Induction of DNA and chromosomal damages by UV-C and solar UV and its photoreactivation in *Crepis capillaris* cells

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The photoreactivation (PR) of chromosomal aberrations (CA) induced by UV-C and solar UV light and the induction *in vitro* and *in vivo* of DNA sites sensitive to the action of UV-endonuclease (SSE) have been researched. A decrease in the number of UV-induced CA after PR points out that their induction was caused by the DNA photoproducts. The exposure of roots to solar light (300–380 nm) immediately after exposing to UV-C radiation reduced the CA formation by 70% and 40% respectively in 2n and 4n cells including the chromatid-type aberrations in the second cycle after UV radiation. There were some photoproducts that have not been removed by DNA repair and were capable of inducing chromatid-type aberrations in the second cell cycle after irradiation. PR did not reduce CA formation if roots were photoreactivated 3 h after exposing to UV radiation. Solar light (290–360 nm) induced SSE in the DNA solutions up to 180 SSE/10^8 Da, in June twice more effectively than in October. Solar UV-B and UV-A, B light induced CA formation. The formation of CA was reduced after irradiation with both solar UV-A or visible light.

Key words: chromosomal aberrations, photoreactivation in plants, UV

INTRODUCTION

In the present work, we review our investigations of relationships between DNA damage and chromosomal aberrations inducted by UV-C and solar UV. Plants are exposed to UV-radiation which is present in the sunlight. Solar UV (SUV) action on the chromosomes of higher plants has been poorly studied [1]. We supposed that SUV action on DNA and chromosomes of higher plants could involve some not yet elucidated processes such as induction of photoproducts by SUV irradiation, repair of these photoproducts. Later some new photoreactivating enzymes were found; they specifically repair UV-induced (6–4) photoproducts in higher plants and lesions induced by UV-B [2–8].

We supposed that DNA lesions inducted by solar UV could be important for mutation process and could take place in chromosomal mutation. This supposition was based on forgotten data [9] which show that solar UV can induce pyrimidine dimers in cell DNA. Literature data on the photochemistry, photophysics of the mechanisms of DNA pyrimidine dimer and other photoproducts repair [10–13] allowed to suppose that solar UV can induct a sufficient number of DNA photoproducts in the plant cells. These damages could be repaired or not repaired and later involved again in the formation of mutations.

MATERIALS AND METHODS

The heterogenous cell population of *Crepis capillaris* root tips was used to study the induction of chromosomal aberrations [14] and the influence of photoreactivating light on the frequency of aberrations induced by UV-C [15–17].

DNA lesions – thymine dimers (TD) were estimated as sites sensitive to the action of UV-endonuclease (SSE) in labeled DNA [18]; irradiation of DNA, roots by SUV was done in Vilnius region, Lithuania [19–20].

RESULTS AND DISCUSSION

SSE and CA induction curves depending on UV irradiation dose showed that when the UV irradiation dose was low, SSE were induced linearly (Figure). Comparison of the dose–response curves of barley and *Crepis* helped to show better that irradia-

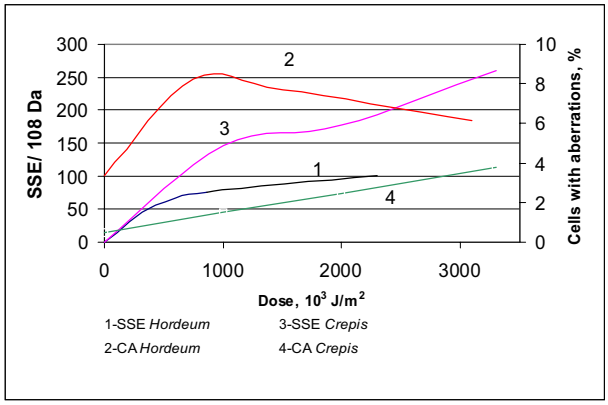

Figure. Induction of SSE and CA by UV-C irradiation. Comparison of dose–response curves of *Hordeum* and *Crepis*

tion with high doses decreased the frequency of CA, whereas DNA lesions at the same doses increased.

Induction of SSE by SUV-irradiation in DNA *in vitro*. We studied SSE induction with SUV in the DNA of barley, *B. subtilis* (in collaboration with K. Ambrozaitis, K. Žukas and E. Česnavičienė, Vilnius State University) and of *Crepis*. DNA solutions, labeled and frozen barley seedlings were irradiated with a whole (total) spectrum of solar radiation, or only with a visible light, or only with UV. Absorption filters with known transmission rate at different wave length and absorption coefficients were used.

Dose–response curves, one for the whole solar spectrum and one for the UV spectral range (transmitted by UVS-1 filter) were established [19]. The solar radiation of the whole spectrum and only of the UV region induced a high number of SSE in DNA solutions – UV-endonuclease degraded such DNA. The SSE induction dependence on SUV irradiation dose was linear. Summer solar UV light induced a higher SSE number if compared with that of autumn light: approximately about 80 SSE/10^8 Da DNA in summer and 20 SSE/10^8 Da DNA in autumn after $60 \cdot 10^3$ J/m^2 of SUV irradiation. Having learned the level of day's energetic exposure of solar UV-B irradiation, SSE induction dependence on SUV irradiation dose, we could estimate that the probability of DNA damage by SUV in plant cells was quite real.

A relationship between the intensity of pyrimidine dimers with SUV-B, UV-C induction and formation of chromosomal aberrations in apical meristems, as well as between the frequency of CA and the number of SSE depending on UV dose was noticed [18].

Induction of chromosomal aberrations and its photoreactivation. The frequency of UV-induced CA in two cell cycles of *Crepis capillaris* cells was investigated. The main attention was paid to the level of chromatid-type aberrations. Both chromosome-type and chromatid-type mutations were observed. The frequency dependence upon irradiation dose was linear both in diploid and tetraploid cells. Symmetric and asymmetric translocations were the major type of chromosome aberrations in diploid and tetraploid cells. Chromatid-type deletions, isochromatid deletions, symmetric and asymmetric translocations were observed in diploid and tetraploid cells

Single fragments (mainly terminal deletions) in tetraploid cells were detected. If they had been formed from pyrimidine dimers not excised in the previous cell cycle, the photoreactivation had to decrease the frequency in tetraploid cells. The photoreactivating light was found to decrease the frequency of UV-induced chromosomal aberrations in tetraploid cells and the number of SSE.

Therefore, it is possible to suppose that pyrimidine dimers in plant cells can be the initial DNA lesions, which later turn into chromosomal mutations. This conclusion was confirmed in the second experiment of PR with a greater number of fixations in UV-irradiated roots of *Crepis capillaris*. In this experiment PR was fully confirmed in two cell cycles. The sunlight photoreactivated about 70% of CA in 2 n cells and about 65% in 4 n cells [21].

The PR light decreased the frequency both chromosome-type and chromatide-type aberrations in 2 n and 4 n cells. This means that in higher plants PD can pass over replication and realize into CA in the next cell cycle after UV.

The frequency of UV-induced chromosomal aberrations in G_1 and G_2 cells was decreased by the photoreactivating light [15–16].

The PR light would not decrease the aberration frequency if it was used not immediately after UV irradiation, but some hours later [21]. That is why the influence of PR light was effected after UV within a sliding period of time. It was confirmed in our two experiments in 1996 and 1997. In 1996, UV inducted 4.83 CA in 2n cells. The PR light immediately after UV decreased the CA frequency to 0.98%, 0.5 h later – to 0.33%, 1 h later – 0.89%, 2 h later – 0.86%, 3 h later – 3.92% CA.

In 1997, a repeated experiment was performed in order to gain more precise data. Should PR light not act 4 hours later as well? It was shown that UV light induced 4.7% of CA. If PR light was used immediately after UV, it decreased the CA frequency to 0.81%, 1 h later – to 1.19%, 2 h later – to 0.95%. After 3 or 4 hours after UV irradiation the PR effect disappeared. The frequency of CA was 4.78% and 4.24%, respectively.

So, all our PR experiments confirm the supposition that part of TD can cause CA formation. Besides, it is possible that lesions caused by solar UV

can pass over DNA replication (like the lesions caused by UV-C irradiation) and be realized into CA within the second cell cycle after UV action. This was proved by the presence of single fragments within this cycle. Existence of CA as a chromatide type in 4n cells of *Crepis capillaris* after UV-C-irradiation shows that the latter type of CA was formed within the second cell cycle after irradiation [21]. This shows that the cells have been already divided after UV irradiation, *i.e*. DNA has been completely replicated. That is why it is possible to affirm that nonexcised solar UV-induced TD give rise to the formation of aberrations in the second cycle of UV-irradiated cells. This means that SUV-inducted DNA lesions can be left in DNA chain and induce aberrations in tetraploid cells. Irradiation by PR light immediately after UV action decreased the number of aberrations also within the second mitosis after UV-irradiation, *i.e*. in 4n cells. On the other hand, PR experiments showed that the number of photoreactivated TD was incomparably greater than the number of CA [19]. A question arises why only one-thousandth part of the total number of dimers had induced CA. Probably CA were induced by another product, maybe some different products (not only TD) and there were much less of them, but they were photoreactivated as well. Considering the above-mentioned facts, it is possible to say that there is not one or two photolyases as it was proposed before, but several of them, and that was confirmed by literature data [3, 7, 11, 22].

Induction of chromosomal aberrations by solar UV and its photoreactivation. In two different experiments during two summers it was shown that solar UV-B and UV-B+A could induce a detectable level of chromosomal aberrations approximately about twice more frequently than in control cells. Irradiation with solar photoreactivating light (UV-(B+A)+visible or only visible) decreases the CA number to a control level [20].

These results confirmed our supposition that SUV-induced photoproducts could induce the formation of chromosomal aberrations. Some of them could be cyclobutane-pyrimidine dimers, and this genetical danger for plants, defined by increasing UV-B radiation, is quite real.

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DNR IR CHROMOSOMŲ PAŽAIDŲ INDUKCIJA UV-C IR SAULĖS UV SPINDULIAIS IR JŲ FOTOREAKTYVACIJA *CREPIS CAPILLARIS* LĄSTELĖSE

S a n t r a u k a

Apibendrintais darbų ciklo rezultatais aiškinama, kaip DNR pažaidos, indukuotos saulės ultravioletiniais spinduliais, gali dalyvauti susidarant chromosomų aberacijoms. Tokio proceso schema pagrįsta 4 faktų grupėmis: 1) aberacijų ir DNR pažaidų dozinės priklausomybės kreivėmis; 2) DNR pažaidų indukcija saulės UV spinduliais; 3) chromosomų aberacijų fotoreaktyvacija; 4) chromosomų aberacijų indukcija saulės UV spinduliais ir jų fotoreaktyvacija.