CHANGES OF ISOFLAVONOID LEVELS IN PEA (*PISUM SATIVUM* L.) SEEDS, SEEDLINGS AND PLANTS INFLUENCED BY UV-A AND γ-IRRADIATION*

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In two pea cultivars (Lantra, Menhir) changes in isoflavonoid levels caused by UV-A and γ-irradiation were investigated. The changes were determined in seeds, seedlings and plants. UV-A irradiation was performed for 0 (control), 24, 48 and 72 hours at λ = 365.5 nm, P = 125 W, γ-irradiation with ^{60}Co at doses 0 (control), 10, 20 and 40 Gy. Cultivars responded differentially to various doses of irradiation in both types of irradiation. Daidzein and genistein are the main isoflavones of biological interest belonging to the group of naturally occurring isoflavonoid phytoestrogens. Daidzein (Dai) and genistein (Gen) were determined by a selective and sensitive radioimmunoassay 7- and 4'-cross-reactive method. In lower amounts biochanin A (Bio A) was determined by means of 7-cross-reactivity. The highest levels among isoflavonoids were found for daidzein-7 cross-reactivity in both pea varieties (on average in Lantra seeds 6 562.4 $\mu g.kg^{-1}$ DM, in Menhir seeds 3 481.3 $\mu g.kg^{-1}$ DM). During sprouting of seeds and developing of plants the isoflavonoid content significantly increased. UV-A irradiation caused apparent increase of isoflavonoids at high levels (72 hours) of irradiation, especially in seedlings and plants. In all analysed isoflavonoids the higher effect on isoflavonoid increase was found in seedlings compared with plants, esp. for daidzein-7 and 4' and genistein-7 immunoreactivity. At lower doses of UV-A irradiation the effect of irradiation on the increase of isoflavonoids was much lower. A little effect was found in the seeds. On the contrary γ-irradiation was the most efficient at low doses of 10 Gy and 20 Gy. Seedlings and plants differed in doses of γ-irradiation causing increase or decrease of isoflavonoid levels. In seedlings of cv. Lantra the highest increase of isoflavonoids was observed at dose of 20 Gy, while the highest increase in cv. Lantra was found at 10 Gy. UV-A irradiation and γ-irradiation (at lower doses) activate protective enzymic plant

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system producing protective polyphenols and esp. isoflavonoids with the result of enhancing their content. On the other hand γ-irradiation at higher doses could damage it. Resultant content depends on the rate of these two changes in a given part of plant.

UV-A irradiation; γ-irradiation; pea cultivars; isoflavonoids; daidzein; genistein; biochanin A; radioimmunoassay

INTRODUCTION

Polyphenolic complex of pea (*Pisum sativum* L.) comprises many structures, such as phenolcarboxylic acids, flavonoids, anthocyanins, pterocarpans, coumestrols and isoflavonoids (Lachman et al., 1995). Some identified structures are shown in Table I (Duke, 1992a, b). Based on the results obtained, the antioxidant activity of leguminous extracts appears to be generated by a great number of phenolic compounds and does not depend directly on their total content in the seeds (Amarowicz, Raab, 1997). The main polyphenolic compounds of legumes are glycosides of quercetin, isoflavonoids, isoflavonoid phytoalexins, and anthocyanins. Special structures present phytoalexins, such as pisatin (Perrin, Bottomley, 1961, 1962). Their content is affected by many factors, such as deterioration of seeds or by attack of numerous fungi (Lachman et al., 1997).

An important and specific group of flavonoids represents isoflavonoid phytoestrogens naturally occurring usually in glycosidic form, esp. in some legumes and pulses belonging to *Fabaceae* (Lachman et al., 1990; Xu et al., 1994). The precursors of these diphenolic compounds have been found

I. Polyphenolic compounds identified in pea (*Pisum sativum* L.).

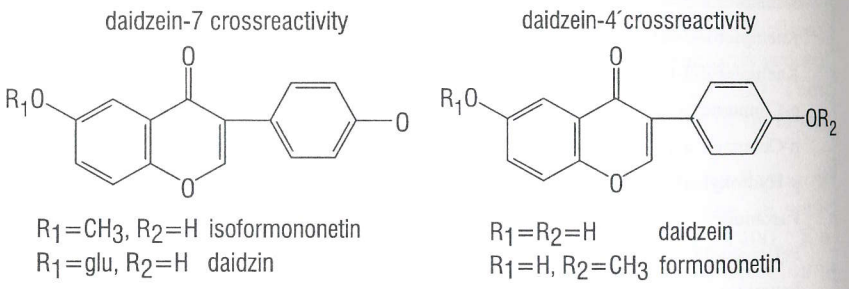
Compound	Organ
1-O-Feruloyl-β-D-glucose	petiole
1-O-p-Coumaroyl-β-D-glucose	petiole
1-O-Sinapoyl-β-D-glucose	petiole
3-Hydroxy-2,9-dimethoxypterocarpan	embryo
4,4'-Dihydroxy-2'-methoxychalcone	plant
4-Hydroxy-2,3,9-trimethoxypterocarpan	embryo
Caffeic acid	plant
Caffeic acid 4-O-β-D-glucoside	fruit

Continuation of Table I

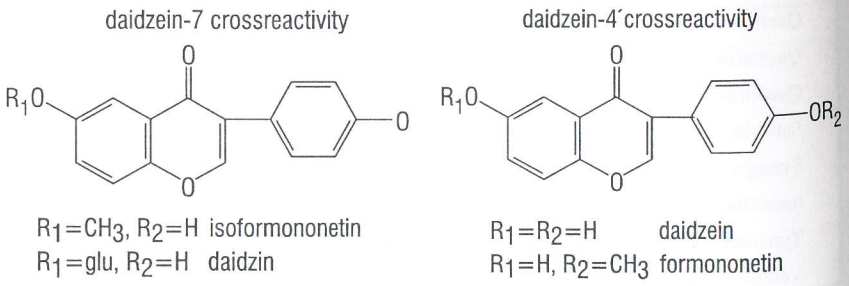
Compound	Organ
Cis-Sinapic acid	seed
Coumestrol	fruit 300*, seed 0.6*
Cyanidin	flower
Cyanidin-3-sambubioside-5-glucoside	hull husk
Cyanidin-3-sophoroside-5-glucoside	leaf
Delphinidin	flower
Delphinidin-3,5-diglucoside	flower
Delphinidin-3-glucoside	flower
Ferulic acid	seed
Genistein	shoot
Gentisic acid	seed
Kaempferol	tissue culture 500
Kaempferol-3-coumaroyl-triglucoside	leaf
Kaempferol-3-feruloyl-triglucoside	leaf
Kaempferol-3-triglucoside	leaf
o-Coumaric acid	seed
p-Coumaric acid	seed
p-Hydroxybenzoic acid	root
Paeonidin	flower
Pelargonidin	flower
Petunidin	flower
Pisatin	sprout seedling
Quercetin-3-coumaroyl-triglucoside	leaf
Quercetin-3-feruloyl-triglucoside	leaf
Quercetin-3-triglucoside	leaf
Quercitrin	seed
Salicylic acid	plant
Syringic acid	root
trans-Sinapic acid	seed
Tyrosine	fruit 990–9 792*, seed 1 130–5 345*
Vanillic acid	root, seed

S – seeds, SDL – seedlings, P – plants, * – content ($mg.kg^{-1}$)

in fibre rich in unrefined grain products, various seeds, cereals, foods of plant origin (Mazur et al., 1996). The main isoflavones of biological interest occurring in plants are the glycosides of genistein (4',5,7-trihydroxyisoflavone) and daidzein (4',7-dihydroxyisoflavone). They have antiinflammatory, antitumor (breast, stomach, prostate) or antioxidant activity (Duke, 1992a, b). Since there have been lately developed very sensitive novel radioimmunoassays for daidzein, genistein (Figs. 1 and 2) and their 4'- (formononetin, biochanin A) and 7-derivatives (daidzin, isoformononetin, genistin, prunetin) for their simultaneous determination (Lapčík et al., 1996, 1997, 1998a, b) that has not been carried out previously. The method is very useful for screening of metabolic studies. We have used the advantage of this method to determine the changes in the daidzein and genistein contents caused by the UV-A and γ-irradiation influencing their levels in pea seeds. Orsák et al. (2000) studied already the influence of UV-A and γ-irradiation on the content of total polyphenols (TP), compounds of catechol, resorcinol and phloroglucinol type (CRP) and phenolcarboxylic acid content in pea. During pea



1. Daidzein-7 and 4'-crossreactivity



2. Genistein-7 and 4'-crossreactivity

sprouting content of total polyphenols increased. UV-A irradiation caused an increase of total polyphenol content in investigated plants, in γ-irradiation the change of total polyphenol content depended on the dose of irradiation. Statistical dependence of TP content on growth phases of plants and on doses of irradiation was demonstrated. CRP in sprouting seeds decreased, but in further development of plants it increased. UV-A and γ-irradiation decreased CRP content in pea. 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 were 2,3-dihydroxybenzoic, sinapic, m-hydroxybenzoic, veratric and vanillic acid. UV-A irradiation caused apparent increase of these acids in pea, whereas γ-irradiation caused only little changes in the content of these compounds.

There exists tremendous variability in plant species to UV-radiation (Teramura, 1983). Some species show sensitivity to present levels of UV radiation (Bogenrieder, Klein, 1978), while the others are apparently unaffected by rather massive enhancements (Becwar et al., 1982). Because the earth's protective stratospheric ozone layer is depleting, the effect of UV and gamma irradiation on the plants is enhancing (Teramura and Sullivan, 1991). The aim of this work was to complete our knowledge about the response of pea against irradiation in special isoflavonoid protective compounds that are typical for *Fabaceae* and to compare the results obtained with changes in total polyphenols, phenolcarboxylic acids and polyphenols of resorcinol, phloroglucinol and catechol type that were reported previously (Orsák et al., 2000).

MATERIAL AND METHODS

Pea cultivars Lantra and Menhir obtained from the Department of Plant Production of Czech University of Agriculture in Prague in the year 1999 were irradiated with UV-A radiation (λ = 365.5 nm, P = 125 W for 0 [control], 24, 48 and 72 hours) and γ-irradiation (^{60}Co, 0 [control], 10, 20 and 40 Gy at the Faculty of Medicine, Hradec Králové).

The following three variants were investigated:

1. Dry seeds (S): were stored after two month-radiation at the laboratory temperature before analyses.
2. Seedlings (SDL): for the preparation of seedlings the seeds were soaked for 10 hours in distilled water and then sown on the soil surface or sand. After 5 days of cultivation at laboratory temperature 22 °C the seedlings were exposed to irradiation and then cultivated for other 7 days.

3. Plants (P): the seeds were sown directly into the soil in the pot. After the cultivation for 21 days at laboratory temperature 22 °C the plants were irradiated and then cultivated for other 7 days.

Preparation of extracts: Weighed ground seeds (approx. 25 g pea) were extracted in the Soxhlet apparatus with ethanol-water mixture (80 : 20 V/V) for 20 hours. The extract was adjusted to 250 mL volume and from this volume 5 µL aliquots were pipetted for determination.

Pea seedlings and plants were dried in a drying box at 500 °C for 20 hours and then the weighed ground pea seedlings (approx. 7–9 g) were extracted in the Soxhlet apparatus with ethanol-water mixture (80 : 20 V/V) for 22 hours continuously. The extracts were adjusted to 250mL volumes in volumetric flasks.

Determination of isoflavonoid content was performed with radioimmunoassay method developed by Lapčík et al. (1996, 1997, 1998a, b) and Hampl et al. (1997). Radioimmunoassays with these specifies were used:
1. daidzein and its 4'- derivatives (e.g. formononetin),
2. daidzein and its 7-derivatives (e.g. daidzin, isoformononetin),
3. genistein and its 4'-derivatives (e.g. biochanin A),
4. genistein and its 7-derivatives (e.g. genistin, prunetin).

Immunoreactivities were measured either in crude extracts, or after chromatographic fractionation by HPLC (reversed phase, octadecylsilica) described by Lapčík et al. (1998a, b). The HPLC system consisted of an LC-6A pump (Shimadzu, Japan), a column oven LCO 100, an UV detector LCD 2082 (Ecom, Czech Republic) and a fraction collector FC-203 B (Gilson, France). An ET 250/4 Nucleosil 100-5 C 18 (Macheray-Nagel, Germany) was used. The mobile phase was methanol : water (60 : 40 v/v), the flow rate 0.8 mL.min^{-1} and the temperature was 35 °C (Lapčík et al., 1998a, b). Chromatographic mobilities of immunoreactive fractions were compared to those of daidzein, daidzin, formononetin, genistein, genistin and biochanin A standards. Standards of isoformononetin and prunetin were prepared from daidzein and genistein, respectively, using diazomethane as the methylation agent, as it is described elsewhere (Furniss et al., 1989). The pea extracts were fractionated by TLC on Merck PSC silica plates (Art. 13794) and Merck DC-Alufolien (Art. 5583) with developing system dichloromethane : methanol 95 : 5 (v/v) after Lapčík et al. (1998a, b).

Determination of dry matter (DM) was performed after usual method at 105 °C (Davídek et al., 1977).

RESULTS AND DISCUSSION

As it was reported previously, some selected factors could affect polyphenol and isoflavonoid contents in pea (Lachman et al., 1999). Accelerated ageing caused increase of TP contents in pea (on average by +28.41 %$_{rel.}$ in the years 1996 and 1997). Similar trend was found in isoflavonoid contents. The highest content was found for daidzein-7 activity (3 850 µg.kg^{-1} in 1997, 1 610 µg.kg^{-1} in 1996) followed with genistein-7 activity (1 130 µg.kg^{-1} in 1997 and 240 µg.kg^{-1} in 1996). For isoflavonoid contents were significant origin and variety effects and treatment only in several cases, as daidzein-4'and genistein-4'.

Results of influence of *E. purpurascens* on TP and isoflavonoid contents in pea seeds showed that elicitors of *E. purpurascens* have caused increase of TP content both in sterilised and unsterilised media (Lachman et al., 1999). Analogously, the increase of genistein-7, daidzein-7 and -4'cross-reactivities after cultivation in sterile water, pea and potato extracts with addition of glucose was determined. The highest concentrations were found for daidzein-7 (on average 26 500 µg.kg^{-1} in control) and genistein-7 (6 250 µg.kg^{-1}). The highest increase of daidzein-7 (from 25 370 to 136 090 µg.kg^{-1}) in comparison with the control was found after cultivation in pea extract with addition of glucose and barley and potato extracts with addition of glucose. High levels were found also for genistein-7 (from 8 800 to 28 690 µg.kg^{-1} in pea extract with addition of glucose and from 4 060 to 21 170 µg.kg^{-1} in potato extract with addition of glucose), as it was reported previously (Lachman et al., 1999).

Results obtained after UV-A and γ-irradiation are given in Tables II and III. The highest levels among isoflavonoids were found for daidzein-7 cross-reactivity in both pea varieties (on average in Lantra seeds 6 562.4 µg.kg^{-1} DM, in Menhir seeds 3 481.3 µg.kg^{-1} DM). It is apparent that during sprouting of seeds and developing of plants the isoflavonoid content was significantly increasing, as it has been demonstrated in total polyphenols and phenolcarboxylic acids recently (Orsák et al., 2000). The significant increase in seedlings was the highest for these different isoflavonoids: in cv. Lantra Dai-4' by +10 707.7 %$_{rel.}$, Dai-7 by +2 694.4 %$_{rel.}$, Gen-7 by +1 923.0 %$_{rel.}$, in cv. Menhir Gen-7 by +5 134.4 %$_{rel.}$, Dai-4' by +4 777.9 %$_{rel.}$, Bio-A-7 by +4 616.2 %$_{rel.}$ and Dai-7 by +2 094.6 %$_{rel.}$. The increase in plants in comparison with seeds was a little lower – the highest values were found for cv. Lantra for Dai-4'(by +4 195.3 %$_{rel.}$) and for cv. Menhir for Dai-7 (by +1 101.5 %$_{rel.}$), Dai-4'(by +3 313.4 %$_{rel.}$) and Bio A-7 (by +1 596.1 %$_{rel.}$). During sprouting and developing of plants the enzymic system is activated and the production of polyphenols and esp. that of isoflavonoids increases at great rate.

II. Effect of UV-A irradiation on the isoflavonoid content in pea

Part	Variety	Dose (h)	Daidzein-7 ($\mu g.kg^{-1}$ DM)	Daidzein-4' ($\mu g.kg^{-1}$ DM)	Genistein-7 ($\mu g.kg^{-1}$ DM)	Biochanin A-7 ($\mu g.kg^{-1}$ DM)
S	Lantra	0	6 667.6	688.7	2 945.5	2 089.7
S	Lantra	24	5 802.0	514.3	7 588.6	150.7
S	Lantra	48	5 421.5	502.2	6 084.5	88.4
S	Menhir	0	4 048.6	907.3	2 699.0	49.2
S	Menhir	24	4 332.8	791.7	3 375.8	57.4
S	Menhir	48	3 571.8	470.5	3 222.8	66.4
SDL	Menhir	0	24 656.0	22 018.3	8 371.6	x
SDL	Menhir	24	43 604.7	47 577.5	4 554.3	96.9
SDL	Menhir	48	108 956.7	53 858.3	18 563.0	1 003.9
SDL	Menhir	72	98 026.3	186 184.2	17 171.1	4 868.4
P	Lantra	48	80 357.1	13 809.5	24 523.8	x
P	Lantra	72	188 466.5	17 139.2	126 095.4	663.7
P	Menhir	0	39 062.5	14 015.2	6 865.5	636.8
P	Menhir	24	32 907.7	13 998.0	5 255.4	1 733.8
P	Menhir	48	35 214.2	11 820.4	8 340.2	2 930.4
P	Menhir	72	57 990.8	19 924.5	12 814.6	3 104.0

DM – dry matter, x – under limit of detection

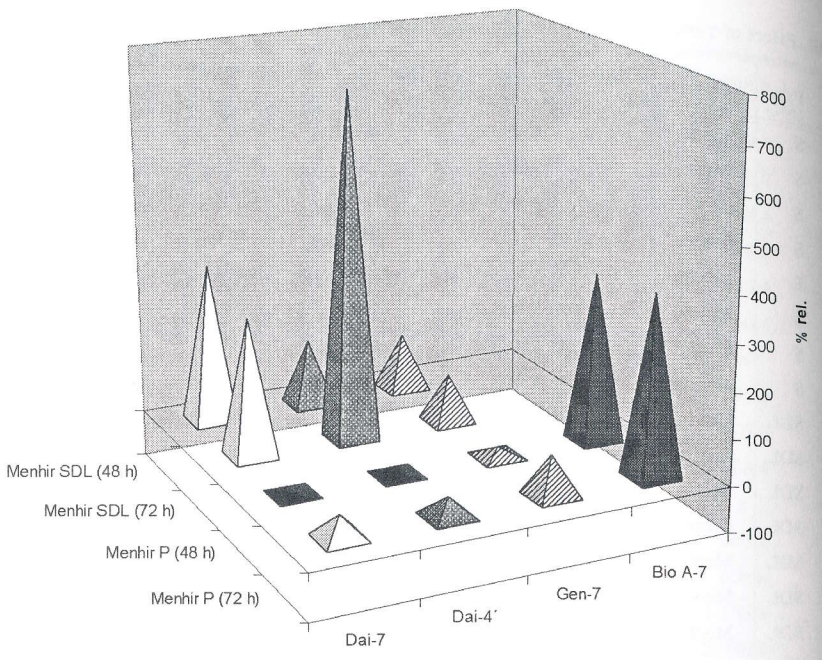
UV-A irradiation caused apparent increase of isoflavonoids at high levels (48 and 72 hours) of irradiation, esp. in seedlings and plants. In cv. Menhir increase of daidzein-7 immunoreactivity was by +297.6 $\%_{rel.}$ in seedlings and by +48.5 $\%_{rel.}$ in plants, similarly for daidzein-4'immunoreactivity by +745.6 $\%_{rel.}$ and by +42.2 $\%_{rel.}$, for genistein-7 immunoreactivity by +105.1 $\%_{rel.}$ and by +86.7 $\%_{rel.}$, respectively, and for biochanin A-7 immunoreactivity by +387.4 $\%_{rel.}$ in plants (Fig. 3). In all analysed isoflavonoids the higher effect on isoflavonoid increase was found in seedlings in comparison with plants, esp. for daidzein-7 and 4' and genistein-7 immunoreactivity. At lower doses of UV-A irradiation the effect of irradiation on the increase of isoflavonoids was much lower, esp. at level of 24 hours of irradiation. A little effect was found in the seeds.

On the contrary, γ-irradiation was the most efficient at low doses of 10 Gy and 20 Gy (Fig. 4). In cv. Lantra seeds increase for daidzein-7 immunoreactivity was by +21.9 $\%_{rel.}$, for daidzein-4' immunoreactivity by +9.0 $\%_{rel.}$,

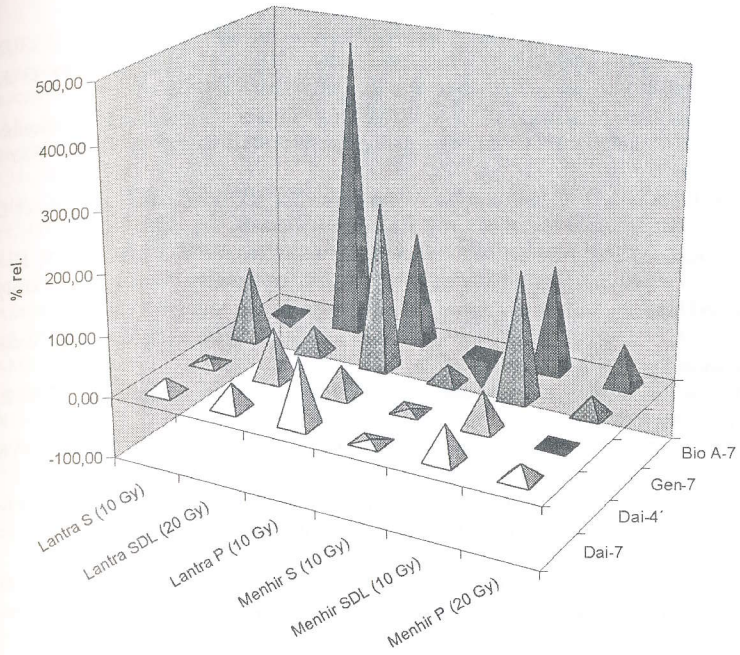
III. Effect of γ-irradiation on the isoflavonoid content in pea

Part	Variety	Dose (h)	Daidzein-7 ($\mu g.kg^{-1}$ DM)	Daidzein-4' ($\mu g.kg^{-1}$ DM)	Genistein-7 ($\mu g.kg^{-1}$ DM)	Biochanin A-7 ($\mu g.kg^{-1}$ DM)
S	Lantra	0	6 457.2	482.9	1 956.6	69.6
S	Lantra	10	7 868.8	526.1	4 265.3	51.1
S	Lantra	20	4 964.7	340.6	1 131.1	58.8
S	Lantra	40	8 191.7	677.6	1 776.6	113.9
S	Menhir	0	2 913.9	510.5	621.1	49.3
S	Menhir	10	3 015.2	525.4	799.3	20.2
S	Menhir	20	2 156.7	422.5	477.5	22.7
S	Menhir	40	2 492.3	546.3	625.5	21.5
SDL	Lantra	0	180 439.9	52 190.4	39 581.8	261.6
SDL	Lantra	10	98 486.8	32 842.1	19 657.9	740.8
SDL	Lantra	20	253 609.2	96 005.8	56 917.7	1 507.5
SDL	Lantra	40	118 459.3	35 610.5	13 711.2	998.1
SDL	Menhir	0	110 151.5	33 441.1	63 095.6	2 325.1
SDL	Menhir	10	172 871.9	53 758.7	191 290.3	6 325.0
SDL	Menhir	20	90 222.2	34 042.6	41 927.4	4 239.7
SDL	Menhir	40	97 624.3	33 793.9	26 096.5	2 624.3
P	Lantra	0	39 148.4	20 741.8	5 975.3	590.7
P	Lantra	10	81 404.7	30 678.1	21 932.2	1 660.4
P	Lantra	20	21 590.9	6 818.2	5 909.1	1 000.0
P	Lantra	40	21 505.4	9 274.2	3 414.0	x
P	Menhir	0	52 923.4	23 951.6	5 866.9	989.9
P	Menhir	10	38 783.5	16 043.5	5 147.9	694.8
P	Menhir	20	65 456.1	20 945.9	7 742.1	1 672.3
P	Menhir	40	48 622.2	21 685.3	7 687.7	1 733.2

genistein-7 by +118.0 $\%_{rel.}$, in cv. Menhir seeds by +3.5 $\%_{rel.}$, by +2.9 $\%_{rel.}$ and by +28.7 $\%_{rel.}$, respectively. Seedlings and plants differed in doses of γ-irradiation causing increase or decrease of isoflavonoid levels. In seedlings of cv. Lantra the highest increase of isoflavonoids was observed at a dose of 20 Gy (Dai-7 by +40.6 $\%_{rel.}$, Dai-4' by +84.0 $\%_{rel.}$, Gen-7 by +43.8 $\%_{rel.}$, Bio A-7 by +476.3 $\%_{rel.}$), in cv. Menhir at dose of 10 Gy (Dai-7 by +56.9 $\%_{rel.}$, Dai-4' by +60.8 $\%_{rel.}$, Gen-7 by +203.2 $\%_{rel.}$, and Bio A-7 by +172.0 $\%_{rel.}$,



3. Changes of isoflavonoids in pea seedlings and plants cv. Menhir after UV-A irradiation for 48 or 72 hours



4. Changes of isoflavonoids in pea after γ-irradiation at 10 or 20 Gy doses

respectively). Similar tendency was found in plants, where the highest increase in cv. Lantra was found at 10 Gy (Dai-7 by +107.9 $\%_{rel.}$, Dai-4' by +47.9 $\%_{rel.}$, Gen-7 by +267.1 $\%_{rel.}$, and Bio A-7 by +181.1 $\%_{rel.}$) and in cv. Menhir at 20 Gy (Dai-7 by +23.7 $\%_{rel.}$, Gen-7 by +32.0 $\%_{rel.}$, and Bio A-7 by +68.9 $\%_{rel.}$, respectively). These doses seem to be important for rate equilibrium between the induction of the production of isoflavonoids as defence compounds and response to damage effect of irradiation and the depletion of the enzymes responsible for their biosynthesis. At higher doses of irradiation (40 Gy or more) the destruction of these enzymes already prevails as compared with lower isoflavonoid levels in pea seedlings and plants irradiated with this dose.

CONCLUSIONS

The highest levels among isoflavonoids were found for daidzein-7 cross-reactivity in both pea varieties. During sprouting of seeds and developing of plants the isoflavonoid content significantly increased. UV-A irradiation caused apparent increase of isoflavonoids at high levels (48 and 72 hours) of irradiation, esp. in seedlings and plants. In all analysed isoflavonoids the higher effect on isoflavonoid increase was found in seedlings in comparison with plants, esp. for daidzein-7 and 4' and genistein-7 immunoreactivity. At lower doses of UV-A irradiation the effect of γ-irradiation on the increase of isoflavonoids was much lower. A little effect was found in the seeds. On the contrary, γ-irradiation was the most efficient at low doses of 10 Gy and 20 Gy. Seedlings and plants differed in doses of γ-irradiation causing increase or decrease of isoflavonoid levels. In seedlings of cv. Lantra the highest increase of isoflavonoids was observed at dose of 20 Gy, while the highest increase in cv. Lantra was found at 10 Gy. UV-A irradiation and γ-irradiation activate protective enzymic plant system producing protective polyphenols and esp. isoflavonoids with the result of enhancing their content, but on the other hand γ-irradiation at higher doses could damage it. Resultant content depends on the rate of these two changes in a given plant part.

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Změny obsahu izoflavonoidů v semenech, naklíčených semenech a rostlinách hrachu setého (*Pisum sativum* L.) způsobené vlivem UV-A záření a γ-záření.

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U dvou odrůd hrachu (Lantra, Menhir) byly sledovány změny obsahu izoflavonoidů způsobené UV-A a γ-ozářením. Změny byly sledovány v semenech, naklíčených semenech a rostlinách. UV-A záření bylo provedeno v dávkách 0 (kontrola), 24, 48 a 72 hodin celkového ozáření při λ = 365,5 nm, P = 75 W, γ-záření s ^{60}Co v dávkách 0 (kontrola), 10, 20 a 40 Gy. Odrůdy vykázaly různou odezvu na různé dávky ozáření u obou druhů záření. Daidzein a genistein jsou hlavními izoflavony z hlediska biologického zájmu a patří do skupiny přírodních izoflavonoidních estrogenů. Daidzein (Dai) a genistein (Gen) byly stanoveny selektivní a citlivou radioimunoanalýzou 7- a 4'-křížovou reaktivitou. V menším množství byl zastoupen biochanin A (Bio A) stanovený pomocí 7-křížové reaktivity. Mezi sledovanými izoflavonoidy byly nejvyšší hladiny stanoveny u daidzein-7-křížové reaktivity u obou odrůd (průměrně 6 562,4 $\mu g.kg^{-1}$ sušiny v semenech odrůdy Lantra a 3 481,3 $\mu g.kg^{-1}$ sušiny v semenech odrůdy Menhir). Během klíčení semen a vývoje rostlin se obsah izoflavonoidů výrazně zvýšil, přičemž nárůst u naklíčených semen byl vyšší než u rostlin při srovnání se semeny (nejvyšší byl u odrůdy Lantra pro Dai-4'+10 707,7 $\%_{rel.}$ a u odrůdy Menhir Gen-7 +5 134,4 $\%_{rel.}$). UV-A záření způsobuje výrazné zvýšení obsahu izoflavonoidů při vysokých dávkách ozáření, zejména v naklíčených semenech a rostlinách. U od-

růdy Menhir byl nárůst daidzein-7-imunoreaktivity +297,6 %rel. v naklíčených semenech a +48,5 %rel. v rostlinách; podobně u daidzein-4'-imunoreaktivity +745,6 %rel. a +42,2 %rel., u genistein-7-imunoreaktivity +105,1 %rel. a +86,7 %rel. a u biochanin A-7-imunoreaktivity o +387,4 %rel. při ozáření po dobu 72 hodin. U všech analyzovaných izoflavonoidů byl větší vliv na zvýšení obsahu izoflavonoidů nalezen u naklíčených semen ve srovnání s rostlinami, zvláště pro daidzein-7 a 4'- a genistein-7-imunoreaktivitu. Při nižších dávkách UV-A záření byl vliv záření na zvýšení obsahu izoflavonoidů mnohem menší, zvláště při ozáření po dobu 24 hodin byl nalezen malý vliv u semen. Naproti tomu γ-záření bylo nejúčinnější v dávce 10 Gy (u semen odrůdy Lantra byl nárůst daidzein-7-imunoreaktivity +21,9 %rel., daidzein-4'-imunoreaktivity +9,0 %rel., genistein-7-imunoreaktivity +118,0 %rel.). U odrůdy Menhir byly odpovídající hodnoty +3,5 %rel., +2,9 %rel. a +28,7 %rel.. Naklíčená semena a rostliny se lišily v dávkách γ-záření způsobujících nárůst nebo pokles obsahu izoflavonoidů. U naklíčených semen odrůdy Lantra byl největší nárůst obsahu izoflavonoidů zjištěn při dávce 20 Gy (Dai-7 +40,6 %rel., Dai-4'+84,0 %rel., Gen-7 +43,8 %rel., Bio A-7 +476,3 %rel.), u odrůdy Menhir při dávce 10 Gy (Dai-7 +56,9 %rel., Dai-4'+60,8 %rel., Gen-7 +203,2 %rel. a Bio A-7 +172,0 %rel.). Podobný trend byl zjištěn také u rostlin – nejvyšší nárůst u odrůdy Lantra byl nalezen při dávce 10 Gy (Dai-7 +107,9 %rel., Dai-4'+47,9 %rel., Gen-7 +267,1 %rel. a Bio A-7 +181,1 %rel.) a u odrůdy Menhir při 20 Gy (Dai-7 +23,7 %rel., Gen-7 +32,0 %rel. a Bio A-7 +68,9 %rel.). UV-A i γ-záření mohou aktivovat obranný enzymový systém rostlin syntetizující ochranné polyfenoly a zejména izoflavonoidy, avšak γ-záření může na druhé straně poškozovat enzymovou dráhu jejich výstavby. Poměr mezi těmito dvěma změnami rozhodne o zvýšení nebo snížení obsahu izoflavonoidů v dané části rostliny.

UV-A záření; γ-záření; kultivary hrachu; daidzein; genistein; biochanin A; radioimunoanalýza

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