G_1 ir G_2 ląstelės ciklo fazėse. Fotoreaktyvacija buvo veiksminga 15–30 °C temperatūros intervale pirmajame ląstelės cikle po švitinimo UVC.