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The effect of cherry fruit extracts on meristematic plant cells

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Abstract

Protective, antimutagenic and anticancerogenic action of plant pigments anthocyanins is well researched for human and animal cells, but significantly less for plant cells. It was shown that anthocyanin-rich extracts from cherry fruits of cv. 'Lotinė' and 'Pandy' produce positive effect on mitosis of the model plants in cytogenetic studies of onion (*Allium cepa* L.) and *Crepis capillaris* (L.) Walrr. root cells and slight antimutagenic action against UVB irradiation in meristematical root cells of *C. capillaris*. Concentration of cells in prophase and partially in metaphase or, specifically for cv. 'Pandy' in telophase, may also be attributed to the protective action of anthocyanin-rich fruit extracts as time for repair processes is prolonged. Effects of anthocyanin-rich fruit extracts on the cell proliferation depended upon the cultivar and concentration of tested extracts. In ripe fruits the SOD isoforms were determined. They may have a protective role for cherry fruits themselves and clearly depended upon the tested cherry genotype.

Key words: cherry anthocyanins, protection in mitosis, antimutagenic action, UVB irradiation, *Crepis capillaris*, *Allium cepa*, SOD in fruits.

Introduction

Anthocyanins are a group of natural pigments, responsible for the red-blue colour of different plant organs. However, their biological role mainly lies in their anti-oxidant action as free radicals scavengers /Kong et al., 2003; Pascual-Teresa, Sanchez-Ballesta, 2008/. Antimutagenic effect of anthocyanins has been proven experimentally with a range of different groups of mutagens: reactive oxygen species (ROS) /Sarma, Sharma, 1999; Lazze et al., 2003/, directly acting mutagen methane sulfonate /Yoshimoto et al., 1999/, metals /Posmyk et al., 2008/, and various indirectly metabolically activated mutagens such as benzo(a) pyrene /Gosiorowski et al., 1997; Yoshimoto et al., 1999/ or 2-amino fluorene /Gosiorowski et al., 1997/. Anthocyanins have also been effectively used against clastogenic mutagens /Gosiorowski et al., 1997; Azevedo et al., 2003; 2007/. It is very important that juice from various intensely coloured berries possesses antioxidant and antimutagenic activity: *Aronia melanocarpa* /Gosiorowski et al., 1997/; strawberry, raspberry /Hope-Smith et al., 2004; Krupa, Tomala, 2007/; blueberry, black

currant /Meller et al., 2004/; blackberry /Ding et al., 2006/. Especially strong antioxidant action is exerted by fruits of sour (tart) cherry (*Prunus cerasus*), effectively used as an anticancerogen /Kang et al., 2003; Blando et al., 2004; Kirakosyan et al., 2009/.

The colour of plant organs is the main property in selecting plants as the source of biologically active anthocyanins. It holds true for the aforesaid *Prunus*, *Aronia*, also for black skin of sweet potato (*Ipomoea batatas*) /Yoshimoto et al., 1999/ or eggplant (*Solanum melanogena*) /Azevedo et al., 2007/, grape cultivars with intensively coloured fruit /Kataoka et al., 2003/, black beans (*Phaseolus vulgaris*) /Azevedo et al., 2003/, and red cabbage /Glinska et al., 2007/.

It is known that ultraviolet irradiation induces additional synthesis of anthocyanins in plants, and that effect is validated as plant protective response /Balakrishnan et al., 2005; Zhang, Björn, 2009/. Diffuse sunlight gives opposite effect: it reduces synthesis of covering materials, including anthocyanins, which prevent UV light from penetrating plant tissues /Kataoka et al., 2003/.

Several investigators /Paskual-Teresa, Sanchez-Ballesta, 2008; Wang, Stoner, 2008/ assumed that antimutagenic action of the berry juice is due not only to anthocyanins and other antioxidants, but also to enzymes involved in DNA repair or acting as antioxidants, such as SOD, catalase and others. This assumption has been confirmed experimentally. Various proteins (even though in low concentrations), including SOD, were detected by means of different methods in the juice of *Vitis vinifera* and other grape species /Rani et al., 2004; Sarry et al., 2004/, in gooseberry, orange, tomato juice /Rani et al., 2004/ or juice from mesocarp tissues from divergent *Cucumis melo* L. genotypes /Lester et al., 2009/. It is unlikely that proteins in juice are not digested by proteolitic enzymes in human or animal stomach, but the enzymes in ripe berries may have a protective role for the berries themselves. For these reasons, the SOD isoforms were analysed electrophoretically in the whole fruit, peels and mesocarps of cherry cultivars.

The aim of the present work was to investigate the SOD isozymes spectra in ripe cherry fruit and action of anthocyanin-rich fruit extracts on proliferation of the meristematic root cells and antimutagenic/anticlastogenic activity on the background of chromosome damages, induced by UVB irradiation.

Materials and methods

Berries of cv. ‘Lotinė’ and ‘Pandy’ were picked from cherry trees planted in the orchard of the Lithuanian Institute of Horticulture. Anthocyanin-rich fractions were extracted from berries reached 50% maturity. Conditions for extraction of anthocyanin-rich fraction are standardized and were the same as for the black currant studies /Rančelienė et al., 2009/. Antioxidant activity of anthocyanin-rich fraction was determined according to Brand-Williams et al. (1995).

Mitotic activity was investigated in two plants – onion (*Allium cepa* L.) and *Crepis capillaris* (L.) Walrr. The latter plant has only 6 chromosomes in diploid cells. The treatment of onion and *Crepis capillaris* with cherry anthocyanins differed.

Onion bulbs of cv. ‘Stutgarten Riesen’ from the Lithuanian Institute of Horticulture were placed in distilled water for three days for rooting, and then were transferred to anthocyanin-rich fruit extracts of different concentrations – 125, 250, and

500 μM for 3 h. After that the root tips were fixed with an acetic acid and ethanol (1:3) mixture and were stored in 70% ethanol in a freezer until used. Cells were stained with acetocarmine. Separate mitotic phases were analysed according to Glinska et al. (2007).

Seeds of *C. capillaris* (population maintained at the Institute of Botany) were germinated in a thermostat at +25ºC in the dark on distilled water in Petri dishes for 36 h until root tips reached 3–5 mm in length. Part of roots were rinsed with a solution of anthocyanin-rich fruit extracts, while control roots were placed in distilled water. For *C. capillaris*, significantly lower concentrations of anthocyanin-rich extracts must be used because of the higher sensitivity of *C. capillaris* to anthocyanin-extracts. The concentration 10 μM was chosen after preliminary investigations. After 2 hours, part of roots were irradiated with the 1 500 J m^{-2} (max. 2.9 mW cm^{-2}) dose of UVB lamp (Vilber-Lourmat) (λ max. 312 nm). After that the roots were treated with colchicine (100 mg l^{-1}) and fixed with acetic acid and ethanol (1:3) mixture for 3 h. All manipulations with roots before and after irradiation, till fixation, were carried out in the red light to avoid photoreactivation of DNA damages by visible light. The fixed roots were stored in 70% ethanol in a freezer until used. Mitotic index and chromosome aberrations (CA) were studied on temporary preparations, stained with acetocarmine. The methaphase cells were examined. CAs were observed with Nikon Eclipse 80i microscope. Most of CAs were presented by chromatid and chromosome fragments.

Superoxide dismutase (SOD, EC 1.15.1.1) was investigated in ripe fruits of cherry cultivars ‘Pandy’, ‘Lotinė’, ‘Vytėnų žvaigždė’, ‘Rovesnica’, and breeding line No 23 according to Beauchamp and Fridovich (1971). All fruit material was of the same origin – from the Lithuanian Institute of Horticulture. Fruits (0.5 g) were homogenized in 1 ml chilled potassium buffer (pH 7.8) with 2% PVP (polyvinyl pyrrolidone) to bind phenolic substances. Conditions of SOD preparation and electrophoresis were the same as for black currant berries /Vyšniauskienė et al., 2009/.

Statistical analysis. The mean values ± standard deviation are given in Figures and Tables.

Results and discussion

The positive effect of cherry anthocyanin-rich fruit extracts on cell proliferation was revealed by increased mitotic index in meristematical cells of *A. cepa* root tips (Table 1).

However, in positive concentrations of anthocyanin extracts the mitotic index was nearly equal for both cherry cvs ‘Lotinė’ and ‘Pandy’, while in our previous investigation of the black currant anthocyanins dependence of extract activity upon the cultivar was well expressed /Rančelienė et al., 2009/. Despite this difference, for both sources of anthocyanin extracts – black currant or cherry fruits – the “dose-effect” relation was well expressed. The maximum positive effect was after exposure of roots to the 250 μM concentration of anthocyanin extracts. Differences between anthocyanin extracts from various cherry cultivars were clearly revealed after exposure of *A. cepa* cells to 500 μM concentration. Significant decrease in mitotic activity was observed after treatment with 500 μM concentration of anthocyanin extracts from cv. ‘Lotinė’, while only return to control level was recorded if root tips were exposed to 500 μM concentration of anthocyanin extracts from fruits of cv. ‘Pandy’ (Table 1).

Table 1. The influence of anthocyanin-rich extracts from fruits of cherry cvs 'Lotinė' and 'Pandy' on mitotic activity of onion (*Allium cepa*) root cells

1 lentelė. *Antocianinų ekstraktų, išskirtų iš vyšnių veislių 'Lotinė' ir 'Pandy' uogų, poveikis valgomojo svogūno (Allium cepa) šaknų ląstelių mitoziniam aktyvumui*

Concentration of anthocyanins *Antocianinų koncentracija* µM	Cell number *Ląstelių skaičius*						Mitotic index *Mitozės indeksas* %	
	Dividing *Dalijasi*		Undividing *Nesidalija*		Total *Iš viso*			
	'Lotinė'	'Pandy'	'Lotinė'	'Pandy'	'Lotinė'	'Pandy'	'Lotinė'	'Pandy'
0	291	998	1983	6702	2274	7700	12.8±0.70	13.0±0.38
125	318	906	1992	4990	2310	5896	13.8±0.72	15.2±0.47
250	509	1461	2573	7488	3082	8949	16.5±0.67	16.3±0.39
500	315	398	3126	2597	3441	2995	9.2±0.49	13.3±0.62

The comparison of the effect of anthocyanin-rich extracts on different phases of mitosis in *A. cepa* cells allowed us to reveal the dependence of the effect upon the cherry cultivar even more clearly than it was possible to judge from mitotic index analysis (Table 2).

Table 2. The effect of anthocyanin-rich extracts from the fruits of different cherry cultivars on separate phases of mitosis in the dividing cells of onion (*Allium cepa*) roots

2 lentelė. *Antocianinų ekstraktų, išskirtų iš vyšnių skirtingų veislių uogų, poveikis besidalijančių valgomojo svogūno (Allium cepa L.) šaknų ląstelių mitozės fazėms*

Concentration of anthocyanins *Antocianinų koncentracija* µM	Number of cells *Ląstelių skaičius*				Phases of mitosis / *Mitozės fazės* %			
	Prophase *Profazė*	Metaphase *Metafazė*	Anaphase *Anafazė*	Telophase *Telofazė*	Prophase *Profazė*	Metaphase *Metafazė*	Anaphase *Anafazė*	Telophase *Telofazė*
'Lotinė'								
0 (Control *Kontrolinis variantas*)	155	66	36	34	53.3±2.93	22.7±2.46	12.4±1.94	11.7±1.89
125	159	89	39	31	50.0±2.80	28.0±2.52	12.3±1.84	9.7±1.66
250	297	103	66	43	58.3±2.19	20.2±1.78	13.0±1.49	8.4±1.23
500	203	46	35	31	64.4±2.70	14.6±1.99	11.1±1.77	9.8±1.68
'Pandy'								
0 (Control *Kontrolinis variantas*)	609	156	122	111	61.0±1.54	15.6±1.15	12.2±1.04	11.1±1.00
125	531	163	66	146	58.6±1.64	18.0±1.28	7.3±0.86	16.1±1.22
250	903	265	79	214	61.8±1.27	18.1±1.01	5.4±0.59	14.6±0.93
500	179	67	25	127	45.0±2.49	16.8±1.88	6.3±1.22	31.9±2.34

The concentration of cells in the prophase and partially in the methaphase was observed if anthocyanin-rich extract was applied from cv. 'Lotinė' (Table 2). Such effect was recorded also for anthocyanin-rich extracts from the black currant, and it was evaluated as protective, i.e. extending time for DNA and protein repair processes /Rančelienė et al., 2009/, a phenomenon well investigated in cell division biology and genetics. However, the unexpected and unique effect was obtained after exposure of roots to the 500 µM concentration of anthocyanin-rich extracts from cv. 'Pandy' fruits: more cells were concentrated in the telophase. It may be suggested that anthocyanin-rich extract from cv. 'Pandy' blocked final steps of cell division.

In the majority of works referred to in the Introduction, the antimutagenic action of anthocyanins was revealed on the background of mutations, induced by a concrete mutagen. For plants UV irradiation is a very important environmental mutagen. Considering this, the UVB irradiation was used in the present work to reveal antimutagenic action of the anthocyanin-rich extracts from cherry fruits. It should be particularly mentioned that the antimutagenic action of anthocyanin-rich extracts from cv. 'Lotinė' on meristematical cells of *Crepis capillaris*, irradiated with UVB, was examined in conditions of low 10 µM concentration of anthocyanins, irradiation with UVB being performed with relatively low 1 500 J m^{-2} dose of UV (Figure 1). That dose is closer to the natural doses of UV in the plant environment.

The latter presumption was confirmed also by the fact that treatment with both factors, anthocyanin-rich extract or UVB, did not modify cell proliferation activity in such doses (Figure 1 A). Even after combined action of the 10 µM anthocyanins and 1 500 J m^{-2} UVB, the level of proliferating cells was nearly the same as in control, untreated *C. capillaris* cells.

A relatively low UVB dose used was sufficient to reveal only slight antimutagenic action of anthocyanin extract from the fruits of cv. 'Lotinė', despite the fact that irradiation with 1 500 J m^{-2} UVB dose increased the level of chromosome aberrations almost three times (Figure 1 B). If *C. capillaris* root tips were preliminary exposed to anthocyanin-rich extracts, the level of CA decreased after exposure to extracts almost to the natural (control) level (Figure 1 B). However, our investigations may be of interest to future investigations, because UVB dose was relatively low, close to the one plants are exposed to in the natural conditions.

To investigate the protective role of antioxidant enzymes for protection of fruits themselves from free radical damage, the SOD isoforms were analysed electrophoretically in the whole fruits, peels and mesocarps of cherry cultivars 'Vytėnų žvaigždė', 'Pandy', 'Rovesnica', 'Lotinė', and breeding line No 23 (Figure 2). In general, several SOD isozymes were recorded in whole ripe cherry fruits, mainly in the mesocarps. However, very high polymorphism of the tested cherry cultivars was revealed according to this property. Like in the work of Sarry et al. (2004), in fruits of investigated cherry cultivars SOD concentrated mostly in mesocarp. However, in the mesocarp and peels of cv. 'Lotinė' only traces of SOD were revealed on the electrophoregrams (Figure 2 C). The result is not accidental because it was observed in the three separate electrophoresis.

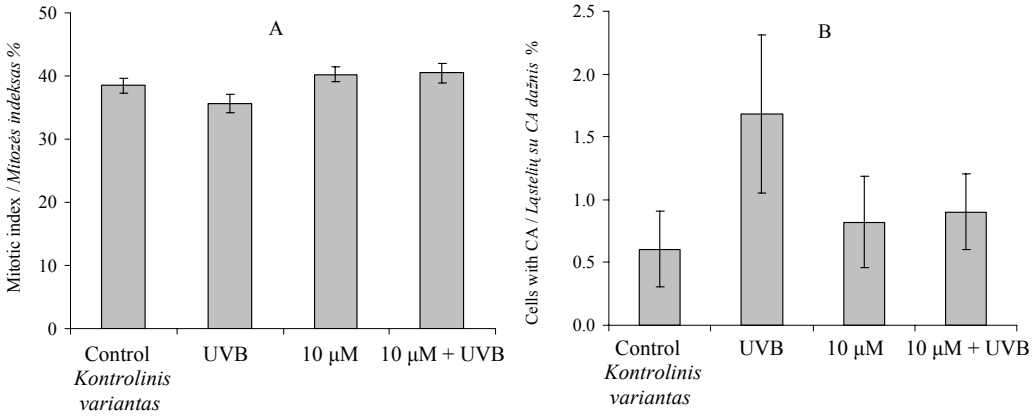

Figure 1. The influence of 10 µM concentration of anthocyanin-rich extract from cherry cv. 'Lotinė' fruits on mitotic index (A) and chromosome aberration frequency (B) in *Crepis capillaris* root cells, irradiated with 1 500 J m^{-2} UVB dose

1 paveikslas. *Antocianinų ekstrakto (10 µM koncentracijos), išskirto iš vyšnių veislės 'Lotinė' uogų, poveikis mitozės indeksui (A) ir chromosomų aberacijų dažniui (B) kreisvės (Crepis capillaris) šaknų ląstelėse, apšvitintose 1 500 J m^{-2} UVB doze*

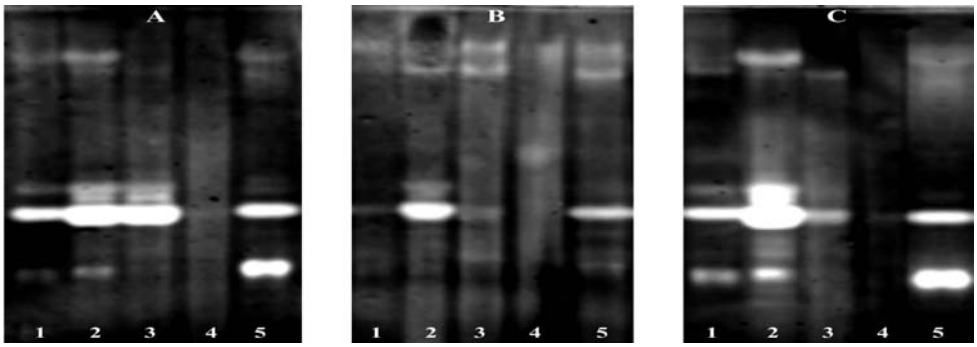

Figure 2. Superoxide dismutase isozymes, fractionated with 10% PAG gel, in the mesocarps with peels (A), only in the peels (B) or only in the mesocarps (C) of different cherry cultivars: 1 – 'Vytėnų žvaigždė', 2 – 'Pandy', 3 – 'Rovesnica', 4 – 'Lotinė', 5 – No 23

2 paveikslas. *Superoksido dismutazės, frakcionuotos 10 % PAG geliu, izoformos mezokarpe kartu su luobele (A), tik luobelėse (B) arba tik mezokarpe (C) iš įvairių veislių vyšnių: 1 – 'Vytėnų žvaigždė', 2 – 'Pandy', 3 – 'Rovesnica', 4 – 'Lotinė', 5 – Nr. 23*

Conclusions

1. Anthocyanin-rich extracts from the ripe cherry fruits positively affected the dividing meristematical root cells of onion (*Allium cepa*) by increasing frequency of dividing cells and by prolonging time for the repair processes.

2. Anthocyanin-rich extracts from both tested cherry cultivars – ʻLotinėʼ and ʻPandyʼ – were efficient, but several peculiarities were observed in response to the highest tested concentration of anthocyanin-extracts and to the mode of cell concentration in mitosis phases. The cv. ʻPandyʼ anthocyanin-rich extracts concentrated cells not in prophase but in telophase.

3. The slight antimutagenic action of anthocyanin-rich extracts from the cv. ʻLotinėʼ fruits was observed in UVB irradiated *Crepis capillaris* root cells. After application of anthocyanin-rich extracts the frequency of chromosome aberrations decreased in UVB irradiated cells to the spontaneous level.

4. In the mesocarps of the ripe cherry fruits (with exception of cv. ʻLotinėʼ) SOD isoforms were detected. It is suggested that SOD has protective role for fruits themselves.

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Vyšnių uogų ekstraktų poveikis augalų meristeminėms ląstelėms

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Santrauka

Antocianinų apsauginis, antimutageninis ir antikancerogeninis poveikis geriau yra ištirtas žmonių ir gyvūnų ląstelėse. Antocianinų biologinė reikšmė augalams yra mažiau tyrinėta, nors juos sintetina patys augalai ir antocianų sudėtis yra savita konkretiems augalams. Nustatyta, kad daug antocianinų turinčių ekstraktų, išskirtų iš vyšnių veislių 'Lotinė' bei 'Pandy' uogų, teigiamas poveikis svogūno (*Allium cepa* L.) šaknų meristeminių ląstelių mitozei pasireiškia dviem būdais: padidėja besidalijančių ląstelių dažnis ir stabdomas mitozės ciklas, taip pratęsiamas laikas, būtinas reparacijos procesams. Efektyvumas bei poveikis priklauso nuo veislės ir daug antocianinų turinčio ekstrakto koncentracijos. Veislės 'Lotinė' uogų ekstraktai ląsteles kaupė profazėje, o veislės 'Pandy' – telofazėje. Nustatytas silpnas antimutageninis (antiklastogeninis) veislės 'Lotinė' uogų antocianinų poveikis chromosomų aberacijų, kurias indukavo UVB spinduliuotė modelinio augalo kreisvės (*Crepis capillaris* (L.) Walrr.) meristeminėse šaknų ląstelėse, dažniui. Sunokusiose vyšnių uogose aptiktos superoksido dismutazės izoformos (SOD), kurios gali būti svarbios pačių vaisių saugai. SOD izoformų sudėtis priklausė nuo vyšnių genotipo.

Reikšminiai žodžiai: vyšnių antocianinai, mitozės apsauga, antimutageninis poveikis, UVB spinduliuotė, *Crepis capillaris*, *Allium cepa*, vaisių SOD.