POLYPHENOL AND ISOFLAVONOID LEVELS IN BARLEY AND PEA SEEDS AND SEEDLINGS INFLUENCED BY THEIR DETERIORATION AND *EPICOCCUM PURPURASCENS* EHRENB. EX SCHLECHT. ELICITORS*

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Secondary metabolites – polyphenols contained in seeds – play an important role in the control of dormancy and sprouting, in decrease oxygen consumption rates and uncoupled oxidative phosphorylation. In three barley cultivars from different localities in 1996–1997 years changes in total polyphenols (TP), catechol, resorcinol and phloroglucinol polyphenols (CRP) and in three pea cultivars changes in TP and daidzein and genistein type isoflavonoids caused by accelerated ageing (AA) were investigated. Daidzein and genistein are the main isoflavones of biological interest belonging to the group of naturally occurring isoflavonoid phytoestrogens. Their activities were also determined in pea samples influenced by *Epicoccum purpurascens* elicitors after 3 days germination on filtrates of different media and water obtained after 14 days incubation with *E. purpurascens*. Daidzein (Dai) and genistein (Gen) were determinated by a selective and sensitive radioimmunoassay 7- and 4'-cross-reactive method. Total polyphenols (TP) were determined with Folin-Ciocalteau's reagent and CRP polyphenols with p-dimethylaminocinnamaldehyde (p-DMASA). In pea seeds significant differences were in TP and isoflavonoid contents caused with variety, growing conditions and accelerated ageing. The highest TP content was found in cv. Komet (in average from two years 70.54 mg/100 g), the lowest in cv. Menhir (av. from two years 50.75 mg/100 g). There were significant differences among localities. AA caused enhancing of TP av. by +28.41 %rel. In isoflavonoids the highest contents were found in daidzein-7 activity (2.73 μg/g) and genistein-7 activity (0.69 μg/g). In barley seeds were significantly influenced TP and CRP contents by origin and variety (cv. Forum had TP content 139.61 mg/100 g, cv. Amulet 126.71 mg/100 g). Elicitors of *E. purpurascens* have caused increase of TP and isoflavonoids in sterilised or

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unsterilised media after inoculation with *E. purpurascens*. The highest increases were found in asparagine medium (+53.79 %rel.) and pea extracts with glucose (+60.26 %rel.). In isoflavonoid content the highest increase in comparison with control was found in pea extract with glucose for daidzein-7 (from 25.37 to 136.09 μg/g) and for genistein-7 (from 8.80 to 28.69 μg/g) as well as in potato extract with glucose (from 4.06 to 21.17 μg/g).

seeds barley; pea; polyphenols; variety environmental conditions isoflavonoids; daidzein; genistein; radioimmunoassay deterioration; *Epicoccum purpurascens* elicitors

INTRODUCTION

The outer layer coats of the seeds of plants contain different polyphenolic compounds, e.g. flavonoids, phenolic acids, coumarins, anthocyanins. Their content and composition is strongly influenced by different stress factors (Weidner et al., 1996). Phenolic compounds, especially free phenolic acids are regulating factors that retard precocious germination processes (Weidner, Paprocka, 1996). The process of deterioration with increased temperature and moisture cause significant increase both total polyphenols and catechol, resorcinol and phloroglucinol type polyphenols in barley seeds and total polyphenols in pea seeds (Lachman et al., 1997). It was found a positive correlation between dormancy levels and the contents of phenolic acids in the process of after-ripening, supporting the idea of involvement of phenolic compounds in the control of dormancy and sprouting in cereal caryopses. Phenolics with high activity (germination inhibition 80%) include m-coumaric, p-coumaric, ferulic, ellagic, salicylic, p-hydroxybenzoic and gentisic acids, and also resorcinol and quercetin. Their changes in dormant seeds have been reported both among free and soluble bound phenolic acids during several months storage in dry state (Weidner et al., 1995, 1996). In germinating barley seeds, benzoquinones decrease oxygen consumption rates, uncoupled oxidative phosphorylation and inhibit amino acid activation (Van Sumere et al., 1975), endogenous concentrations of scopoletin and scopolin (7-O-glucoside) regulate longitudinal growth in germinating oats (Pollock et al., 1954).

An important and specific group of flavonoids represent 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 phenolic compounds have been found in fibre rich 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). Since there have been lately developed very sensitive novel radioimmunoassays for daidzein, genistein and their 4'-(formononetin, biochanin A) and 7- derivatives (daidzin, isoformononetin, genistin, prunetin) for their simultaneous determination (Lapčík et al., 1996, 1997, 1998) 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 deterioration stress factors (high temperature and moisture) and *Epicoccum purpurascens* Ehrenb. ex Schlecht. elicitors influencing their levels in pea seeds.

MATERIAL AND METHODS

The pea seeds were obtained from the harvests 1996 and 1997: cultivars Komet, Lantra and Menhir from Staňkov, Jaroměřice, Šumperk (1996, 1997), Chrlice (1996) and Žatec (1997) localities. The barley seeds were obtained from the harvest 1996 and 1997: cvs. Forum, Akcent and Amulet – from Branišovice, Krásné Údolí, Staňkov and Jaroměřice localities.

Accelerated ageing (AA): Seeds were weighed and placed on a screen tray that was inserted into an inner box containing 50 mL of water. The inner chamber was placed into an accelerated ageing chamber and the seeds were aged at 41 °C for 72 hours. The AA was performed after Te Krony (1985, 1995).

Preparation of extracts: Weighed ground seeds (approx. 25 g pea, or 20 g barley, resp.) 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 mL aliquots were pipetted for determination.

Samples of pea seedlings (cv. Komet) and barley seedlings (after 14 days cultivation) were obtained from the Department of Plant Protection of the Czech University of Agriculture in Prague.

From 1997 harvest sets of sterile water were performed, liquid media prepared by Prokinová: pea extract with glucose, asparagine medium, barley extract with glucose and potato extract with glucose. Concentration of glucose was 10 g in 1 L of medium, concentration of asparagine was 1 g in 1 L of medium. All these media were used in three variants as sterile control, filtrate after cultivation with *E. purpurascens* sterilised before application on seeds and as filtrate after cultivation with *E. purpurascens* (Table I). Volumes of media were 250 mL in each flask. Into every flask a disk of Sabourand cultivation agar (diameter 10 mm) inoculated with fungal mycelium was inserted. Cultivation was performed in dark at 24 °C for 14 days. After cultivation mycelium was centrifuged and medium filtered. One half of ob-

I. Influence of *E. purpurascens* on total polyphenol content in barley and pea seedlings and isoflavonoid content in pea seedlings in 1997

	Barley	Pea				
	TP	TP	isoflavonoids			
	(mg/100 g)	(mg/100 g)	(μg/g)			
	TP	TP	daidzein-4'	daidzein-7	genistein-4'	genistein-7
Sterile water	1271.3	245.1	2.2	7.0	0.6	2.7
Sterile water, filtrate after cultivation with *E. purpurascens* sterilised before application on seeds	1104.2	311.1	2.7	9.6	0.6	2.9
Sterile water, filtrate after cultivation with *E. purpurascens*	1492.8	343.1	1.6	2.6	0.4	1.4
Pea extract with glucose	932.7	306.2	9.3	25.4	1.6	8.8
Pea extract with glucose, filtrate after cultivation with *E. purpurascens* sterilised before application on seeds	745.2	391.1	5.2	36.3	1.6	11.2
Pea extract with glucose, filtrate after cultivation with *E. purpurascens*	1494.7	349.5	10.9	136.1	2.9	28.7
Asparagine medium	1013.5	278.1	6.4	42.7	2.2	10.3
Asparagine medium, filtrate after cultivation with *E. purpurascens* sterilised before application on seeds	1027.7	352.4	3.5	32.0	0.8	9.0
Asparagine medium, filtrate after cultivation with *E. purpurascens*	1125.8	427.7	7.0	50.0	2.1	8.7
Barley extract with glucose	1342.0	353.1	5.9	35.8	1.9	5,4
Barley extract with glucose, filtrate after cultivation with *E. purpurascens* sterilised before application on seeds	934.8	284.6	4.1	27.4	1.2	8.2
Barley extract with glucose, filtrate after cultivation with *E. purpurascens*	1008.1	275.5	5.9	66.1	1.3	8.4
Potato extract with glucose	890.5	361.1	3.5	21.6	1.1	4.1
Potato extract with glucose, filtrate after cultivation with *E. purpurascens* sterilised before application on seeds	1211.8	297.9	6.0	61.9	1.9	21.2
Potato extract with glucose, filtrate after cultivation with *E. purpurascens*	1060.1	276.5	3.0	28.0	1.2	7.8

tained filtrate was used immediately, the other part after sterilisation (100 °C, 152 kPa, 20 min). 10 mL of obtained filtrate with the content of secondary metabolites of fungus was aseptically inserted on filtration paper into Petri dishes (diameter 110 mm). In such way prepared dishes were inserted pea and barley seeds disinfected on their surface (for 1 min 5% $NaClO_2$, 3x washed with sterile water. After 3 days cultivation pea and barley seedlings were dried in a drying box at 50 °C for 20 hours and then the weighed ground 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 250 mL volumes in volumetric flasks.

Determination of isoflavonoid content was performed with radioimmunoassay method developed by Lapčík et al. (1996, 1997, 1998a, b, c). 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).

daidzein-7 crossreactivity

R_1=CH_3, R_2=H isoformononetin

R_1=glu, R_2=H daidzin

daidzein-4'crossreactivity

R_1=R_2=H daidzein

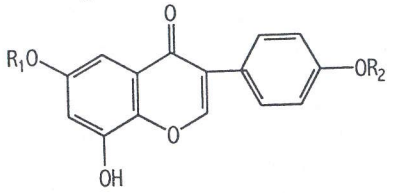
R_1=H, R_2=CH_3 formononetin

Immunoreactivities were measured either in crude extracts, or after chromatographic fractionation by HPLC (reversed phase, octadecylsilica). 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, resp., using diazomethane as the methylation agent. The pea extracts were fractionated either by TLC on silica or by ion exchange TLC on aminosilica.

HPLC: HPLC and TLC were performed after modified methods (Lapčík, 1998a, b, c). The HPLC system consisted of 1C-6A pump (Shimadzu, Japan), column oven 1CO100 and UV detector LCD 2082 (Ecom, Czech Republic),

fraction collector FC-203B (Gilson, France). ET 250/4 Nuleosil 100-5 C18 (Macheray – Nagel, Germany) column was used. Mobile phase A: 40% methanol in water, mobile phase B: 100% methanol. Gradient (all steps linearly): 0 min: A = 100%, B = 0%, 10 min: A = 80%, B = 20%, 25 min: A = 50%, B = 50%, 30 min: A = 0%, B = 100%, next 10 min: B = 100%, then step to A = 100%, reconditioning of the column 10 min. Flow rate: 1.0 mL/min, temperature 40 °C. UV detection at 254 nm.

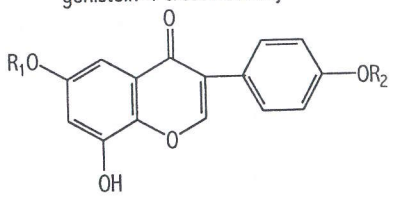
genistein-7 crossreactivity



R_1=CH_3, R_2=H prunetin

R_1=glu, R_2=H genistin

genistein-4´crossreactivity



R_1=H, R_2=CH_3 biochanin A

R_1=R_2=H genistein

TLC: Alugram Nano-Sil G/UV254 aluminium sheets Art. 818 Macherey – Nagel (Duren, Germany) were developed in dichlormethane-isopropanol 95 : 5 V/V. In ion-exchange TLC aluminium sheets NH_2F_{254S} from Merck (Darmstadt, Germany) were developed twice in the same system. After detection under UV the corresponding zones were eluted with ethanol and after evaporation in speedvac a redissolution in the assay buffer used for radioimmunoassay.

Determination of total polyphenols (TP): total polyphenols were determined with Folin-Ciocalteau's reagent (Lachman et al., 1997). Relative standard deviation of this method was about ±1.96 %rel.

Determination of catechol, resorcinol and phloroglucinol (CRP) type polyphenols: CRP polyphenols were determined with p-dimethylaminocinnamaldehyde (p-DMASA) according to Lachman et al. (1997). Relative standard deviation of this method was about ±2,5 %rel.

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

RESULTS AND DISCUSSION

There were investigated differences in TP and CRP contents in barley seeds and TP and isoflavonoid contents in pea seeds as influenced by variety, growing conditions and accelerated ageing. Influence of elicitors of *Epicoccum purpurascens* on the TP content in barley and TP and isoflavonoid contents in pea seedlings was also investigated.

In pea seeds there are significant differences in TP and isoflavonoid contents as influenced by variety, growing conditions and accelerated ageing (Tables II and III). The highest TP content has the variety Komet (58.16 mg/100 g in 1997, 82.91 mg/100 g in 1996), the lowest variety Menhir (45.94 mg/100 g in 1997, 63.56 mg/100 g in 1996). From the results obtained significant influence of the year of cultivation could be seen (average TP content in 1996 was 73.89 mg/100 g). As well as significant was the effect of the growing area (e.g. Staňkov locality 45.72 mg/100 g in 1997 and 72.03 mg/100 g in 1996 vs. Jaroměřice locality – 61.02 mg/100 g in 1997 and 73.19 mg/100 g in 1996). Accelerated ageing caused in pea increase of TP contents (in average by +28.41 %rel. in both years). Similar trend could be observed in isoflavonoid contents. The highest content was found for daidzein-7 activity (3.85 µg/g in 1997, 1.61 µg/g in 1996) followed with genistein-7 activity (1.13 µg/g in 1997 and 0.24 µg/g in 1996). For isoflavonoid contents were significant origin and variety effects and treatment only in several activities, as daidzein-4'and genistein-4'.

In barley seeds are significant influences of origin and variety on TP and CRP contents (Table IV). The highest TP content had cv. Forum (134.08 mg/100 g in 1996, 144.42 mg/100 g in 1997), the lowest cv. Amulet (109.00 mg/100 g in 1996, 144.42 mg/100 g in 1997). Among localities Krásné Údolí had the highest values. Accelerated ageing treatment influenced significantly only TP content in 1996 (average increase was +10.67 %rel.).

Results of influence of *E. purpurascens* on TP and isoflavonoid contents are summarised in Table I. In pea seeds elicitors of *E. purpurascens* have caused increase of TP content both in sterilised and unsterilised media: sterile water, pea extract with glucose and asparagine medium (increase +53.79 %rel. was the highest in asparagine unsterilised medium). Similarly elicitors of *E. purpurascens* have caused increase of TP contents in unsterilised filtrates after cultivation with *E. purpurascens* – sterile water, pea extract with glucose, asparagine medium and potato extract with glucose. The highest increase was in pea extract with glucose (+60.26 %rel.). No increase was observed in barley extract with glucose. In isoflavonoid content there could be observed increase in genistein-7, daidzein-7 and -4'crossreactivities in sterile water, pea extract with glucose and potato extract with glucose. The highest concentrations were found for daidzein-7 (average 26.50 µg/g in control) and genistein-7 (6.25 µg/g). The highest increase in comparison with control was found in pea extract with glucose for daidzein-7 (from 25.37 to 136.09 µg/g) and barley and potato extracts with glucose. High levels were found also for

II. Average values of polyphenol content in pea seeds in 1997

Locality	Variety	TP (mg/100 g)		Dai-4'(µg/g)		Dai-7 (µg/g)				Gen-7 (µg/g)	
		C	AA	C	AA	C	AA	C	AA	C	AA
AVG	Komet	58.16b	69.00NS	0.57b	0.64ab	4.09b	5.14ab	0.19ab	0.25a	0.84a	0.90a
AVG	Lantra	51.23ab	73.83	0.59b	0.76b	5.23c	6.55b	0.28b	0.35b	1.54b	2.05b
AVG	Menhir	45.94a	71.35	0.24a	0.57a	2.22a	3.33a	0.16a	0.25a	1.01a	1.68ab
Čáslav	AVG	58.98b	90.57c	0.69c	1.14c	6.87c	9.98b	0.35b	0.45b	2.07c	2.79c
Jaroměřice	AVG	61.02b	69.40b	1.24d	1.27c	9.43d	10.58b	0.45b	0.58b	1.88c	2.17bc
Staňkov	AVG	45.72a	73.21b	0.20b	0.54b	1.87b	3.38a	0.12a	0.20a	0.92b	1.46abc
Šumperk	AVG	51.83ab	67.76b	0.11ab	0.21a	0.83ab	1.38a	0.07a	0.11a	0.58ab	1.00ab
Žatec	AVG	41.35a	56.02a	0.07a	0.12a	0.235a	0.39a	0.05a	0.08a	0.20a	0.29a
Average		51.78a	71.39b	0.46a	0.66b	3.85a	4.95b	0.21a	0.28b	1.13NS	1.541NS
F-test	origin	11.675	36.931	452.262	113.191	222.372	40.074	56.087	67.329	97.216	7.420
Sg.l.		0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0017
F-test	variety	10.336	2.301	109.145	5.984	51.377	10.273	11.476	7.436	33.012	4.380
Sg.l.		0.0015	0.1344	0.0000	0.0123	0.0000	0.0018	0.0009	0.0057	0.0000	0.0318
F-test	treatment	106.637		26.927		12.162		22.19		5.430	
Sig.l.		0.0000		0.0000		0.0013		0.0000		0.0252	

III. Average values of polyphenol content in pea seeds in 1996

Locality	Variety	TP (mg/100 g)		Dai-4'(µg/g)		Dai-7 (µg/g)		Gen-4' (µg/g)		Gen-7 (µg/g)		Bioch-7 (µg/g)	
		C	AA	C	AA	C	AA	C	AA	C	AA	C	AA
AVG	Komet	82.91b	86.96NS	0.34c	0.66b	1.58b	1.75b	0.12NS	0.17NS	0.19a	0.18a	0.03b	0.03a
AVG	Lantra	75.21b	94.52	0.16b	0.30a	2.45c	2.29c	0.11	0.16	0.32b	0.33b	0.04b	0.05b
AVG	Menhir	63.56a	88.45	0.12a	0.30a	0.80a	0.81a	0.08	0.13	0.22ab	0.21a	0.02a	0.02a
Chrlice	AVG	64.80a	85.71ab	0.05a	0.13a	0.46a	0.66a	0.05a	0.06a	0.12a	0.20a	0.02a	0.03a
Jaroměřice	AVG	73.19a	80.46a	0.27c	0.46c	1.31b	1.43b	0.14b	0.16b	0.27b	0.22a	0.03b	0.03a
Staňkov	AVG	72.03a	95.11bc	0.19b	0.24b	1.15b	1.08b	0.08a	0.11ab	0.25ab	0.20a	0.03ab	0.04ab
Šumperk	AVG	85.55b	98.62c	0.32d	0.86d	3.16c	3.31c	0.15b	0.28c	0.33b	0.34b	0.03b	0.04b
Average		73.89a	89.98b	0.21a	0.42b	1.61NS	1.62NS	0.10NS	0.15NS	0.24NS	0.24NS	0.03NS	0.03NS
F-test	origin	13.411	8.197	111.366	851.803	142.666	201.721	21.372	31.89	9.221	8.615	4.714	7.848
Sg.l.		0.0004	0.0031	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0019	0.0025	0.0213	0.0037
F-test	variety	22.883	2.515	160.444	474.619	101.68	109.763	3.828	2.325	7.213	13.801	18.143	27.364
Sg.l.		0.0001	0.1224	0.0000	0.0000	0.0000	0.0000	0.0518	0.1401	0.0088	0.0008	0.0002	0.0000

IV. Average values of polyphenol content in barley seeds

Variety	Locality	1996				1997			
		TP (mg/100 g)		CRP (mg/100 g)		TP (mg/100 g)		CRP (mg/100 g)	
		C	AA	C	AA	C	AA	C	AA
AVG	Branišovice	125.88b	139.26NS	16.57a	15.35a	122.93a	131.97a	1.26	1.74
AVG	Jaroměřice	112.51a	128.47	27.57c	34.41d	127.41a	137.35b	6.83	4.05
AVG	Krásné Údolí	127.06b	132.68	24.56b	29.68c	159.72c	149.44c	3.37	4.22
AVG	Staňkov	120.02ab	136.84	27.93c	22.52b	152.23b	152.73c	5.22	2.43
Akcent	AVG	121.02b	130.42a	21.48b	25.62b	144.68b	139.81b	3.55	2.65
Amulet	AVG	109.00a	122.25a	18.92a	18.60a	132.61a	134.89a	5.89	5.19
Forum	AVG	134.08c	150.26b	31.93c	32.25c	144.42b	153.92c	3.05	2.71
Average		121.36a	134.31b	24.11NS	25.49NS	140.57NS	142.87	4.17	3.29
F-test	origin	7.664	1.167	100.242	542.834	247.617	174.104	102.664	23.26
Sig.l.		0.00097	0.3628	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
F-test	variety	36.277	14.306	231.85	484.112	47.752	234.489	54.557	45.222
Sig.l.		0.0000	0.0007	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

genistein-7 (from 8.80 to 28.69 μg/g in pea extract with glucose and from 4.06 to 21.17 μg/g in potato extract with glucose).

CONCLUSIONS

In deteriorated barley and pea seeds there were significant varietal and locality differences in total polyphenol contents. In pea seeds were significant differences in some isoflavonoid contents, esp. in daidzein-7 and genistein-7 activities. In barley seeds CRP content was also influenced with origin and variety. In pea seeds accelerated ageing significantly increased TP and isoflavonoid contents, in barley seeds this trend could be found only in TP content. Elicitors of *E. purpurascens* increased TP contents both in barley and pea seedlings after cultivation on sterile water, pea extract with glucose and asparagine medium obtained after incubation with *E. purpurascens*. In pea seedlings increase of genistein-7, daidzein-7 and -4'activities in sterile water, pea and potato extracts with glucose obtained after incubation with *E. purpurascens* was observed.

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Ovlivnění obsahu polyfenolů a isoflavonoidů v semenech a naklíčených semenech ječmene a hrachu jejich deteorací a elicitory houby *Epicoccum purpurascens* Ehrenb. ex Schlecht.

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Sekundární metabolity – polyfenolické látky – obsažené v semenech mají důležitou úlohu při kontrole dormance a klíčení, při snižování spotřeby kyslíku a při oxidační fosforylaci. Na třech kultivarech ječmene a třech kultivarech hrachu z různých lokalit byly v letech 1996–1997 sledovány změny v obsahu celkových polyfenolů (CP), polyfenolů typu resorcinolu, katecholu a floroglucinolu (RFK) a biologicky významných isoflavonoidů daidzeinu a genisteinu v obilkách ječmene a semenech hrachu po urychleném stárnutí. CP byly stanoveny s Folin-Ciocalteuovým činidlem, CRP s p-dimethylaminoskořicovým aldehydem (p-DMASA) a isoflavonoidy 7- a 4'-specifickým crossreaktivním radioimunoassayem. V semenech hrachu byly statisticky významné změny v obsahu celkových polyfenolů (CP) a isoflavonoidů ovlivněny odrůdou, podmínkami pěstování a urychleným stárnutím. Nejvyšší obsah CP vykazovala odrůda Komet (58,16 mg/100 g v roce 1997 a 82,91 mg/100 g sušiny v roce 1996), nejnižší odrůda Menhir (45,94 mg/100 g v r. 1997, 63,56 mg/100 g v r. 1996). Ze získaných výsledků vyplývá významný vliv ročníku (průměrný obsah CP byl v r. 1996 73,89 mg/100 g, v r. 1996 51,78 mg/100 g) a rovněž lokality (např. lokalita Staňkov 45,72 mg/100 g v r. 1997 a 72,03 mg/100 g v r. 1996 oproti lokalitě Jaroměřice – 61,02 mg/100 g v r. 1997 a 73,19 mg/100 g v r. 1996). Urychlené stárnutí způsobilo nárůst obsahu CP u hrachu (v průměru o +28,41 %rel.) v obou sledovaných ročnících. Podobné trendy byly zjištěny i v obsahu isoflavonoidů. Nejvyšší obsah byl nalezen u crossreaktivity 7-daidzeinu (3,85 µg/g v r. 1997, 1,61 µg/g v r. 1996) a 7-genisteinu (1,13 µg/g v r. 1997 a 0,24 µg/g v r. 1996). U obsahu isoflavonoidů byly statisticky významné původ a odrůda, urychlené stárnutí pouze u některých isoflavonoidů typu 4'-daidzeinu a 4'-genisteinu. V obilkách ječmene mají statisticky významný vliv na obsah CP a RFK původ a odrůda. Nejvyšší obsah CP měla odrůda Forum (134,08 mg/100 g v r. 1996, 144,42 mg/100 g v r. 1997), nejnižší odrůda Amulet (109,00 mg/100 g v r. 1996, 144,42 mg/100 g v r. 1997). Nejvyšší hodnoty vykazovala lokalita Krásné Údolí. Urychlené stárnutí ovlivnilo významně pouze obsah CP v roce 1996 (průměrný nárůst byl +10,67 %rel.). V semenech hrachu způsobily elicitory *E. purpurascens* zvýšení obsahu CP ve sterilizovaných a nesterilizovaných médiích: sterilizované vodě, hrachovém extraktu s glukózou a asparaginovém médiu (nejvyšší nárůst +53,79 %rel. byl v nesterilizované asparaginové živné půdě). Podobně elicitory *E. purpurascens* způsobily zvýšení obsahu CP v nesterilizovaných filtrátech po kultivaci s *E. purpurascens* – sterilizované vodě, hrachové živné půdě s glukózou, asparaginové půdě a bramborové živné půdě s glukózou. Nejvyšší nárůst byl v hrachové živné půdě s glukózou (+60,26 %rel.). U isoflavonoidů byl zjištěn nárůst u 7-genisteinové a 7- a 4'-daidzeinové aktivity ve sterilizované vodě, hrachové a bramborové živné půdě s glukózou. Nejvyšší koncentrace byly nalezeny u 7-daidzeinové (průměrně 26,50 µg/g u kontroly) a 7-genisteinové crossreaktivitě (6,25 µg/g). Nejvyšší nárůst oproti kontrole byl nalezen u hrachové živné půdy s glukózou pro 7-daidzein (z 25,37 na 136,09 µg/g) a ječné a bramborové živné půdy a také pro 7-genistein (z 8,80 na 28,69 µg/g u hrachové živné půdy s glukózou a z 4,06 na 21,17 µg/g u bramborové živné půdy s glukózou).

obilky ječmene; semena hrachu; odrůda; pěstební podmínky; polyfenoly; isoflavonoidy; daidzein; genistein; stanovení radioimunoanalýzou; deteorace; vliv elicitorů *Epicoccum purpurascens*

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