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REGULAR ARTICLE

Modification of UV-B radiation effect on *Crepis capillaris* by antioxidant and environmental conditions

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Abstract

Stability of cell division and genetic structures has special significance for plant development and productivity. On the basis of literature data about the very low photolyase activity in roots, the action of UV-B radiation and its putative modifying factors were studied on the root meristematic cells of model plant *Crepis capillaris*. It is really a very sensitive system for UV-B radiation. Action of UV-B was investigated in a wide range of doses (0; 0.75;1.00; 1.13; 1.50; 2.00; 2.50; 3.00 kJ m^{-2}. The dose of 0.75 kJ m^{-2} already decreased the cell division and up to 1 kJ m^{-2} UV-B dose induced the chromosome aberrations (CAs). The supplementary B chromosome did not show any effect on CA induction, but plants with B chromosome had a more stable mitotic activity of cells. The strongest protective effect on CA induction was revealed by salicylic acid (10-4 M). Photoreactivation also showed certain decrease of the CA level, and the lowest effect was of ascorbic acid.

Key words: B-chromosome, Chromosome aberrations, Mitotic activity, Photoreactivation, Salicylic acid, UV-B radiation

Introduction

UV-B radiation, an inevitable environmental factor, has dualistic impact for plants. Stress inducing damaging effects of UV-B radiation on genome stability and physiological processes are well known, and sunlight UV-B radiation increasing the latter time is relevant not only for the human health but also for the plant productivity and production quality (Caldwell et al., 2007). Opposite to it, UV-B radiation acts also as a regulatory factor of plant life processes, even in the stress inducing conditions (Kakani et al., 2003; Ristilä et al., 2011; Robson and Aphalo, 2012).

Many stress-inducing environmental factors, including UV-B radiation, cause oxidative stress. That plant condition, induced by UV-B radiation, may be successfully changed by the exogenous anthocyanins (Woodall and Stewart, 1998; Tsoyi et al., 2008), ascorbic (Athar et al., 2008) or salicylic (Mahdavian et al., 2008) acids. In our study, ascorbic (AA) and salicylic (SA) acids decreased chromosome aberration level induced by the sunlight UV-B radiation in the meristematic root tip cells of *Crepis capillaris* (L.) Walrr. (Rančelienė et al., 2007). AA also protected *Vicia faba* plants against ozone (Turcsanyi et al., 2000). Anthocyanins from the cherry fruits revealed positive action on mitosis of onion (*Allium cepa*) and *C. capillaris* root cells and antimutagenic action against UV-B radiation in the meristematic root cells of *C. capillaris*. Concentration of cells in the prophase may be also attributed to protective action of anthocyanins as prolonging time for repair processes (Rančelienė et al., 2009).

As SA also elevates negative action of other stress-inducing factors (Horvath et al., 2007; Hayat et al., 2010) and, as demonstrated in several other studies did not included in the referred reviews, of salt stress (Gunes et al., 2007; He and Zhu, 2008; Szepesi et al., 2008), drought (Bechtold et al., 2010), metals (Ivanova et al, 2008; Popova et al., 2008), it is supposed that exogenous antioxidants may have wider application as means for the simultaneously increasing resistance not to one but to several stress-inducing factors. It may be applied in agriculture for increasing the plant production quality. The same may be attributed to AA because it activates defence-signalling genes regulating responses to ozone and pathogens (Conklin and Barth, 2004).

It was supposed that regulating action of UV-B radiation, like its interaction with modifying factors, may be effectively shown on root meristematic cells of *C. capillaris*. Information on

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the action of UV-B radiation as well as of endogenous or exogenous antioxidants on the meristematic cells is very limited (Perennes et al., 1999; Potters et al., 2000).

In the present study, we investigated the effect of UV-B radiation on meristematic root cells in a wide range of UV-B doses, compared two different *C. capillaris* karyotypes with and without supplementary B-chromosome, and action of the exogenous AA and SA on UV-B radiation effects in the meristematic root tip cells of *C. capillaris*. Effect of SA was compared with the action of photoreactivating light on the UV-B irradiated cells. Mitotic activity and induction of chromosome aberrations were studied. *C. capillaris* is very suitable for chromosome structure studies because it has only six chromosomes in diploid cells.

Meristematic root cells were chosen for two reasons. First, it allowed to carry out all treatments in the dark to escape photoreactivation and in other fully controlled environmental conditions. Second, it was believed that the root tip cells are very sensitive to UV radiation, because root cells have the lowest photolyase activity. It has been determined on different plants: rice (Hidema and Kumagai, 1998; Iwamitsu et al., 2008), *Arabidopsis* (Waterworth et al., 2002), spinach (Yoshihara et al., 2005). Maybe, other protective factors against UV action are also less expressed in root tip cells. Consequently, it was believed that in such system various modifications of UV-B radiation effects are easier to detect.

The aim of the present work was to show that meristematic root tip cells of *C. capillaris* are sensitive and useful as the test system for investigation of UV-B radiation action on plant meristematic cells as well as to investigate factors significant for UV-B radiation effect: dependence on UV-B dose, supplementary B chromosome, time of root fixation, photoreactivation, action of ascorbic and salicylic acids.

Materials and methods

A heterogeneous meristematic cell population of *C. capillaris* root tips was exposed for artificial UV-B radiation. Seed material for all experiments was grown in natural out-door conditions, typical for temperate altitude of Vilnius city (54º45´N 25º16´E) and humid continental climate. For separate experiments only fresh seeds of the same year reproduction were used. Seeds of plants with B-chromosome were obtained from J. Maluszynska through Neil R. Jones; the plants were preliminary propagated in the same conditions as the plants without B-chromosome.

Root tip irradiation

Seeds of *C. capillaris* were germinated in thermostat at 25 °C in the dark and root tips of 2-3 mm length 36 h after beginning of germination were irradiated with UV-B (312 nm, max. 2.7 mW cm^{-2}, Vilber Lourmat, France) lamp. The UV-B doses were measured with radiometer VLX-3 and sensor CX-312 (Vilber-Lourmat, France). Dose dependence was studied in a wide range – 0; 0.75;1.00; 1.13; 1.50; 2.00; 2.50; 3.00 kJ m^{-2}. Duration of irradiation from 28 sec to 1 min 50 sec, respectively UV-B dose. For examination of SA or AA (both from Sigma) effects on UV-B radiation, seeds were germinated for 36 h on distilled water or on 10^{-4} M SA or AA solutions in Petri dishes with following irradiation by UV-B lamp. Concentrations of SA and AA were chosen after preliminary studies. For photoreactivation the part of root tips were immediately irradiated with visible light.

Determination of mitotic cell activity and chromosome aberration (CA) level

Mitotic activity (MA) was determined and CAs were studied on temporary preparations stained with acetocarmine. The root tips were treated with colchicine (100 mg/l) and fixed with an acetic acid and ethanol (1:3) mixture for 3, 6, 9 hours after irradiation. The fixed root tips were stored in 70 % ethanol in a freezer until used. All cells were analyzed for MA, while CAs were determined only in the metaphase cells, as only in such cells all chromosome arrangements are clearly seen. Most of CAs are presented by chromatid and chromosome fragments.

Statistical analysis

Results were statistically evaluated as the two alternatives expressed in percentage. The mean values ± S.D. are given in Figures and Table. The significance of differences between UV-unirradiated and UV-irradiated cells were analyzed by Student′s *t*-test.

Results and discussion

UV-B dose effect

Certainly, the first question, which arises regarding the action of UV-B radiation on meristematic cells, is dose dependence of mitotic activity and chromosome stability of *C. capillaris* root cells. It was expected that the doses which are usually applied to the above-ground parts of plants are too high for the meristematic root cells. That assumption is grounded on the comparative studies of the DNA repair enzyme activity in different plant organs (Hidema and Kumagai, 1998;

Waterworth et al., 2002; Yoshihira et al., 2005). Consequently, the action of UV-B radiation on the root tip cells was studied in a wide range of UV-B doses (0; 0.75;1.00; 1.13; 1.50; 2.00; 2.50; 3.00 kJ m^{-2}). The used doses were at least three times lower than those usually used for UV-B irradiation of the above-ground plant organs. Interval between the UV-B doses was also not large, only 0.25-0.5 kJ m^{-2}. The levels of UV-B radiation on the Earth′s surface during the vegetation season are anywhere between 2 and 12 kJ m^{-2} day (UNEP, 2002; Kakani et al., 2003).

Exclusive sensitivity of *C. capillaris* root meristem cells was confirmed experimentally by UV-B radiation effects on cell division (Figure 1).

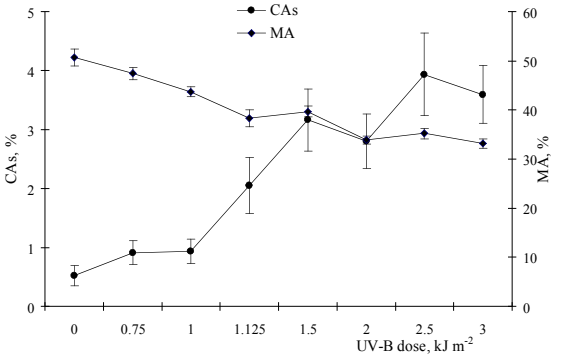

Figure 1. Mitotic activity (MA) and chromosome aberration (CA) frequency after irradiation of meristematic root cells with a wide range of UV-B doses.

Stimulation effect of the low doses on cell division may be expected. However, even the very low 0.75 kJ m^{-2} dose decreased cell division. Starting from 1 kJ m^{-2} the effect became statistically significant, and MA decrease was rather proportional to UV-B dose. CA analysis proved additionally the high sensitivity of *C. capillaris* root meristem to UV-B radiation (Figure 1), but effective doses were above 1 kJ m^{-2}. The CA doubling dose must be in the range between 1.0 and 1.13 kJ m^{-2} doses.

CAs are usually divided into two types – chromosomal and chromatid-type. Their ratio depends on the character of genotoxic factor, but increase of chromosomal type in the pool of CAs also shows the increase of the genotoxic effect. Slight prevalence of chromosomal CAs was observed even in control (UV-B unirradiated) cells, but increase of the part of chromosomal CAs proportionally to UV-B dose was also obvious (Figure 2). As it was shown with artificial UV-C radiation (Cieminis et al., 1987), CAs correlate with the level of DNA lesions.

Plants with and without B-chromosomes

The supplementary chromosomes are generally assumed to be genetically empty (Jones and Houben, 2003). However, the B chromosome in *C. capillaris* karyotype primarily discovered by Maluszynska and Schweizer (1989) is exceptional, because 45S rRNA genes are not only located in it (Jamilena et al., 1994), but also transcribed (Leach et al., 2005).

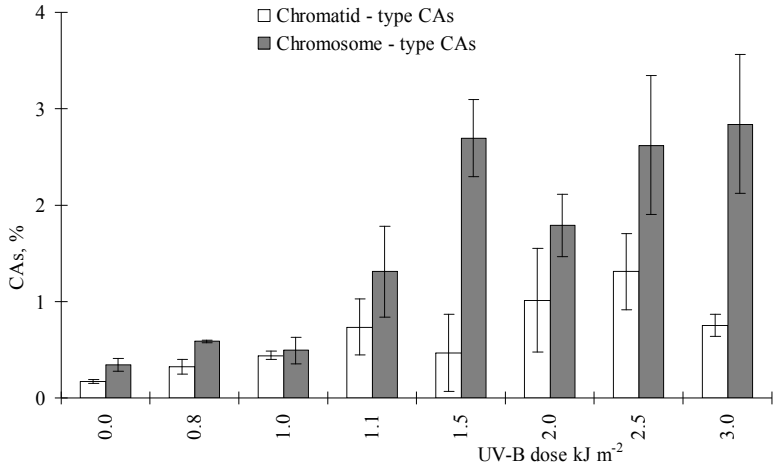

Figure 2. Relation of chromatid-type (white columns) and chromosomal-type (black columns) aberrations upon the UV-B dose.

UV radiation effects on plants with B chromosomes are not yet studied, and we expected to find differences between two groups of cells – with (B+) and without (B-) supplementary chromosome. However, results were partially disappointing, because no differences between the action of UV-B radiation on CA induction in B+ or B- cells was revealed. However, effects on mitotic activity were observed. As in the previous experiment (see Figure 1), the 1.2 kJ m^{-2} UV-B dose reduced MA in meristematic cells without B chromosome, while cells with B-chromosome were more resistant to UV-B radiation (Table). It is a noteworthy fact because cell division is coupled with the repair of DNA lesions and, in general, until DNA lesions are not eliminated, the cell division is blocked by p53-like proteins (De Veylder et al., 2007). On the other hand, the B chromosome of *C. capillaris* has 45S rRNA genes which are very important for the cell activity (Jamilena et al., 1994; Leach et al., 2005).

Therefore, it is relevant to compare mitotic activity at various time intervals after UV-B radiation (Table). Certain increase of MA after 9 h was observed even in control, i.e. UV-B unirradiated cells. We could not observe direct relation of the same effect exclusively in UV-B irradiated cells with B chromosome, but difference between the 3rd and 9th hours of fixations is much higher in the UV-B irradiated cells with B-chromosome than in cells without B-chromosome (Table).

Table. Comparison of mitotic activity (MA) and chromosome aberration (CAs) level in *Crepis capillaris* root cells with (B+) chromosome irradiated with 1.2 kJ/m².

Experimental conditions	Time after UV-B, h	Metaphases		MA	
		n	with CAs, %	n	%
UVB-/B-	3	340	0.59	638	40.0
	6	342	2.92	523	42.6
	9	268	0.37	1358	47.6
Total		950	1.37 ± 0.38	2519	44.7 ± 1.0
UVB+/B-	3	231	4.33	1115	30.6
	6	490	3.47	684	38.5
	9	151	2.65	359	38.4
Total		872	3.56 ± 0.63	2158	34.4 ± 1.1
UVB-/B+	3	233	0.43	382	40.1
	6	412	1.46	556	47.8
	9	272	1.47	1041	41.3
Total		917	1.20± 0.33	1979	42.9 ± 1.1
UVB+/B+	3	362	3.87	642	33.0
	6	413	2.66	1035	50.0
	9	274	3.28	510	54.7
Total		1049	3.24 ± 0.55	2187	46.1 ± 1.0

n – number of investigated cells.

Effects of photoreactivation, ascorbic and salicylic acids on UV-B radiation

SA about twice reduced the CA level induced by 1.2 kJ m^{-2} UV-B radiation, while effect of photoreactivation was lower but statistically significant. Combined action of SA and photoreactivation did not strengthen the protective effect against UV-B effect on chromosome stability (Figure 3).

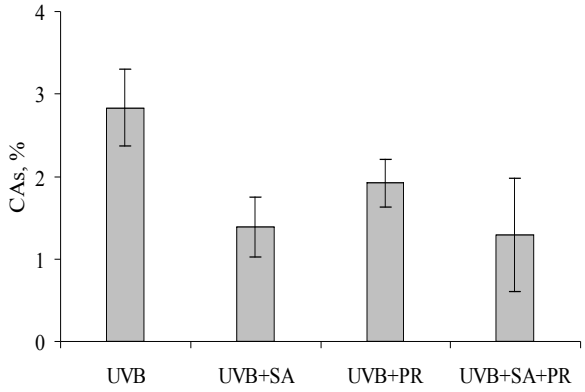

Figure 3. Action of salicylic acid (SA) and photoreactivation (PR) on chromosome aberration (CAs) induction in meristematic root cells irradiated with 1.2 kJ m^{-2} UV-B. Summarized results for three fixations: 3, 6 and 9 h after UV-B irradiation.

Observation of photoreactivation induction is an interesting fact regarding the low photolyase activity in the roots (Hidema and Kumagai, 1998). However, effect of photoreactivating light on CA level induced by the sunlight UV-B or sunlight UV-B+UV-A in *C. capillaris* root meristem was stronger (Rančelienė et al., 2004). Effect of SA on sunlight UV radiation was slightly lower (Rančelienė et al., 2007) than on artificial UV-B in the present work. The effects of photoreactivation and SA on artificial UV-B radiation were observed in our previous work (Rančelienė et al., 2007) similarly as in the present work, but the combined effect of both modifying agents was stronger than their separate actions. The seed quality of plants grown in different years may be caused by such differences.

Slight effect of AA on induction of CAs by artificial UV-B was also revealed. The effect partially depended on the time of root fixation after the UV-B irradiation. Certain effect was revealed only 6-9 h after irradiation with 1.2 kJ m^{-2} UV-B dose (Figure 4).

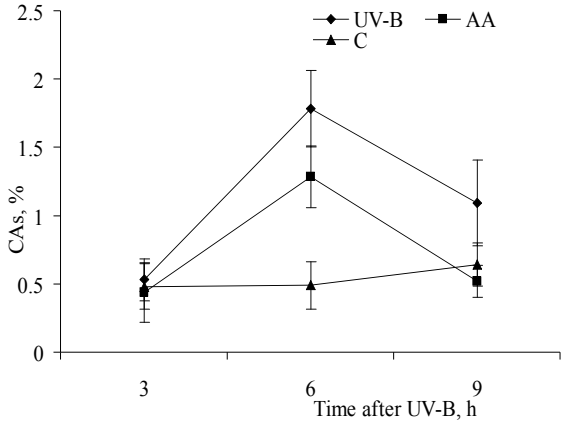

Figure 4. Action of ascorbic acid (AA) on chromosome aberration induction dependently on the root tip fixation time after irradiation with 1.2 kJ m^{-2} UV-B.
C-control, UV-B unirradiated tips

It should be noted that anthocyanin-rich extracts from cherry fruits also showed protective effect on chromosome stability and cell division in UV-B irradiated *C. capillaris* meristematic root cells (Rančelienė et al., 2009). Despite that anthocyanins-rich extracts from fruits are presented by mixture of compounds, we suggest that generally for both agents, anthocyanins and ascorbic acid, effects are due to antioxidant action. Both agents act as powerful antioxidants (Conklin and Barth, 2004; Athar et al., 2008; Tsoyi et al., 2008). Common feature of both substances is a delayed effect on mitosis (Potters et al., 2000; Rančelienė et al., 2009).

Conclusion

The root meristem of *Crepis capillaris* is a useful and very sensitive system for investigation of UV-B radiation and its various modifying agents. Low number of chromosomes in diploid karyotype allows investigating the effects on chromosome stability. Effects of UV-B action on meristematic root cells depended on the dose, presence or absence of the supplemental B chromosome or partially on salicylic or ascorbic acids, as well as photoreactivation. Modifying agents eliminate only part of chromosome lesions, and the nature of remaining chromosome aberrations needs further investigation.

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