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# Modification of genotoxic action of sunlight UV with antioxidants – ascorbic and salicylic acids

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Cells of *Crepis capillaris* root tips were irradiated with full sunlight, UVB, UV(B+A), or with artificial UVB. The modifying effect of ascorbic acid (AA) and salicylic acid (SA) on the genotoxic action of UV radiation expressed by chromosome aberration (CA) frequency has been studied. Both substances were shown to decrease CA frequency, but the effect was more pronounced if the artificial source of UVB was used. It was proposed that variations in microconditions on the day of UV irradiation as well as the different quality of seeds may be the main cause of variations in the results of investigations.

**Key words:** sunlight UVB, UVA+UVB, genotoxic action, chromosome aberrations, modifiers, salicylic acid, ascorbic acid, *Crepis capillaris*

## INTRODUCTION

For the vital activity of plants solar irradiation is a necessity. A very important part of the solar light spectrum is photosynthetic active radiation (PAR; 400–700 nm). Seven percent of the electromagnetic radiation emitted from the sun is in the UV range (200–400 nm). As it pases through the atmosphere, the total UV flux transmitted is greatly reduced and the composition of the UV radiation is modified. Short-wave UVC radiation (200–280 nm) is completely absorbed by atmospheric gases. UVB (280–320 nm) is additionally absorbed by stratospheric ozone and thus only a very small proportion is transmitted to the Earth's surface, whereas UVA radiation (320–400 nm) is hardly absorbed by ozone and reaches the Earth's surface [1].

UVB radiation is a small but biologically significant portion of the solar spectrum reaching the Earth's surface. The predominantly UVB-induced DNA lesions are various types of pyrimidine dimers (PDs). Simultaneously PDs are reversed to initial state by photoreactivation in the range of 300–420 nm light with photolyases [2–7].

The features of sunlight action on plants are: (1) all parts of the sunlight spectrum exerting a different effect act simultaneously, including the photoreactivating part of the sunlight spectrum (PHL; 320–400 nm); (2) a significant genotoxic effect of UVA has been shown recently [8–13]; (3) intensive solar irradiation in the PAR part of the spectrum is also (geno)toxically hazardous, including oxidative burst production of reactive oxygen intermediates (ROI) of the excited chlorophyll [14]. Hence the real solar UVB hazard can be fixed only after PHL, and the results of its interaction with UVA must be also determined.

The employment of PHL has an important advantage because photoreactivation removes only PDs by monomerisation, and a reduction in the biological effect after PHL means that PDs are involved in that biological effect of UV irradiation [15]. That strategy of investigation was successfully applyed to show that PDs induced by artificial UVC are realized to chromosome aberrations (CAs) in meristematical cells of barley root tips [16].

Many-year investigation of sunlight UV action on meristematical cells of root tips of the model plant *Crepis capillaris* (L.) Wallr. shows that the solar UVB, and, especially UV(B+A), increase the CA level, respectively, 1.73–4.75 and 2.23–10.5 times. The solar light spectrum was dissected into UVB, UVA and PHL ranges by special filters. However, only about half of CAs were removed by PHL [17, 18].

The two features are intriguing with regard to the solar UV effect on CA induction: the nature of CAs remaining after PHL and the higher level of CAs after irradiation with UV(B+A) in comparison with UVB alone. The PHL test has revealed that the remaining genotoxic effect of solar UV after PHL, leading to CAs, may be a result of DNA lesions of some other nature, not only cyclobutane pyrimidine dimers (CPDs) or (6-4) pyrimidine pyrimidone photoproducts (PPs). Both these types of DNA lesions are effectively removed by the photoreactivating enzymes photolyases [2–8]. However, it is also known that UVB, in addition to CPDs and PPs, induces other types of DNA lesions such as cytosine hydrates, DNA–DNA and DNA–protein crosslinks, DNA strand breaks [19, 20].

As regards UVB and UVA, both have been described as mutagenic, but the processes by which they alter DNA are mostly different. Despite convincing works [9,

21, 22] that UVA also induces CPDs, since UVA is so weakly absorbed by DNA, its mutagenic effect has generally not been attributed to pyrimidine photoproducts but rather to exication of non-DNA chromophores leading to production of reactive oxygen species (ROS) who, in turn, attack the DNA double helix to yield oxidized bases and DNA strand breaks [23, 24]. UVA induces photooxidation products of guanine, most notably the highly mutagenic adduct 8-oxo-7,8-dihydroguanine (8-oxoG) [7, 21, 25–27].

We supposed that CAs remaining after PHL may be conditioned by UVB-induced oxidative stress. The higher frequency of CAs after UV(B+A), in comparison to UVB action alone, strengthens that assumption.

The genotoxic consequences of oxidative stress are effectivelly removed by antioxidant treatment. Ascorbic [28–30] and salicylic [1, 31–35] acids show such effect. Salicylic acid serves also as a signaling molecule for induction of several gene groups, including *PR* genes, common for responses to various stresses. We proposed that the part of CAs that is induced by ROS may be effectivelly eliminated by ascorbic (AA) and salicylic (SA) acids. In the present work, this assumption was checked out experimentally and a positive effect of SA and AA has been obserwed.

## MATERIALS AND METHODS

A heterogeneous meristematic cell population of *Crepis capillaris* (L.) Wallr. root tips was used to study the chromosome aberrations (CAs) induced by sunlight (full spectrum), solar UV(B+A) or artificial UVB, and the effect of ascorbic (AA) and salicylic (SA) acids on the level of induced CAs. Seed material for all experiments was grown in different years. Fresh seeds were used.

**Treatment with ascorbic and salicylic acids**. Seeds of *C. capillaris* were germinated in a thermostat at 25° C in the dark either on distilled water or on $10^{-4}$ M solutions of AA or SA in Petri dishes for 36 h until root tips reached 3–5 mm in length. Such root tips were irradiated or used as control without treatment with sunlight or artificial UVB.

**Irradiation by solar light, solar UV(B+A), UVB or PHL**. Roots were placed into Petri dishes and put into special chambers equipped with filters that passed only the UVB, UV(B+A), or PHL part of the sunlight spectrum. For UVB the filters were ZS-20 and UFS-2, for UV(B+A) – UFS-2, and for photoreactivation – SZS-23 (made in Russia). In one experiment root tips were irradiated in open air with a full sunlight spectrum. The maximum sunny days were specially selected. The choice of these days was very difficult. Several faulty attempts were made each year.

A comparison of SA effect on the full sunlight spectrum or only its UV(B+A) part was made on 21 May 2002. It lasted 5 h, beginning from 10 a.m. The exposition to sunlight UV(B+A) alone or after SA treatment was made on 22 May 2002 and lasted 5 h, beginning from 9.30 a. m. The next experiment with sunlight UV(B+A) alone or after SA treatment was performed on 26 May 2003; the exposition lasted 5 h, beginning from 11 a. m. In 2002, separate experiment was made with ascorbic acid-treated material. Root tips exposed to sunlight UV(B+A) on 8–9 June 2002 for 3 h, beginning from 11 h a. m.

In each experiment, after UV irradiation part of roots were immediately treated with photoreactivating solar light. Exposition to PHL lasted 1 h.

In 2005, the effect of SA was tested on root tips irradiated with artificial UVB. A Vilber–Lourmat lamp was used (max. 312 nm), the dose was 1200 $Jm^{-2}$ (0.185 mW $cm^{-2}$).

**Chromosome aberration (CA) test**. All manipulations 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 3, 6, 9 hours after irradiation. The fixed root tips were stored in 70% ethanol in a freezer until used. CAs were studied on temporary preparations stained with acetocarmine. The metaphase cells were examined. CAs were observed with a PZO microscope (Warsaw). Most of CAs were presented by chromatid and chromosome fragments.

**Statistical analysis** The mean values ± S. D. are given in Figures and Table. The significance of differences among the means was analysed by Student's t test.

## RESULTS AND DISCUSSION

Different sources and conditions of UV irradiation were tested in four separate experiments with SA and in two experiments with ascorbic acid (AA). The source of irradiation was from a full sunlight spectrum to irradiation with the UV(B+A) part of sunlight or only with UVB from sunlight or artificial source – a Vilber–Lourmat lamp (max. 312 nm). Experiments with SA were made in different years: 2002, 2003, 2005, with AA – only in 2002, and the different seed material growing conditions in each parental generation, as well as microconditions in the different days of irradiation might have been of significance.

The SA protective effect was most pronounced if root tips had been irradiated with UVB alone from an artificial source (Vilber–Lourmat lamp) (Table). SA treatment decreased the CA level nearly twice. The results of that experiment confirmed also our previous conclusion [17, 18] that part of CAs is due not to CPDs, but is related with ROS and DNA lesions induced by oxidative stress [21, 23, 24]. A comparison of results after photoreactivation (PHL) and treatment with SA alone or in combination with PHL showed that the part of CAs repaired by SA treatment comprised about 20% of the common pool of CAs induced by artificial UVB.

Unlike in the case of artificial UVB, the ecological microconditions on the day of sunlight UV-irradiation may have a significant influence on the biological ef-

fectivity of UV and its modifiers such as SA or AA. It is very difficult, or even impossible, to escape differences in microconditions on days of irradiation with a natural source of UV. For that reason, the effect of SA on sunlight UV(B+A) was tested in two different years – 2002 and 2003 and, indeed, differences were observed between the results of both experiments, despite the fact that both experiments, in 2002 and 2003, were made at about the same time of the year, respectively on 22 and 26 May.

In 2002, a significant decrease in CA frequency induced by sunlight UV(B+A) was observed only if for CA repair SA was used in combination with sunlight photoreactivating light – SUV(B+A)+SA+PHL (Fig. 1). SA treatment decreased slightly also the spontaneous CA level observed in control root tips without any treatment with sunlight and planted always in the same conditions, but in the dark. Under UV (B+A) irradiation of root tips treated with SA, the CA level was about the same as for UV (B+A)+PHL.

In 2003, both protective factors, PHL or SA, were equally effective on root tip meristematical cells irradiated with UV (B+A). However, the simultaneous action of both protective factors, SA and PHL, was not so effective as in the previous year (Fig. 2). A slight protective effect of SA on spontaneous mutagenesis was also observed in that experiment (Fig. 2).

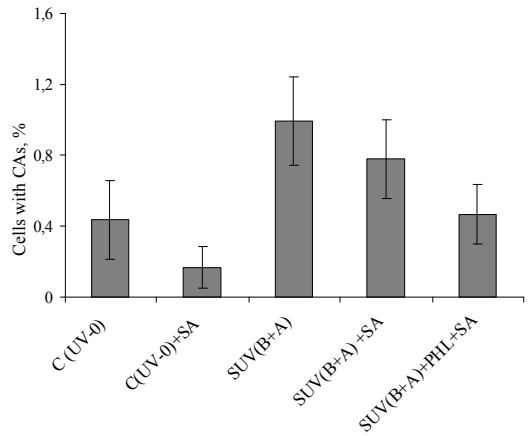


**Fig. 1**. Modifying action of salycilic acid (SA) on chromosome aberration (CA) frequency (%) induced in *Crepis capillaris* root tip meristematical cells irradiated with sunlight UV(B+A), experiment of 2002:
C(UV-0) – untreated control cells; PHL – photoreactivating light

In 2002, an additional experiment was made, in which the mutagenic effects of the full sunlight spectrum were compared with the mutagenic action of the UV(B+A) part of the sunlight (Fig. 3). The purpose of this experiment was to ascertain that only the UV(B+A) part of sunlight is genotoxic. Both experiments in 2002 were made on the same seed material and with time difference of in only one day. Only a slight protective effect of SA was observed for full sunlight and for UV (B+A). On the other hand, results of that experiment showed that the mutagenic effect of sunlight is determined only by the UV (B+A) part of sunlight. The CA

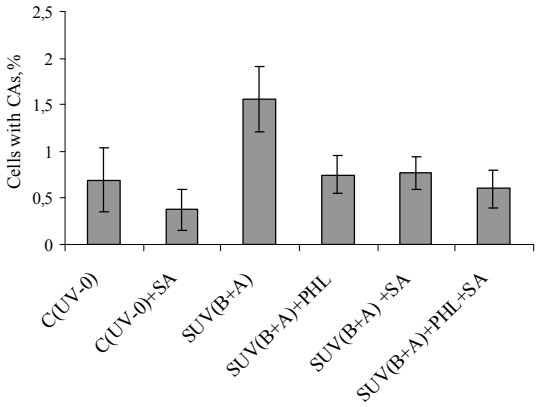


**Fig. 2**. Modifying action of salycilic acid (SA) on chromosome aberration (CA) frequency (%) induced in *Crepis capillaris* root tip meristematical cells irradiated with sunlight UV(B+A), experiment of 2003. Abbreviations as in Fig. 1.

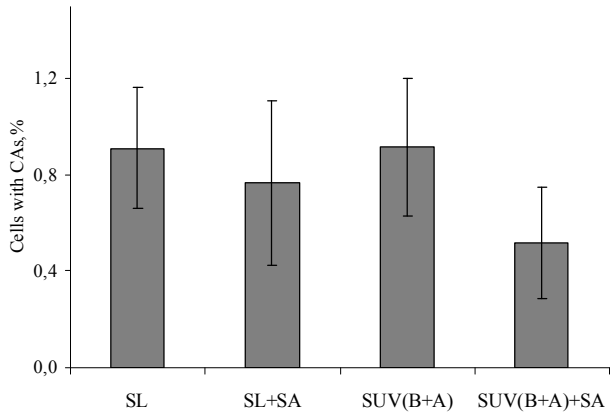


**Fig. 3**. Comparison of mutagenic action of full sunlight (SL) spectrum with SUV(B+A) part alone. Action of salicylic acid as a modifier, experiment of 2002

Table. **Protective effect of photoreactivating light (PHL) and salicylic (SA) on chromosome aberration frequency in meristematic cells of *Crepis capillaris* root tips after irradiation with artificial UVB (year 2005)**

| Experimental conditions | Number of methaphases | Chromosome aberrations | | | Effect in comparison with UVB, % |
|---|---|---|---|---|---|
| | | Number | % | t | |
| UVB | 1272 | 36 | 2.83 ± 0.46 | - | 100.0 |
| UVB+PHL | 1457 | 28 | 1.92 ± 0.36 | 1.6 | 67.8 |
| UVB+SA | 288 | 4 | 1.39 ± 0.69 | 1.7 | 49.1 |
| UVB+SA+PHL | 1476 | 19 | 1.29 ± 0.29 | 2.9 | 45.6 |

level for full sunlight or only its UV(B+A) part in that experiment was nearly equal (Fig. 3).

It is necessary to note that in the effective combinations, SUV (A+B)+SA+PHL (Figs. 1 and 2) or SUV (B+A)+SA and SUV (B+A)+PHL (Fig. 2), CA frequency decreased to the control level (without irradiation with UV) (Fig. 2).

No such effect was observed when ascorbic acid (AA) was used as a modifying factor. Not only PHL, but also AA were unable to remove completely CAs induced by sunlight UV(B+A) (Fig. 4) or UVB (Fig. 5). It shows a certain difference in the action of SA and AA on (1) CA induction with UV or (2) realisation of DNA lesions to CAs, or (3) both. We suggested the SA action to be wider. As is known from the literature, SA acts also as an inducer of PR-proteins [36, 37] and increases plant immunity to pathogens [39, 40], improves the general state of a plant as a signaling molecule [40, 41]. On the other hand, SA acts in tobacco cell suspension culture as a ROS inducer. In that case the endogenous peroxidases showed a protective action [42]. So, the interaction of the endogenous and exogenous antioxidant systems is one of the problems to be studied in the future. We propose that the dependence of that interaction on environmental conditions is among the main causes of the varying effect of SA and AA (see Figs. 4 and 5) in different experiments. It is true also for a varying action of SA and AA on the spontaneous level of CAs. Variations in environmental microconditions at the moment of sunlight UV irradiation or SA and AA treatment may cause also a different relative effect of PHL in comparison to SA or AA effect on CA level. PHL was ineffective in both experiments (Figs. 4 and 5) in which AA was used. It is necessary to emphasize that both experiments with AA were performed with a difference of one day only.

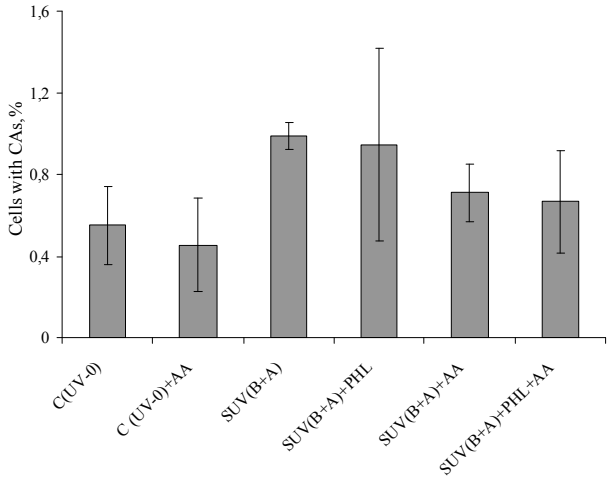


**Fig. 4**. Action of ascorbic acid (AA) on chromosome aberation (CA) frequency (%) induced by sunlight UV (B+A), experiment of 2002

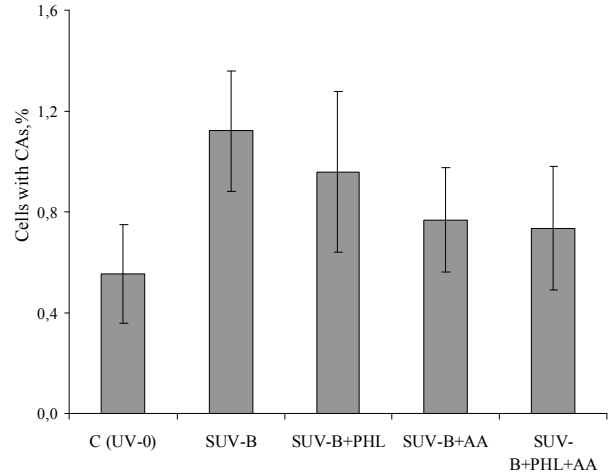


**Fig. 5**. Action of ascorbic acid (AA) on chromosome aberration (CA) frequency (%) induced by sunlight UVB, experiment of 2002

As to AA, its protective effect on CA induction by sunlight UV(B+A) was not more effective if AA and PHL were used simultaneously (Fig. 4).

Despite the well known fact that the mutagenic action of UVB is defined mainly by PDs [3–8], the more perceptible protective effect of AA, as well as of SA, was observed on the UVB (Fig. 5) part of the sunlight spectrum alone, while ROS induction was attributed mainly to UVA [23–27].

In conclusion, it should be noted that the investigation of the effect of exogenous SA or AA on UV irradiation was restricted. Exogenous SA and AA were used mostly for reducing the stressogenic effect of ozone [28, 30, 34, 43], heat shock [35, 44, 45] or cold [46 as review, 35]. Furthermore, the protective effect of AA [47–50] or SA [49–51] was shown on artificial sources of UVB [47-50] or even UVC [51]. The latter investigation, as well as [50], is of significance in using UV irradiation for cosmetic purposes.

The present work on AA or SA effects has shown that irradiation with an artificial UVB source gives more convincing results than sunlight UV. In the latter cases the additional microecological conditions are escaped. However, investigation of the sunlight UV radiation is more significant ecologically.

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## SAULĖS UV GENOTOKSINIO VEIKIMO MODIFIKAVIMAS ASKORBO IR SALICILO RŪGŠTIMIS

S a n t r a u k a

Žaliosios kreisvės (*Crepis capillaris)* meristeminės šaknų ląstelės buvo apšvitintos viso saulės spektro šviesa, saulės UVB ir UV(B+A) arba dirbtiniu UVB. Ištirtas modifikuojantis askorbo ir salicilo rūgščių poveikis genotoksiniam UV spinduliuotės poveikiui, kuris įvertintas pagal chromosomų aberacijų dažnį apšvitintose ląstelėse. Modifikuojantis poveikis buvo didesnis, jeigu buvo naudojamas dirbtinis UVB šaltinis. Manoma, kad skirtumai tarp bandymų atsiranda dėl nevienodų sėklų, išaugintų įvairiais metais, ir skirtingų aplinkos sąlygų eksperimentinės medžiagos apšvitinimo metu. Palyginus genotoksinį viso saulės spektro ir tik jo UV dalies veikimą, įsitikinta, kad genotoksinis saulės šviesos poveikis priklauso tik nuo UV spektro dalies.

**Raktažodžiai:** saulės UVB + UVA, genotoksinis veikimas, chromosomų aberacijos, modifikatoriai, askorbo rūgštis, salicilo rūgštis, žalioji kreisvė (*Crepis capillaris*).