Chromosome lesions induced by solar UV(A+B) radiation and their photoreactivation by solar light in *Crepis capillaris* cells

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Crepis capillaris roots were grown in the dark and irradiated by solar UVB in special chambers with UV filters. Part of the UV(A+B)-irradiated roots were exposed to solar photoreactivating light (PHL). Another part of roots were exposed to solar PHL (340–600) for 0.5 h immediately after UV irradiation. The number of chromosome aberrations (CA) was analysed in the meristematical cells of root tips.

Results in 2001 confirmed the general conclusions made in the previous two years. UVB and especially UV(A+B) increases CA frequency in *C. capillaris* dividing cells. PHL reduced the CA level by about one half. That part of CA must be attributed to pyrimidine dimers. CA remained after PHL is really hazardous for the plant genome. The nature of such CA is discussed.

Key words: solar UV, photoreactivation in plants, chromosome aberrations, *Crepis capillaris*

INTRODUCTION

The stratospheric ozone layer is an effective screen for solar UVC and significantly decreases the dose of UVB rays at the earth's surface. UVA radiation is not changed by the ozone layer. So, only solar UVB increases with destruction of the stratospheric ozone layer [1]. However, it was shown that changing solar UVB doses act on different plant species and cause significant changes of the plant yield, growth and other physiological traits [2, 3]. The action of the solar UVB that reaches the earth's surface on plant genome was also observed. UVB radiation induces DNA lesions in plant cells. It could be cyclobutane pyrimidine dimers (CPDs) or (6–4) photoproducts [4]. Several phenomena were observed to take place simultaneously in field-grown maize. A sizable damage of development, cellular heterogeneity of reaction to UV and to repair was also observed [5]. Photoreactivation of solar UV damage was observed in *Arabidopsis thaliana* [6], alfalfa seedlings [7], rice [8, 9]. In *Arabidopsis thaliana,* photoreactivation of pyrimidine (6–4) pyrimidone adducts (6–4 PPs) also occurs [10]. One UV-sensitive mutant of *Arabidopsis* has a normal CPDs repair but a defective repair of (6–4) photoproducts [11]. So, repair of different types of DNA lesions may be separate processes catalysed by different enzymes. The nature of DNA lesions and the effectivity of their photorepair also depend on the wavelength of solar UVB [12, 13].

In general, the genotoxicity of solar UV in plant genome depends on the balance between the level of DNA damage and, on the other hand, on the intensity of repair of DNA lesions. In previous works [14–16] it has been shown that the balance is significantly disturbed if meristematical cells of *Crepis capillaris* have been irradiated by a wider diapason of UV(A+B) wavelength. It was expressed by a significant increase of CA frequency after UV(A+B) irradiation, in comparison to UVB. UV (A+B) significantly increases the part of CA that is unrepairable by photoreactivation. This result, important for plant genotoxicology, takes several years to be confirmed, because of variations in the complex of natural conditions.

In the present work, the effectivity of UVB and UV(A+B) on CA induction is compared and the level of CA unrepairable by photoreactivation is determined in standardized conditions.

MATERIALS AND METHODS

A heterogeneous cell population of *Crepis capillaris* (L.) Wall-root tips were grown in the dark. The conditions of solar UVB, UV(A+B) and photore-

activation were exactly the same (in a special chamber with filters) as in 2000 [13]. Only the surrounding ecological conditions were different. Root tips were irradiated by UVB (Ž-20 and UFC-5 filters) and UV (A+B) filters (UFC-2). For photoreactivation with 340–600 nm wavelength of solar light, a SZC-23 filter was used. The roots of *C capillaris* were irradiated on 3–4 May 2001. The exposition lasted 2 and 4.5 h to UV (A+B) and 5.5 h to UVB. Exposition to photoreactivating light for 0.5 h followed immediately after UV(A+B) irradiation.

For chromosome aberrations, the root tips were fixed in acetic acid and ethanol (1:3) mixture at 3; 6; 9; 12 h after UV irradiation. All the operations with roots before and after irradiation were carried out in the red light. CA were studied on temporary preparations stained with acetocarmine. The metaphase cells were examined.

RESULTS AND DISCUSSION

Highly energetic UVC rays below 280 nm are very genotoxic and their mutagenic action on DNA was intensively studied also in plants in our laboratory [15], but this part of UV spectrum is effectively absorbed by the ozone layer in the stratosphere and is not present in sunlight at the earth's surface. UVB rays (from 280 to320 nm) and UVA (upper 320 nm) do reach the earth. UVB increases as the stratospheric ozone decreases, and this is the actual problem of ecological genotoxicology. The UVA rays are unaffected by ozone layer reduction. So, their dose is not influenced by the factors that destroy the ozone layer [16].

The use of special chambers equipped with filters cutting off definite region of the sunlight allowed us to discriminate among the action of solar UVB, UV(A+B) and photoreactivating light and to show the stronger mutagenic action of UV(A+B) in comparison to UVB. Also, it has been shown that part of CA is unphotorepairable [13]. However, the genotoxicity of different parts of UV spectrum not equally depends on the environmental factors such as temperature. In plants, the effect of UVB and photoreactivation decreases at a low temperature, while UVC-induced DNA damage is no temperature-depending. The decreasing effect of temperature is unequal for CPDs and 6–4 PPs [17]. So, our findings need confirmation in various years.

On the grounds of the previous work [14], the bulk of attention was paid to the action of UV(A+B). The dose effect was investigated in 2001. The doses were expressed by different duration of UV(A+B) irradiation – 2 and 4.5 h (Figure). The effect of UV(A+B) was dose-dependent. After 2 h of irradiation CA frequency increased about twice against the control level, while after 4.5 h irradiation CA level increased about five times.

In 2001 a stronger effect of UVB was noted. It confirmed the results of 2000.

Results of 2001 confirmed also the main conclusion of 1999 and 2000. Photoreactivation reduced the level of CA in irradiated UV cells by about one half, but it removed not all DNA lesions leading to chromosome aberrations The part of CA that was removed by photoreactivation was due to (CPDs) or (6–4PPs). These DNA lesions are specifically removed by photoreactivation.

However, the part of CA and DNA lesions that cause these CA are insuperable for photoreactivation enzymes. Although, several explanations may hold true for that phenomenon [see 14], the most probable is that the unphotorepairable part of CA arises from DNA lesions other than CPDs or 6–4 PPs.

Although CPDs and 6–4 PPs are the main and most important group of DNA lesions induced by UVC or UVB, also other types of DNA lesions arise after UV irradiation. These types may be attributed to induction of free radicals and oxidative stress or indirectly to photosynthetic machinery [18–20].

A significant contribution to that phenomena can be made by UVA rays. At present, both UVB and UVA are described as mutagenic, but the processes

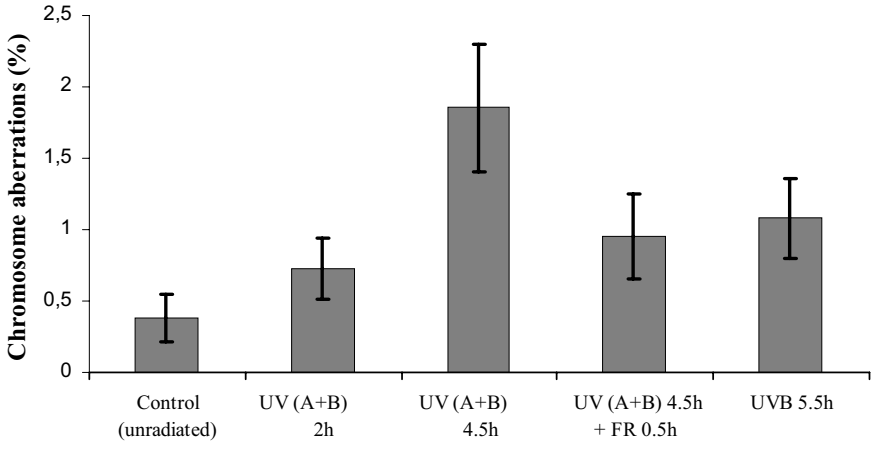

Figure. Action of solar UV on induction of chromosome aberrations and the efficiency of their photorepair (FR) in meristematical cells of *Crepis capillaris* root tips

by which they alter DNA are different. UVB is directly absorbed by DNA molecule and induces CPDs and 6–4 PPs. UVA absorption by DNA is very weak, but UVA interacts directly with other cellular chromophores that can produce reactive oxygen species (ROS) during photosensitization reactions. ROS induced other types of DNA lesions, mainly such as 7,8-dihydro-8-oxo-2-deoxyguanosine (8-OxodG).

Even when UVA and UVB are simultaneous and generate ROS, 8-Oxod-Gs are more often produced by UVA than UVB radiation [21–23].

As regards our works, for UV irradiation of *Crepis capillaris* root tips very sunny days in May–June were chosen [14 and the present work]. It is known that intensive sunlight is also a DNA-damaging factor for plants, also inducing ROS [24, 25].

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V. Rančelienė, K. Šlekytė, K. Cieminis

CHROMOSOMŲ PAŽAIDOS, INDUKUOTOS SAULĖS UV(A+B) SPINDULIUOTĖS, IR JŲ FOTOREAKTYVAVIMAS SAULĖS ŠVIESA *CREPIS CAPILLARIS* LĄSTELĖSE

S a n t r a u k a

Crepis capillaris šaknelės buvo auginamos tamsoje ir apšvitintos UV (A+B) ir UVB specialioje kameroje su UV filtrais. Dalis apšvitintų šaknelių buvo paveikta fotoreaktyvuojančia šviesa (FŠ). Chromosomų aberacijų (CA) dažnis nustatytas šaknelių viršūnėlių meristeminėse ląstelėse metafazių metodu.

Pastebėta, kad UVB ir ypač UV(A+B) sukelia CA. UV (A+B) poveikis priklausė nuo spinduliuotės dozės. Po 4,5 val. UV(A+B) spinduliuotės CA dažnis padidėjo apie 5 kartus. FŠ sumažino CA dažnį apie 2 kartus. Šios CA dalies priežastimi yra pirimidinų dimerai. Kadangi Saulės UV ir FŠ veikia augalus vienu metu, likusios nefotoreaktyvuotos CA kelia realią grėsmę augalų genomui. Straipsnyje yra svarstoma. Šių CA prigimtis.