EFFECT OF UV-A AND GAMMA-IRRADIATION ON THE POLYPHENOL LEVELS IN BARLEY AND PEA SEEDS, SEEDLINGS AND PLANTS*

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In two pea cultivars (Lantra, Menhir) and barley cultivars (Krona, Kompact) changes in total polyphenol content (TP) and major phenolic acids caused by UV-A and γ-irradiation were investigated. The changes were determined in seeds, seedlings and plants. In barley cultivars also the content of catechol, resorcinol and phloroglucinol type compounds (CRP) was estimated. TP content was determined with Folin-Ciocalteau's reagent, CRP with p-dimethylaminocinnamaldehyde, phenolic acids by HPLC. UV-A irradiation was performed for 0, 24, 48 and 72 hours at λ = 351 nm, γ-irradiation with ^{60}Co at doses 0, 10, 20 and 40 Gy. During pea sprouting TP content increased, in barley cultivars TP stagnation was observed. UV-A irradiation caused in investigated plants an increase of TP content, γ-irradiation enhanced TP content in barley but in pea decreased it. It was demonstrated statistical dependence of TP content on growth phases of plants and on doses of irradiation. CRP in sprouting seeds decreased but in further development of plants increased. UV-A and γ-irradiation decreased CRP content in barley. Statistically significant dependence was found between CRP content and the doses of irradiation and the vegetation phase of plants. Cultivars responded differentially to various doses of irradiation in both types of irradiation. The most represented phenolic acids in pea and barley are 2,3-dihydroxybenzoic, sinapic, m-hydroxybenzoic, veratric and vanillic acids. UV-A irradiation caused apparent increase of these acids both in barley and pea, whereas γ-irradiation caused only little changes in the content of these compounds.

UV-A irradiation; γ-irradiation; pea cultivars; barley cultivars; polyphenol content; phenolcarboxylic acids; catechol, resorcinol and phloroglucinol content

* This work was supported by Internal Grant of CUA Prague No. 202/10/36500/0 and Research Project MSM 412100002.

INTRODUCTION

Polyphenols are natural compounds that as secondary metabolites are present in every higher plant and in every its organ. Polyphenols are especially located in outer layers of the seeds where they protect an embryo against ultraviolet irradiation regarding the fact that they have ability to absorb it (Boesewinkel, Bouman, 1995; Lachman et al., 1998). Tannins and other polyphenols such as anthocyanins could form colour components of seeds and can protect them against light exposure, can restrict the sprouting due to the gas diffusion inhibition and deleting of the seed and fruit decomposition in the soil. Plants form them also for their defence against herbivores and diseases because many of them possess significant fungicidal, bactericidal and virocidal activities.

Because the thinning of the stratospheric ozone layer is permitting more UV-B to enter the biosphere, the mechanisms of action of UV-B radiation on plants are of particular current interest. It was determined in *Arabidopsis thaliana* wild-type *Landsberg erecta* (Ler) and the UV-B-sensitive mutant *fahl* (deficient in UV-absorbing esters of sinapic acid) that UV-B exposure (at doses of 6–7 $kJ.m^{-2}.d^{-1}$) decreased dry matter production (Ormrod et al., 1999).

Liu et al. (1995) investigated barley polyphenol levels in plants that were grown with UV-B (280–320 nm) at levels simulating 25 or 5% ozone depletion on the date of the summer solstice at 40° N latitude, with UV-A (320–400 nm), or with no supplemental irradiation. UV-B increased flavonoid (saponarin and lutonarin) accumulation in both the lower epidermis and the mesophyll; about 40% of the saponarin and 20% of the lutonarin were in the lower epidermis under all experimental conditions. Levels of vacuolar ferulic acid esters were significantly higher in UV-B grown plants on days 10 and 15 and this fraction significantly increased in the lower epidermis. UV-A had no significant effects on growth, photosynthesis or ferulic acid, but it slightly increased flavonoid accumulation. Balakumar and Selvakumar (1998) investigated the total phenol content and the polyphenol oxidase activity in the leaves of cowpea and *Crotalaria* seedlings treated with various UV-B irradiance levels. In both the plants tested, the total phenol content has increased proportionately with the dose of UV-B radiation. However, the concentration of total phenols in the leaves of cowpea was higher under UV-B irradiation compared to *Crotalaria* seedlings. The activity of polyphenol oxidase, on the contrary, has shown reduction in both cowpea and *Crotalaria* seedlings. Effects of solar and UV-B radiation on wheat seedlings (Häder, 1996), sensitivity to UV-B of plants growing in different altitudes (Rau, Hofmann, 1996), diurnal variation in UV-protective flavonoids

(Veit et al., 1995) as well as the protective role of plant pigments in *Picea abies* (L.) Karst. is now intensively investigated.

Pendharkar and Nair (1995) studied phenylpropanoid metabolism in γ-irradiated potato tubers by examination the pattern of incorporation of radioactivity from U-^{14}C-phenylalanine into caffeic acid, chlorogenic acid and the coniferyl and sinapyl moities of lignin. During a post-irradiation period of 21 days a depletion in chlorogenic acid was observed. This is a result of its impaired synthesis as well as an accelerated conversion of chlorogenic acid to ferulic and sinapic acids and their deposition in lignin. Changes in potato phenolic and alkaloidal compounds in irradiated potatoes were also referred by Berges (1980), Berges, Kooij (1981) and Thomas (1981). γ-radiation insures the possibility of extending the storage life of different vegetables and fruits, e.g. onions – 0.10 kGy (Agbaji et al., 1981; Khan et al., 1999), garlic (Manniti, 1979). Al-Safadi and Simon (1996) have found that γ-radiation accelerated germination of carrot seed in the M_1 generation at low doses (0.5 and 1 krad, i.e. 5.0 and 10.0 Gy), whereas higher doses delayed germination. Plant size and root weights were by 20% to 35% greater than control plants. Higher doses reduced M_1 plant size by > 50% in germinating seed and tissue culture treatments but less for the dry seed treatment. Irradiation of germinating seed and tissue cultures yielded more M_2 variation than irradiation of dry seed. Massive dosage of γ-irradiation (1–3 kGy) has been found to induce some particular responses in *Arabidopsis thaliana* (Nagata et al., 1999). It was determined the accumulation of anthocyanin in the aerial parts and the formation of new trichomes. The plants stopped their development, too.

The aim of this work was to determine the UV-A and γ-irradiation effect on polyphenol levels in pea (*Pisum* sp.) and barley (*Hordeum* sp.) seeds, seedlings and plants and to determine the changes in total polyphenol content (TP) and catechol, resorcinol and phloroglucinol content (CRP) in barley in relation species x variety.

MATERIAL AND METHODS

Barley cultivars Krona and Kompact and pea cultivars Lantra and Menhir obtained from the Department of Plant Production of the Czech University of Agriculture in Prague in the year 1999 were irradiated with UV radiation (λ = 351 nm, 0, 24, 48 and 72 hours) and γ-irradiation (^{60}Co, 0, 10, 20 and 40 Gy at the Faculty of Medicine, Hradec Králové).

There were investigated three variants:

1. dry seeds (after radiation two months storage at laboratory temperature),

2. seedlings (for 10 hours soaked in distilled water and after 5 days germinating exposed to radiation; seven days after radiation analyzed),
3. plants – after 21 days of cultivation plants were radiated (UV-irradiation for total 72 hours in 9 days, γ-irradiation for 4 hours) and then cultivated for other seven days.

Determination of total polyphenols (TP): Seeds and dried plants were homogenized and extracted in Soxhlet apparatuses with 80% water ethanol solution for 20 hours. After adjusting of extract volume to 250 mL there were 10 mL pipetted into 50 mL flasks. After dilution with distilled water to aprox. 30 mL volume it was 2.5 mL Folin-Ciocalteau's reagent p.a. (Nycom, Prague, CR) added. After agitation and 3 min standing 7.5 mL 20% Na_2CO_3 p.a. solution was added and the volume was adjusted to mark with distilled water. After thorough agitation and two hours standing absorbancy of blue solution in cuvettes 0.5 mL thickness at λ = 765 nm on Spekol 11 (Jena) spectrophotometer against blank was measured. Polyphenol compounds were expressed as gallic acid content on dry matter (DM) basis (in seeds).

Determination of catechol, resorcinol and phloroglucinol type compounds (CRP): 25 mL were dried on water bath to dryness and then redissolved in 15 mL methanol and quantitatively transferred into 25 mL volumetric flasks. In every flask it was 6 mL 3M HCl in methanol added and after agitation adjusted with methanol p.a. to mark. Then 1 mL p-dimethylaminocinnamaldehyde solution, p.a. (Merck-Schuchenhardt, Hohenbrünn bei München, BRD) was added, solution was agitated and left to stand for 30 minutes. After this period absorbancy was measured in 0.5 cm cuvettes at λ = 638 nm on Spekol 11 spectrophotometer against blank. CRP content was expressed as phloroglucinol (British Drug House, Ltd., Bush House London, GB).

Dry matter determination: The procedure was performed after Davídek et al. (1977). 2 g of fine-grained samples were dried to constant mass at 105 °C.

Determination of phenolcarboxylic acids and coumarins by HPLC: For identification and quantification of phenolcarboxylic acids and coumarins in water – ethanolic extracts a HPLC method with gradient elution on Waters(TM) 600S chromatograph with Waters(TM) 717 plus autosampler and Waters(TM) PDA 996 – UV-VIS detector was used. Watrex 250x4 mm Separon SGX C18 column (7 μm) and mobile phase: A – 5% water methanol, B – 40% water methanol (both adjusted with H_3PO_4 to pH 2.5), flow 1 mL.min^{-1}, were used. Standards of phenolcarboxylic acids and coumarins were purchased from Fluka Chemie AG, Buchs, Switzerland).

RESULTS AND DISCUSSION

From the obtained results it could be concluded that UV-A irradiation has statistically significant effect on TP content and that the vegetation phase of plants affects the TP change induced with UV irradiation. Meanwhile seeds contained low TP levels, in seedlings and esp. in plants were found much higher TP contents (Table I). UV-radiation increased significantly TP content (Table II), which is in accordance with the results obtained by Liu et al. (1995), Balakumar and Selvakumar (1998).

The γ-irradiation caused in doses of 40 Gy a decrease of TP in pea cv. Lantra by 9%$_{rel}$, and in barley cv. Kompact by 15%$_{rel}$ (Table II). In other pea and barley cultivars could be observed an increase of TP content (pea cv. Menhir by 15%$_{rel}$, barley cv. Krona by 46%$_{rel}$). Similarly in the seedlings was found a decrease of TP content at 40 Gy doses in pea cv. Menhir by 28%$_{rel}$, in barley cv. Kompact by 5%$_{rel}$. The 20 Gy doses increased TP contents (in barley cv. Kompact by 11%$_{rel}$, Krona 57%$_{rel}$). Irradiation of plants (40 Gy) decreased TP content in pea cultivars (Lantra by 38%$_{rel}$, Menhir by 10%$_{rel}$),

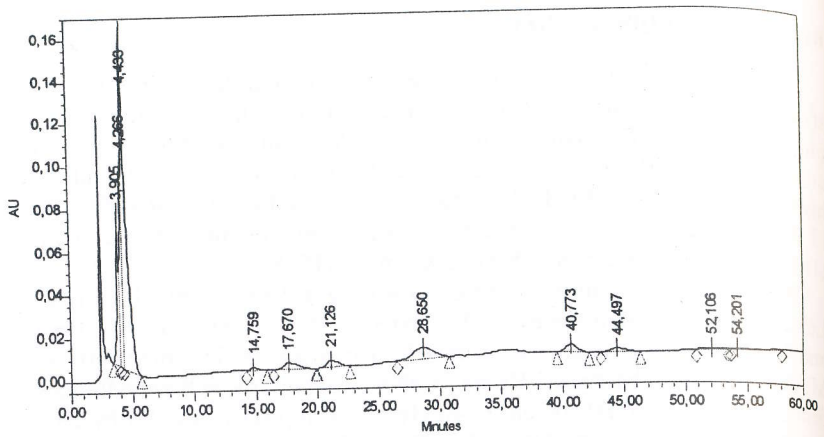
I. TP content (mg.kg^{-1} on a DM basis)

Cultivar	Seeds	Seedlings – sand	Seedlings – soil	Plants
Lantra	841	10,360	–	22,470
Menhir	619	17,662	6,108	11,580
Kompact	1,219	5,450	9,281	8,935
Krona	1,790	6,220	6,230	6,380

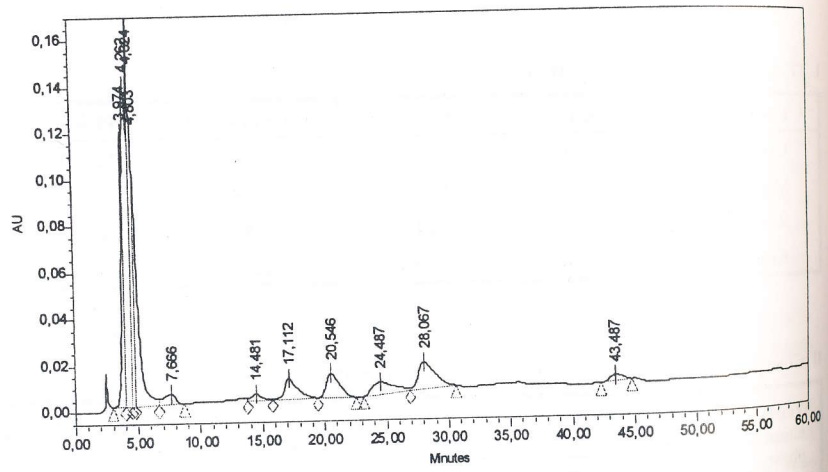
II. Change of TP content (%) induced by UV and γ-irradiation

Vegetation phase	UV-irradiation (Σ hours)			γ-irradiation (Gy)								
	S	Sd	P	S			Sd			P		
Dose	Σ 48 h	Σ 72 h	Σ 72 h	10	20	40	10	20	40	10	20	40
Lantra	–16.0	*	*	–3.0	+43.0	–9.0	+0.9	–1.3	+3.0	–59.0	–32.0	–38.0
Menhir	+12.0	+19.0	+14.0	+14.0	+7.4	+15.4	–3.6	–26.5	–27.5	–19.0	+28.0	–9.0
Kompact	–13.0	+17.0	+6.0	–8.0	–22.4	–15.1	–14.5	+11.2	–4.7	+20.3	+25.9	+29.4
Krona	0.0	+30.0	–49.0	+36.5	+36.7	+46.1	+19.0	+56.8	+27.4	–2.1	+12.0	+56.7

S – seeds, Sd – seedlings, P – plants, * – dead plants



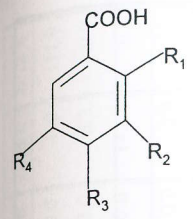
1. Chromatogram of barley cv. Kompact



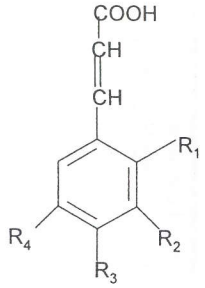
2. Chromatogram of pea cv. Lantra

whereas in barley cultivars was found TP increase (Kompact by 29%$_{rel}$, Krona by 57%$_{rel}$).

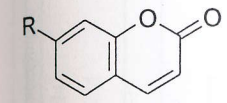
By HPLC (Figs. 1 and 2) 2,3-dihydroxybenzoic acid, m-hydroxybenzoic acid, 3,5-dihydroxybenzoic acid, 3,5-dihydroxybenzoic acid, o-coumaric acid, gallic acid, caffeic acid, sinapic acid, ferulic acid, vanillic acid, veratric



3. Phenolcarboxylic acids derived from benzoic acid



4. Phenolic acids derived from cinnamic acid



5. Coumarins

and cinnamic acid in barley cultivars were identified, and in pea cultivars 2,3-dihydroxybenzoic acid, sinapic acid, vanillic acid, veratric acid, caffeic acid, 3,5-dihydroxybenzoic acid, o-coumaric and ferulic acid were identified (Figs. 3 and 4). In pea coumarin was identified, in barley coumarin and 7-methoxycoumarin (Fig. 5). In both pea cultivars the most present phenolic acids were 2,3-dihydroxybenzoic acid (in cv. Lantra ranged from 230 to 405 mg.kg^{-1} DM of seeds, 8,835 –9,470 mg.kg^{-1} DM of plants, in cv. Menhir 230–1,500 mg.kg^{-1} DM of seeds, 2,230 mg.kg^{-1} DM of seedlings, 3,320–9,058 mg.kg^{-1} DM of plants) and m-hydroxybenzoic acid (in cv. Lantra 35–523 mg.kg^{-1} DM of seeds, in cv. Menhir 617 mg.kg^{-1} DM of seeds, 411 mg.kg^{-1} DM of seedlings). Major phenolic acids identified in barley were 2,3-dihydroxybenzoic acid (in cv. Krona 42–90 mg.kg^{-1} DM of seeds, 308–1,215 mg.kg^{-1} DM of seedlings, 880–1,986 mg.kg^{-1} DM of plants), sinapic acid (62–167 mg.kg^{-1} DM of plants), vanillic acid (17–253 mg.kg^{-1} DM of plants) and veratric acid (350 mg.kg^{-1} DM of plants).

UV-A irradiation caused an increase of the levels of phenolic acids. In pea the highest increase was found in 2,3-dihydroxybenzoic acid (in cv. Lantra seeds by 274%), caffeic acid (in cv. Menhir seeds by 3,000%, seedlings by 410%, plants by 450%) and sinapic acid (in cv. Lantra seeds by 800% and cv. Menhir seedlings by 142% and plants by 320%). In barley the highest increase was determined in sinapic acid (in cv. Kompact seedlings by 1,000%) and vanillic acid (in plants by 730%). In cv. Krona the highest

III. Content of phenolcarboxylic acids and coumarins after γ-irradiation and UV-irradiation in pea seeds, seedlings and plants

Phenolcarboxylic acid and coumarin content after γ-irradiation ($mg.kg^{-1}$ on a DM basis)					Phenolcarboxylic acid and coumarin content after UV-irradiation ($mg.kg^{-1}$ on a DM basis)				
Lantra – seeds	0 Gy	10 Gy	20 Gy	40 Gy	Lantra – seeds	control	Σ 24 h	Σ 48 h	
2,3-dihydroxybenzoic acid	263	884	827	947	2,3-dihydroxybenzoic acid	405.3	462.2	1,517.3	
m-hydroxybenzoic acid	523	570	541	844	3,5-dihydroxybenzoic acid	68.7	66.8	18.8	
o-coumaric acid	2	3	0	2	m-hydroxybenzoic acid	35.4	0.0	0.0	
coumarin	0	6	0	0	caffeic acid	5.5	0.0	2.4	
cinnamic acid	1	1	0	1	o-coumaric acid	2.9	1.6	0.0	
					sinapic acid	1.8	18.7	16.2	
					coumarin	4.6	0.0	0.0	
Menhir – seeds	0 Gy	10 Gy	20 Gy	40 Gy	Menhir – seeds	control	Σ 24 h	Σ 48 h	
2,3-dihydroxybenzoic acid	230	187	186	377	2,3-dihydroxybenzoic acid	1,500.1	1,166.2	432.5	
3,5-dihydroxybenzoic acid	30	27	0	31	3,5-dihydroxybenzoic acid	28.9	1,102.5	51.4	
m-hydroxybenzoic acid	617	732	553	715	caffeic acid	2.3	0.0	70.5	
coumarin	15	9	0	18	o-coumaric acid	1.5	0.0	0.0	
cinnamic acid	1	1	1	1	veratric acid	2.6	0.0	0.0	
					sinapic acid	29.1	0.0	23.8	
					coumarin	2.9	0.0	2.9	
Lantra – seedlings	0 Gy	10 Gy	20 Gy	40 Gy					
2,3-dihydroxybenzoic acid	8,835	8,716	6,000	4,101					
3,5-dihydroxybenzoic acid	0	271	0	0					
3,4-dihydroxybenzoic acid	50	138	57	52					

Menhir – seedlings	*0 Gy*	*10 Gy*	*20 Gy*	*40 Gy*	Menhir – seedlings	control	Σ 24 h	Σ 48 h	Σ 72 h
2,3-dihydroxybenzoic acid	2,23	1,598	1,309	2,15	caffeic acid	21.9	0.0	0.0	111.9
gallic acid	25	20	18	32	vanillic acid	51.4	13.0	5.2	0.0
3,5-dihydroxybenzoic acid	72	38	45	79	sinapic acid	0.0	0.0	142.3	0.0
3,4-dihydroxybenzoic acid	39	30	25	36	cinnamic acid	0.0	0.0	1.6	0.0
m-hydroxybenzoic acid	411	363	53	361					
caffeic acid	193	166	129	139					
sinapic acid	58	40	98	84					
cinnamic acid	3	0	0	0					
Lantra – plants	0 Gy	10 Gy	20 Gy	40 Gy	Lantra - plants	control	Σ 24 h	Σ 48 h	
gallic acid	93	88	90	120	2,3-dihydroxybenzoic acid	8,904.9	9,469.8	0.0	
3,5-dihydroxybenzoic acid	0	46	0	0	caffeic acid	13.5	0.0	278.6	
3,4-dihydroxybenzoic acid	0	12	0	75					
m-hydroxybenzoic acid	0	151	0	0					
caffeic acid	14	45	0	45					
Menhir – plants	0 Gy	10 Gy	20 Gy	40 Gy	Menhir – plants	control	Σ 24 h	Σ 48 h	Σ 72 h
2,3-dihydroxybenzoic acid	9,058	7,426	12,796	9,636	2,3-dihydroxybenzoic acid	3,232.8	4,009.6	5,253.1	4,059.2
gallic acid	70	53	71	34	gallic acid	92.9	137.8	111.9	141.9
caffeic acid	10	17	0	0	3,4-dihydroxybenzoic acid	71.4	0.0	76.1	0.0
ferulic acid	11	20	0	0	caffeic acid	32.2	60.8	180.4	179.5
sinapic acid	68	95	135	56	sinapic acid	137.3	186.2	371.1	577.0
					cinnamic acid	8.8	6.4	12.2	0.0

IV. Content of phenolcarboxylic acids and coumarins after γ-irradiation and UV-irradiation in barley seeds, seedlings and plants

Phenolcarboxylic acid and coumarin content after γ-irradiation ($mg.kg^{-1}$ on a DM basis)					Phenolcarboxylic acid and coumarin content after UV-irradiation ($mg.kg^{-1}$ on a DM basis)			
Kompact – seeds	0 Gy	10 Gy	20 Gy	40 Gy	Kompact – seeds	control	Σ 24 h	Σ 48 h
2,3-dihydroxybenzoic acid	38.3	35.4	24.4	208.4	2,3-dihydroxybenzoic acid	132.9	60.4	51.7
3,4-dihydroxybenzoic acid	4.4	0.0	0.0	3.7	caffeic acid	0.9	0.7	1.3
caffeic acid	3.9	5.0	3.2	4.7	coumarin-3-carboxylic acid	0.7	1.1	0.0
vanillic acid	2.0	2.6	1.8	0.0	o-coumaric acid	0.7	0.0	0.0
o-coumaric acid	2.4	3.9	1.9	1.6	7-methoxycoumarin	0.9	0.0	0.0
coumarin	0.0	4.5	0.0	6.3				
Krona – seeds	0 Gy	10 Gy	20 Gy	40 Gy	Krona – seeds	control	Σ 24 h	Σ 48 h
2,3-dihydroxybenzoic acid	90.4	0.0	0.0	0.0	2,3-dihydroxybenzoic acid	42.6	290.7	101.4
3,5-dihydroxybenzoic acid	30.4	14.0	0.0	0.0	3,5-dihydroxybenzoic acid	6.1	0.0	7.9
3,4-dihydroxybenzoic acid	11.4	4.9	0.0	4.5	caffeic acid	1.8	1.7	1.8
caffeic acid	5.9	1.8	1.9	0.0	vanillic acid	2.8	0.0	0.0
vanillic acid	3.6	1.1	0.0	1.3	sinapic acid	26.0	19.0	22.0
o-coumaric acid	1.6	0.0	0.0	0.0				
sinapic acid	26.3	0.0	0.0	0.0				
7-methoxycoumarin	0.0	2.2	0.0	0.0				
Kompact – seedlings	0 Gy	10 Gy	20 Gy	40 Gy	Kompact – seedlings	control	Σ 24 h	Σ 48 h	Σ 72 h
2,3-dihydroxybenzoic acid	1,990.0	0.0	2,090.3	2,007.1	2,3-dihydroxybenzoic acid	3,688.0	739.9	3,247.3	4,456.3
3,5-dihydroxybenzoic acid	108.9	0.0	0.0	99.1	caffeic acid	26.3	4.2	0.0	0.0
3,4-dihydroxybenzoic acid	33.4	51.0	30.4	66.2	coumarin-3-carboxylic acid	26.7	45.0	0.0	0.0
vanillic acid	106.0	156.6	107.5	137.8	o-coumaric acid	0.0	17.7	126.1	168.7
o-coumaric acid	0.0	38.7	0.0	0.0	veratric acid	12.0	13.2	0.0	0.0
Sinapic acid	*111.6*	*128.3*	*118.2*	*109.2*	sinapic acid	21.2	46.5	264.7	230.7
					7-methoxycoumarin	16.3	10.2	34.4	45.9
Krona – seedlings	0 Gy	10 Gy	20 Gy	40 Gy	Krona – seedlings	control	Σ 24 h	Σ 48 h	Σ 72 h
2,3-dihydroxybenzoic acid	1,214.2	1,410.2	2,576.8	0.0	2,3-dihydroxybenzoic acid	0.0	965.9	0.0	0.0
3,4-dihydroxybenzoic acid	34.6	71.8	74.2	50.9	3,5-dihydroxybenzoic acid	170.4	0.0	94.4	0.0
vanillic acid	112.3	194.0	140.3	168.4	caffeic acid	56.4	38.9	27.0	135.3
m-coumaric acid	7.8	11.8	0.0	0.0	ferulic acid	160.8	123.7	152.6	0.0
o-coumaric acid	23.5	29.0	0.0	0.0	sinapic acid	44.8	0.0	0.0	628.9
sinapic acid	91.9	149.5	112.0	12.1	7-methoxycoumarin	33.1	20.9	29.3	0.0
coumarin	70.1	69.8	57.5	53.5					
Kompact – plants	0 Gy	10 Gy	20 Gy	40 Gy	Kompact – plants	control	Σ 24 h	Σ 48 h	Σ 72 h
2,3-dihydroxybenzoic acid	887.3	564.9	717.5	602.8	2,3-dihydroxybenzoic acid	308.8	320.7	744.5	569.8
vanillic acid	108.9	50.9	38.8	35.9	vanillic acid	22.7	167.2	142.7	188.8
coumarin-3-carboxylic acid	38.7	126.0	0.0	0.0	o-coumaric acid	0.0	12.0	92.6	135.3
o-coumaric acid	84.2	35.6	26.7	24.2	ferulic acid	0.0	29.9	106.3	0.0
coumarin	0.0	45.9	0.0	0.0	sinapic acid	469.4	378.6	174.8	424.6
Krona – plants	0 Gy	10 Gy	20 Gy	40 Gy	Krona – plants	control	Σ 24 h	Σ 48 h	Σ 72 h
2,3-dihydroxybenzoic acid	883.4	458.5	453.1	161.1	2,3-dihydroxybenzoic acid	1,986.2	2,192.6	1,637.7	0.0
3,4-dihydroxybenzoic acid	55.5	0.0	0.0	0.0	vanillic acid	253.1	206.4	146.7	167.5
vanillic acid	17.0	60.9	48.2	24.2	coumarin-3-carboxylic acid	32.4	0.0	0.0	0.0
o-coumaric acid	27.1	69.5	51.4	17.6	o-coumaric acid	142.2	53.0	65.6	67.4
sinapic acid	167.8	291.7	229.7	164.6	veratric acid	350.1	139.9	211.6	189.2
					sinapic acid	62.6	68.1	52.6	43.7

Control – seeds, seedlings and plants unirradiated; Σ 24 h, Σ 48 h, Σ 72 h – total time of irradiation in hours

V. CRP content in barley (mg.kg^{-1} on a DM basis)

Cultivar	Seeds	Seedlings	Plants
Kompact	7,820	2,540	5,825
Krona	4,840	4,090	5,130

VI. Change of CRP content in barley (%) induced by UV-irradiation

	Seeds		Seedlings			Plants		
Dose UV (Σ hours)	Σ 24 h	Σ 48 h	Σ 24 h	Σ 48 h	Σ 72 h	Σ 24 h	Σ 48 h	Σ 72 h
Kompact	–12.8	–39.4	+22.8	+47.9	+92.2	–62.8	–47.6	–65.5
Krona	–4.2	–13.5	–56.3	–56.4	–17.6	+24.1	–13.0	0.0

VII. Change of CRP content in barley (%) induced by γ-irradiation

	Seeds			Seedlings			Plants		
Dose (Gy)	10	20	40	10	20	40	10	20	40
Kompact	–27.0	–31.3	–31.6	+52.9	+81.1	+28.1	+6.6	+27.9	–7.3
Krona	–25.3	+3.0	–10.3	–24.8	–35.7	–9.2	–53.3	–37.9	–82.9

increase was found in 2,3-dihydroxybenzoic acid (in seeds by 130%), sinapic acid (in seedlings by 1,300%) and caffeic acid (in seedlings by 140%). As it was reported recently, UV-B radiation resulted in a decrease of adaxial stomatal conductance increasing stomatal limitation of CO_2 uptake but delayed and reduced the severity of drought stress (Nogués et al., 1998). In addition to UV-B irradiation, ozone fumigation, wounding, aluminium stress and salt stress induced increased transcript levels of the sad genes in pea (Brosché, Strid, 1999). Increase of phenolic acids and other polyphenols are in accordance with the fact that flavonol glycosides and carotenoids are protecting plants from ultraviolet damage (Middleton, Teramura, 1993). UV-B damage could be dependent on the carotenoid concentrations (Götz et al., 1999) and flavonoids can protect DNA from the induction of ultraviolet radiation damage, as it was reported in maize (Stapleton, Walbot, 1994). Phenolic sunscreens are highly responsive to the wavelenghts that are most affected by variations in ozone levels (Mazza et al., 2000). Rao et al. (1996) have shown that UV-B radiation, in contrast to O_3 enhanced the activated oxygen species by increasing membrane-localized NADPH-oxidase activity and decreasing catalase activities. γ-irradiation caused only a little

increase of phenolic acid content in comparison with UV irradiation. In pea cv. Lantra has been found an increase of 2,3-dihydroxybenzoic acid (in seeds by 260%) and caffeic acid (by 50%), in pea cv. Menhir was determined only a little increase of m-hydroxybenzoic acid in seeds, sinapic acid in seedlings and plants (Table III). In barley cv. Kompact was determined after γ-irradiation an increase of 2,3-dihydroxybenzoic acid (in seeds by 440%) and 3,4-dihydroxybenzoic acid in seedlings by 98%. In cv. Krona was found after irradiation an increase of 3,4-dihydroxybenzoic acid in seedlings by 50–100% and vanillic acid in plants by 40–250% (Table IV). Accelerated conversion of conjugated phenolic acids such as chlorogenic acid into simple phenolic acids such as caffeic or ferulic acids were reported by Pendharkar and Nair (1995). Tomoda et al. (1980) noticed changes of sour taste and the composition of carboxylic acids induced in brewed coffee by γ-irradiation. As it was shown on *Arabidopsis*, γ-radiation induced leaf trichome formation on the adaxial surface of mature leaves after massive doses (1–3 kGy) of γ-radiation from ^{60}Co (Nagata et al., 1999). γ-radiation-induced trichome formation was mediated by active oxygen species generated by water radiolysis. Treatment with antioxidants before γ-irradiation suppressed the increase in trichome number.

An average CRP content in barley cv. Kompact was 7,820 mg.kg^{-1} DM of seeds, 2,540 mg.kg^{-1} DM of seedlings and 5,825 mg.kg^{-1} DM of plants. The same trend was observed in Krona cultivar: 4,840 mg.kg^{-1} DM of seeds, 4,090 mg.kg^{-1} DM of seedlings and 5,130 mg.kg^{-1} DM of plants (Table V). It could be concluded that during the process of sprouting CRP content decreased but in plants its content was higher.

UV-A irradiation caused in barley cv. Kompact a decrease of CRP to 68% of their origin content, in the seedlings its accumulation became greater to 128% and in plants a decrease to 93% of its initial content was observed. The similar trend was found in cv. Krona (a decrease to 89% in seeds, 77% in seedlings, and 60% in plants) (Table VI). γ-irradiation produced a decrease of CRP compounds. It was determined statistically significant relation between CRP content and the vegetation phase of the plant (Table VII).

We have recorded increased levels of phenolic content with enhanced doses of UV-A radiation in accordance with data reported earlier (Balakumar et al., 1996, 1997). An enhanced level of total phenols with an apparent inhibition in the PPO activity clearly indicate that under UV treatment, plants as an adaptive strategy tend to maintain the turnover rate of phenols by lowering the PPO activity (Balakumar, Selvakumar, 1998). Phenols might serve as potential non-enzymatic antioxidants under UV radiation that causes generation of reactive oxygen species such as superoxide (O_2) and singlet oxygen ($^1O_2$) which have greater toxicity potentials on biomolecules and membranes.

CONCLUSIONS

During pea sprouting TP content increased, in barley cultivars TP stagnation was found. In investigated plants UV-A irradiation caused an increase of TP content, γ-irradiation enhanced TP content in barley but in pea decreased it. CRP content in sprouting seeds decreased but in further development of plants increased. In barley UV-A and γ-irradiation decreased CRP content. Cultivars responded differentially to various doses of irradiation in both types of irradiation. As the most contained phenolic acids in pea and barley were found 2,3-dihydroxybenzoic, sinapic, m-dihydroxybenzoic, veratric, and vanillic acids. UV-A irradiation caused significant increase of the content of these acids both in barley and pea, whereas γ-irradiation caused only little changes in the content of these compounds.

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Received for publication on April 10, 2000

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Vliv UV-A a gama-záření na obsah polyfenolů v semenech, naklíčených semenech a rostlinách ječmene a hrachu.

Scientia Agric. Bohem., *31*, 2000: 181–196.

Ve dvou odrůdách hrachu (Lantra, Menhir) a ječmene (Krona, Kompact) byly sledovány změny celkového obsahu polyfenolických látek (TP) a dominantně zastoupených fenolických kyselin způsobených UV-A a γ-zářením. Změny byly sledovány v semenech, naklíčených semenech a rostlinách. V odrůdách ječmene byl také sledován obsah látek typu katecholu, resorcinolu a floroglucinolu (CRP). Obsah celkových polyfenolů (TP) byl stanoven spektrofotometricky s Folin-Ciocalteuovým činidlem, obsah polyfenolických látek typu katecholu, resorcinolu a floroglucinolu (CRP) spektrofotometricky s p-dimethylaminoskořicovým aldehydem a fenolické kyseliny pomocí HPLC. UV záření bylo aplikováno po 0, 24, 48 a 72 hod. při vlnové délce λ = 351 nm, γ-záření s ^{60}Co v dávkách 0, 10, 20 a 40 Gy. Během klíčení hrachu došlo ke zvýšení obsahu TP, u odrůd ječmene byla zjištěna stagnace. UV-A záření způsobilo u sledovaných rostlin nárůst obsahu celkových polyfenolů (TP), γ-záření zvýšilo obsah TP u ječmene, avšak u hrachu docházelo k jeho snížení. Byla prokázána statistická závislost obsahu celkových polyfenolů na růstových fázích rostliny a na dávkách záření. Různé odrůdy reagovaly na ozáření různými změnami obsahu celkových polyfenolů i látek typu CRP. UV-A a γ-záření snižovala obsah látek typu katecholu, resorcinolu a floroglucinolu u ječmene. Statisticky významná závislost byla nalezena mezi obsahem CRP a dávkami záření a vegetační fází rostlin. Obsah CRP v naklíčených semenech se snižoval, avšak v dalším vývoji rostlin docházelo k jeho nárůstu. Nejvíce zastoupené fenolické kyseliny u hrachu a ječmene byly 2,3-dihydroxybenzoová, sinapová, m-hydroxybenzoová, veratrová a vanilová kyselina. UV-A záření způsobilo zřetelný nárůst těchto kyselin jak u ječmene, tak i u hrachu, zatímco γ-záření způsobilo pouze malé změny v obsahu těchto látek.

UV-A záření; γ-záření; odrůdy hrachu; odrůdy ječmene; obsah polyfenolů; fenolkarboxylové kyseliny; obsah látek typu katecholu, resorcinolu a floroglucinolu

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