# CHANGES IN THE CONTENT OF POLYPHENOLS IN BARLEY GRAINS AND PEA SEEDS AFTER CONTROLLED ACCELERATED AGEING TREATMENT\*

J. Lachman, V. Pivec, V. Hosnedl

Czech University of Agriculture, Faculty of Agronomy, Prague, Czech Republic

Some of important secondary compounds of seeds are polyphenols which play role in the defense system and in the delicate mechanism of oxygen control and its access to seed embryo. In three barley and three pea cultivars from two different localities changes in total polyphenol content and catechol, resorcinol and phloroglucinol type polyphenols caused by accelerated ageing test with increased temperature and moisture were investigated. The total polyphenols were determined by means of Folin-Ciocalteau reagent and catechol, resorcinol and phloroglucinol type polyphenols with p-dimethylaminocinnamaldehyde. The process of deterioration caused significant increase both total polyphenols and catechol, resorcinol and phloroglucinol type polyphenols in barley grains and total polyphenols in pea seeds, too. There were great differences in total polyphenol contents in barley grains (103.88 mg in 100 g fresh matter) and in pea seeds (32.55 mg in 100 g fresh matter). Particularly high differences were in the catechol, resorcinol and phloroglucinol type of polyphenols, where these compounds are manifested in pea only in negligible amounts (0.717 mg/100g fresh matter) whereas in barley grains their average content was much higher (54.99 mg/100 g fresh matter). The increase of these compounds in barley grains and pea seeds after accelerated ageing treatment was comparable -37.22% rel. of total polyphenols in barley grains and 47.71% rel. in pea seeds, resp., the corresponding values of catechol, resorcinol and phloroglucinol type polyphenols were in barley grains 14.09% rel. and in pea seeds 10.52% rel., resp. There were significant differences in total polyphenols contents and catechol, resorcinol and phloroglucinol contents among barley and pea cultivars and localities where the plants were cultivated.

barley grains; pea seeds; total polyphenols; Folin-Ciocalteau reagent determination; m-dihydroxyl phenolic groups; p-dimethylaminocinnamaldehyde determination; accelerated ageing treatment; deteoriorated changes in polyphenols; varietal and environmental differences

<sup>\*</sup> This work was supported by the Grant Agency of the Czech Republic (Grant No. 521/96/0616).

## INTRODUCTION

The seeds of plants, predominantly their outer layer coats, contain different polyphenolic compounds, e.g. phenolic acids, coumarins, flavonoids, etc. Flavan-3-ols and flavan-3,4-diols as well as phenolic acids could interact and form high molecular weight compounds – tannins. Tannins are formed in tannin vacuoles or in the cytoplasm, or they may be deposited in cell walls.

Tannins are main substances in the chemical defense against seed predators, which range from large vertebrates and insects to fungi and microbes. Tannins form a complex heterogeneous group of phenol derivatives widely distributed in the plant body and are abundant in seed coats. Tannins, as antinutritional factors, have no direct toxic effect but decrease the digestibility of proteins and are difficult to metabolize (Boesenwinkel, Bouman, 1995). Other probable functions of tannins are protection against light, imparting colour to seeds (Chalker-Scott, Krahmer, 1989), restricting germination by hampering gas flow, and delay of the decomposition of seed coats in the soil.

Embryos or seed coats can be rich in the secondary defensive compounds such as alkaloids, amino acids, protease inhibitors, tannins, cyanogennic glycosides, etc. with a direct toxic effect on the living organisms. The seed coat covering of the English walnut embryo is rich in the toxic terpenoid juglone (Janzen, 1983). Several flavonoids in the *Medicago* seeds play an important role in the protection against some pathogenic fungi (Perez-Garcia et al., 1992).

Coats of numerous seeds contain large amounts of phenolic compounds (Bewley, Black, 1982; Côme, 1982), the oxidation of which by polyphenol oxidases reduces the oxygen supply to the embryo (Côme, 1982). In such seeds the inhibitory effect in their coats increases with temperature, since oxygen becomes less soluble and the oxidation of phenolic compounds becomes more intense. This phenomenon plays an important role in the regulation of germination by temperature (Côme, Corbineau, 1992). For example, in dormant barley seeds, oxygen fixed by the phenolic compounds in the glumellae corresponds to 28, 37 and 63% of the total oxygen uptake by the whole seeds at 10, 20, and 30 °C, respectively (Lenoir et al., 1983). Lecat et al. (1992) have shown with dormant oat seeds that gibberellic acid strongly improves germination. They suggest that this substance allows the germination at high temperatures because its germination is allowed even if it is poorly supplied with oxygen.

Polyphenol oxidases occur in most plant tissues, but especially in high concentrations are manifested in some plant tissues, e.g. potato tubers (Lachman et al., 1996). Polyphenol oxidases catalyze two different types

of reactions: the hydroxylation of monophenols to o-diphenols (EC 1.14.18.1., monophenol, dihydroxyphenylalanine: oxygen oxidoreductase or monophenol monooxygenase) and further the oxidation of o-diphenols to o-quinones (EC 1.10.3.1., 1,2-benzenediol: oxygen oxidoreductase) (Matheis, 1987a, b):

monophenol 
$$EC 1.14.18.1. + O_2 > o$$
-diphenol  $O$ -diphen

As it was already reported by Evenari (1949) many seeds, fruits, and other dispersal units contain inhibitors of germination or growth that belong among widely diversified chemical groups. Of phenolic compounds the phytotoxins are coumarins and furocoumarins, e.g. coumarin in *Trigonella arabica* (Lerner et al., 1959), 8-methoxypsoralen in *Ammi majus* (Friedman et al., 1982), heraclenol in *Petroselinium crispum* (Kato et al., 1978), momilactone in rice (Kato et al., 1977), flavonoids, e.g. myricetin in clover (*Trifolium repens*) (Fotrell et al., 1964), glucoflavonoids in beet (Kudrjavcev, 1979) and phenolic acids, e.g. ferulic acid in tomatoes (Akkerman, Veldstra, 1947) or abscisic acid in *Coryllus avelana* (Williams et al., 1973) or in *Fraxinus americana* (Sondheimer et al., 1968).

Phenolic acids have important allelopathic effects. Krogmeier and Bremner (1989) tested the effects of nine phenolic acids derived from crop residues (caffeic acid, chlorogenic acid, p-coumaric acid, ellagic acid, ferulic acid, syringic acid, and vanillic acid) on the seed germination and seedling growth of corn, barley, oats, rye, sorghum, wheat, and alfalfa.

Phenolic compounds act as potential antioxidants because they have the so called "scavenger effect" when they reduce the number of radicals (Richard-Forget et al., 1995). They inhibite in this way e.g. lipoxygenase (phenolic acids, gallates and flavonoids). They mostly have an uncompetitive inhibition activity – e.g. (-)-epicatechol reduces hydroperoxide formation by its radical scavenging activity and thus limits enzyme activation. The exception are flavonol aglycons which are of non-competitive type.

Ridenour et al. (1996) have studied germination and ageing in imbibed Moravian barley grains by solid state nuclear magnetic resonance techniques (<sup>1</sup>H NMR). Whereas magic-angle spinning <sup>1</sup>H NMR spectra reveal the water and lipid components in barley grains, combined rotation and multiple-pulse spectroscopy techniques provide <sup>1</sup>H NMR spectra of grains that reveal the protein and saccharide as well as the water and lipid components. <sup>1</sup>H NMR spectral comparison were made between normal viable grains and artificially aged grains. Oberthur et al. (1995) have investigated the dependence of seed dormancy in six-rowed barley cultivars on different factors and their

results support the statement that dormancy depends on environment, storage conditions, and complex genetic interactions (significant gene x environment and gene x time of postharvest ripening interactions were observed).

Drought is an important stress factor in plant tissues. It causes premature senescence of nodules and disturbs the delicate mechanisms of oxygen control that are essential for active nitrogen fixation (Sprent, 1981). Drought decreases the permeability of the oxygen diffusion barrier of the nodule cortex and thereby restricting oxygen access to the bacteroids. Very little is known about the effects of drought on the metabolism of activated oxygen. In respect to the high potential of nodules to form activated oxygen and their large demand for antioxidant protection to preserve nodule functioning, it seems that drought shifts the balance between formation and scavenging of activated oxygen, leading to oxidative stress. Gogorcena et al. (1995) have studied involvement of activated oxygen in the drought-induced damage of pea (Pisum sativum L. cv. Frilene) nodules, determining various pro-oxidant factors, antioxidant enzymes and relate metabolites, and markers of oxidative damage in nodules of well-watered (nodule water potential approximately -0.29 MPa) and water-stressed (nodule water potential approximately -2.03 MPa) plants. Water-stressed nodules have shown senescence – it causes a 30% decrease in leghemoglobin and total soluble protein, a decrease in the activities of catalase (25%), ascorbate peroxidase (18%), dehydroascorbate reductase (15%), glutathione reductase (31%), and superoxide dismutase (30%), and in the contents of ascorbate (59%), reduced (57%) and oxidized (38%) glutathione, NAD+ and NADH (43%), nADP+ (31%), and NADPH (17%) (Tab. I).

Also the composition of polyphenolic complex of barley seeds is showing on their strong antioxidant activity (Maillard et al., 1996). Depending on the variety, the antioxidant activity of barley is in relationship with the content of the three main phenolic groups – flavan-3-ols (more than 85%), hydroxycinnamic acids (approx. 10%) and flavones (less than 5% from the total content of polyphenol). The major antioxidant activity has flavan-3-ols.

In present study the influence of the accelerated deterioration test on the content of polyphenols and catechol, resorcinol and phloroglucinol type phenols in deteriorated and non-deteriorated barley grains and pea seeds regarding different cultivars and locality has been investigated.

# MATERIAL AND METHODS

The pea seeds were obtained from the harvest 1996: cultivars "ALAN" (great-sized seed), "KOMET" and "LANTRA" from the Chrlice and Staňkov

I. Comparative changes of antioxidant defenses and markers of senescence and oxidative stress caused by nitrate and drought or accelerated ageing test in pea nodules or seeds (Values are percent changes relative to control plants)

Parameter	Nitrate	Drought
Senescence marke	rs	
Nitrogenase	-98	n.d. <sup>+</sup>
Leghemoglobin	-57	-33 <sup>+</sup>
Soluble protein	-32	-31 <sup>+</sup>
Total lipids	0	-5 <sup>+</sup>
NAD <sup>+</sup>	-76	-43 <sup>+</sup>
NADH	-74	-44 <sup>+</sup>
NADP <sup>+</sup>	-44	-31 <sup>+</sup>
NADPH	-63	-17 <sup>+</sup>
Antioxidants		
Ascorbate	-31	-59 <sup>+</sup>
Glutathione	-48	-57 <sup>+</sup>
Ascorbate peroxidase	-46	-18+
Dehydroascorbate reductase	-28	-15 <sup>+</sup>
Malondialdehyde reductase	-17	0+
GSSG reductase	-22	-31 <sup>+</sup>
Catalase	-16	-25+
Pro-oxidants and oxidant	damage	
Low-molecular-mass Fe	+53	+102+
Catalytic Fe	+45	+++
Lipid peroxides	-21	+143**
Oxidized proteins	+37	+40*+
Total polyphenols	n.d.	+16.7***
Catechol, resorcinol, phloroglucinol polyphenols	n.d.	+5.5*++

<sup>&</sup>lt;sup>+</sup> Durand et al. (1987)

localities. The barley grains were obtained from the harvest 1996: cvs. "FO-RUM", "AKCENT" and "AMULET" – from the Chrlice and Krásné údolí localities.

<sup>++</sup> Gogorcena et al. (1995)

<sup>+++</sup> Lachman et al. (1995) - seeds, AAT, cv. "Bohatýr"

Accelerated ageing test (AAT): Seeds were weighed and placed on a screen tray which 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 AAT was performed after Te Krony (1985, 1995).

Determination of the total polyphenol content (TP): 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 of this volume 5 ml aliqouts were pipetted into 50 ml volumetric flasks. After dilution with 80% water-ethanolic solution to the approx. 30 ml, 2.5 ml of Folin-Ciocalteau reagent was added, agitated and mixed. Then 7.5 ml of 20% sodium carbonate solution was added, the volume adjusted with distilled water to the mark and after thorough agitation it was left 2 hours for a quantitative formation of blue colour. The same procedure was used for blank where instead of 5 ml of sample solution 5 ml 80% ethanol was used. After two hours standing the solutions were centrifugated on the Janetzki T 30 centrifuge at 2 000 cycles per minute for 12 minutes. Absorbancy values were determined on the Spekol 11 spectrophotometer against blank at 765 nm wave length and expressed as gallic acid.

Determination of m-dihydroxyl phenolic groups in catechol, resorcinol and phloroglucinol type polyphenols (CRP): 25 ml aliquots were evaporated on the water bath to dryness, then redissolved in 15 ml methanol, quantitatively displaced into 25 ml volumetric flasks. Into every flask 6 ml 3M HCl in methanol was added and after agitation the volume was adjusted to the mark. Then it was 1 ml of p-dimethylaminocinnamaldehyde (p-DMASA) reagent (Merck-Schuchardt, Hohenbrunn bei München) added and after thorough agitation the solution was left to stand for 30 min. Finally, the absorbancy on the Spekol 11 spectrophotometer was measured against blank at 638 nm wave length and expressed as phloroglucinol (sample C<sub>6</sub>C<sub>3</sub>(OH)<sub>3</sub>.2H<sub>2</sub>O, The British Drug House, Ltd.).

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

#### RESULTS AND DISCUSSION

One of the most important effects of the deterioration of seeds caused by water deprivation or by high temperature and seed moisture is the change in the oxygen availability to the embryo and oxygen solubility. The imbibed coats become a barrier to oxygen. In such seeds the inhibitory effects of the coats increase with temperature, since oxygen becomes less soluble and the

oxidation of phenolic compounds becomes more intense. The oxidation process could be observed on the oxidized forms of proteins (Gogorcena et al., 1995, Escuredo et al., 1996) as well as on the higher formation of phenolic compounds (Lachman et al., 1995). Oxidized proteins contain a higher number of total carbonyl groups due to oxidation of amino acids containing hydroxyls (L-serine, L-threonine, L-tyrosine). In the L-tyrosine molecule o-position can be oxidized regarding 4-OH group and further it could be oxidized to o-quinone form. Enhanced formation of L-tyrosine is due the oxidation of L-phenylalanine. The formation of the oxidized protein forms ensures also the formation and increase of the polyphenol content since amino acid L-tyrosine is a key metabolite in the biochemical process of the origin of phenolic compounds.

The increase of the number of hydroxyl groups determined with Folin-Ciocalteau reagent could be observed and a possibility of further oxidation reactions to o- and p-quinone forms (Escuredo et al., 1996). It was confirmed both – the increase of the number of total hydroxyl groups and the increase of the free m-dihydroxyl phenolic groups in catechol, resorcinol and phloroglucinol structures (Lachman et al., 1995). Tendency to higher formation of these compounds is dependent on variety and genus.

In the barley seeds antioxidant eficiency is caused mainly by flavan-3-ols and flavan-3,4-diols, resp. (more than 85 %), i.e. with gallocatechol and (-)-epicatechol and with leucoanthocyanidins of procyanidin and prodelphinidin type which could be converted by oxidation to anthocyanidins or condense to high molecular weight phlobaphene and condensed tannin fractions. Gallocatechol could originate by the (-)-epicatechol oxidation (hydroxylation), prodelphinidin from procyanidin. 10% of the total polyphenol content form phenolcarboxylic acids: p-hydroxybenzoic acid (its hydroxylation leads to gallic acid and esterification to m-galloylgallic acid), vanillic acid, o-hydroxycinnamic acid, ferulic acid, sinapic acid and chlorogenic acid.

Pea seeds contain stepwise oxidized flavone and flavonol glycosides and pterocarpans.

The barley grains contain in the main higher TP content (103.88 mg in 100 g fresh matter) in comparison with the average content in the pea seeds (32.55 mg in 100 g fresh matter) where their content forms about one third of the barley content (Tabs. II and III). Especially high difference is in the catechol, resorcinol and phloroglucinol type polyphenol contents where their share in the barley grains is nearly half – 52.93% (54.99 mg in 100 g fresh matter) from the total polyphenol content whereas their content in pea seeds is negligible (0.717 mg in 100 g fresh matter, i.e. 2.20% from the total polyphenol content). These results also confirm the statement that flavan-3-ols form the dominant group of the barley polyphenolic complex (Maillard et al., 1996).

22

II. The total polyphenol content (TP) and the catechol, resorcinol and phloroglucinol type polyphenol content (CRP) in barley grains of different cultivars and localities

	TP content	TP content	CRP content	CRP content
Sample (Cultivar)	mg/100 g fresh matter	mg/100 g dry matter	mg/100 g fresh matter	mg/100 g dry matter
FORUM <sup>+</sup>	84.03	92.45	52.15	57.37
FORUM DT <sup>+</sup>	139.49	153.19	61.59	67.64
AKCENT <sup>+</sup>	104.39	114.85	57.20	62.93
AKCENT DT <sup>+</sup>	133.36	146.46	69.29	76.10
AMULET*	76.91	84.62	43.88	48.28
AMULET DT <sup>+</sup>	131.88	144.83	53.78	59.06
FORUM <sup>++</sup>	109.61	120.55	74.47	81.91
FORUM DT <sup>++</sup>	147.61	161.91	74.98	82.24
AKCENT <sup>++</sup>	125.97	138.42	56.53	62.12
AKCENT DT <sup>++</sup>	147.87	162.20	58.53	64.20
AMULET**	122.38	134.47	45.69	50.20
AMULET DT <sup>++</sup>	129.73	142.18	54.14	59.34

<sup>&</sup>lt;sup>+</sup> Krásné údolí locality (647 m above sea level)

DT - deteriorated grains

24

Accelerated ageing test by means of influence of higher moisture and temperature in all investigated samples recorded a significant increase of TP and CRP contents. This increase was in the barley grains and pea seeds comparable – an average increase of TP in barley grains was 37.22% rel. and in pea seeds 47.71% rel., for CRP the corresponding values were 14.09 or 10.52% rel. (Tabs. IV and VI). High levels of significance were confirmed by Tukey's HSD test (Tabs. IV and V).

All results when calculated for dry matter have shown the same tendency as those calculated for fresh matter.

Both in the pea seeds and in the barley grains apparent varietal differences in TP and CRP contents were detected – e.g. the barley cvs."FORUM" contained 96.82 mg TP /100 g and "AKCENT" 115 mg/100 g, the pea cvs. "KOMET" 31.72 mg/100 g and "LANTRA" 33.56 mg/100 g. In comparison with the influence of environmental conditions and localities differences among cultivars were lower. In the barley samples the average TP content from the Krásné údolí locality was 88.44 mg/100 g and from the Chrlice

III. The total polyphenol content (TP) and the catechol, resorcinol and phloroglucinol type polyphenol content (CRP) in pea seeds of different cultivars and localities

Cample	TP content	TP content	CRP content	CRP content
Sample (Cultivar)	mg/100 g fresh matter	mg/100 g dry matter	mg/100 g fresh matter	mg/100 g dry matter
ALAN <sup>++</sup>	27.11	29.37	0.77	0.84
ALAN DT <sup>++</sup>	49.16	53.18	0.80	0.86
KOMET <sup>++</sup>	29.66	32.13	0.88	0.95
KOMET DT <sup>++</sup>	61.85	66.91	0.89	0.97
LANTRA <sup>++</sup>	35.89	39.00	0.84	0.92
LANTRA DT <sup>++</sup>	38.23	41.32	1.22	1.31
ALAN+++	37.66	40.92	0.54	0.58
ALAN DT+++	37.46	40.49	0.54	0.58
KOMET***	33.77	36.61	0.55	0.60
KOMET DT***	37.04	40.03	0.62	0.67
LANTRA***	31.22	33.85	0.72	0.78
LANTRA DT <sup>+++</sup>	56.26	60.82	0.73	0.79

<sup>++</sup> Chrlice locality (190 m above sea level)

DT - deteriorated seeds

IV. The average total polyphenol content (TP) and the catechol, resorcinol and phloroglucinol type polyphenol content (CRP) in barley grains and pea seeds in the control and after AAT

Crop	Average TP content	Average CRP content
Стор	mg/100 g fresh matter	mg/100 g fresh matter
Barley	103.88 a	54.99 a
Barley DT	138.32 b	62.05 b
Pea	32.55 a	0.72 NS
Pea DT	46.67 b	0.80 NS

Significance levels: values with different letters significantly differ at 0.05 according to Tukey's HSD test; NS – no significance

locality 119.32 mg/100 g, for the CRP contents the corresponding values were 51.078 and 58.90 mg/100 g, resp. Similarly, in the pea samples the average TP content from the Chrlice locality was 30.89 mg/100 g and from

<sup>++</sup> Chrlice locality (190 m above sea level)

<sup>+++</sup> Staňkov locality (370 m above sea level)

V. The effect and significance of locality and cultivar on the TP and CRP contents in the barley grains and pea seeds

Locality	Pea	Pea	Locality	Barley	Barley
Staňkov	0.60 a	34.22 NS	Krásné údolí	51.08 a	88.44 a
Chrlice	0.83 b	30.87	Staňkov	58.90 b	119.32 b
Cultivar			Cultivar		
Alan	0.65 NS	32.38 NS	Amulet	44.79 a	99.65 NS
Komet	0.72	33.55	Forum	63.31 b	96.82
Lantra	0.78	31.75	Akcent	56.87 b	115.18

VI. The relative increase of total polyhenol content (TP) and catechol, resorcinol and phloroglucinol type polyphenols (CRP) in deteriorated seeds in comparison with the control

Sample	TP increase (% rel.)	CRP increase (% rel.)
Barley FORUM <sup>+</sup>	66.01	18.12
Barley AKCENT <sup>+</sup>	27.75	21.15
Barley AMULET <sup>+</sup>	71.47	22.54
Barley FORUM++	34.67	0.68
Barley AKCENT <sup>++</sup>	17.39	3.54
Barley AMULET++	6.00	18.51
Barley average values	37.22	14.09
Pea ALAN <sup>++</sup>	81.36	2.71
Pea KOMET <sup>++</sup>	108.53	1.59
Pea LANTRA <sup>++</sup>	6.50	44.30
Pea ALAN+++	-0.54	0.00
Pea KOMET+++	9.66	12.73
Pea LANTRA+++	80.22	1.81
Pea average values	47.62	10.52

<sup>&</sup>lt;sup>+</sup> Krásné údolí locality (647 m above sea level)

the Staňkov locality 34.22 mg/100 g, the corresponding values for the CRP contents were 0.83 and 0.60 mg/100 g. Significance of the influence of locality was higher when compared with the effect of cultivar.

AKKERMAN, A. M. – VELDSTRA, H.: The chemical nature of Knoekemann's blastocholine from *Lycopersicum esculentum* Mill. Recu. Trav. Chimiques du Pays-Bas, *66*, 1947: 411–412. BEWLEY, J. D. – BLACK, M.: Physiology and biochemistry of seeds in relation to germination. Berlin, Springer Verlag 1982.

BOESENWINKEL, F. D. – BOUMAN, F.: Seed structure and function. In: KIGEL, J. – GALILI, G. (eds.): Seed Development and Germination. N. Y., Basel, Hong Kong, Marcel Dekker, Inc. 1995: 1–24.

CHALKER-SCOTT, L. – KRAHMER, R. L.: Microscopic studies of tannin formation and distribution in plant tissues. In: HEMMINGWAY, R. W. – KARCHESY, J. J. – BRAUHAM, S. J. (eds.): Chemistry and Significance of Condensed Tannins. N. Y., London, Plenum Press 1989: 345–368.

CÔME, D.: Germination. In: MAZLIAK, P. (ed.): Croissance et développement. Physiologie Végétale II. Paris, Hermann 1982: 129–226.

CÔME, D. – CORBINEAU, F.: Environmental control of seed dormancy and germination. In: JIARUI, F. – KHAN, A. A. (eds.): Advances in the Science and Technology of Seeds. Beijing, N. Y., Science Press 1992: 288–298.

DAVÍDEK, J. – HRDLIČKA, J. – KARVÁNEK, M. – POKORNÝ, J. – SEIFERT, J. – VELÍŠEK, J.: Laboratorní příručka analýzy potravin (Laboratory manual of food analysis). Praha, SNTL 1977.

DURAND, J. L. – SHEENY, J. E. – MINCHIN, F. R.: Nitrogenase activity, photosynthesis and nodule water deprivation. J. Exp. Bot., 38, 1987: 311–321.

ESCUREDO, P. R. – MINCHIN, F. R. – GOGORCENA, Y. – ITURBE-ORMAETXE, I. – KLUCAS, R. V. – BECANA, M.: Involvement of activated oxygen in nitrate-induced senescence of pea root nodules. Plant Physiol., 110, 1996: 1187–1195.

EVENARI, M.: Germination inhibitors. Botanical Review, 15, 1949 (3): 153-194.

FOTTRELL, P. F. – O'CONNOR, S. – MASTERSON, C. L.: Identification of flavonol myricetin in legume seeds and its toxicity to nodule bacteria. Ir. J. Agric. Res., 3, 1964: 246–249.

FRIEDMAN, J.: Allelopathy, autotoxicity, and germination. In: KIGEL, J. – GALILI, G. (eds.): Seed Development and Germination. N. Y., Basel, Hong Kong, Marcel Dekker, Inc. 1995: 629–644.

FRIEDMAN, J. – RUSHKIN, E. – WALLER, G. R.: Highly potent germination inhibitors in aqueous eluate of fruits of Bishop's Weed (*Ammi majus* L.) and avoidance of autoinhibition. J. Chem. Ecol., 1982 (8): 55–65.

GOGORCENA, Y. – ITURBE-ORMAETXE, I. – ESCUREDO, P. R. – BECANA, M.: Antioxidant defenses against activated oxygen in pea nodules subjected to water stress. Plant Physiol., 108, 1995: 753–759.

JANZEN, D. H.: Physiological ecology of fruits and their seeds. In: LANGE, O. L. – NOBEL, P. S. – OSMOND, C. B. – ZIEGLER, H. (eds.): Physiological Plant Ecology. Berlin, Heidelberg, Springer Verlag 1983.

KATO, T. – KOBAYASHI, M. – SASAKI, N. – YOSHIO, K. – NONINDO, T.: The coumarin heraclenol as growth inhibitor in parsley seeds. Phytochemistry, *17*, 1987: 158–159.

<sup>++</sup> Chrlice locality (190 m above sea level)

<sup>+++</sup> Staňkov locality (370 m above sea level)

KATO, T. – TSUNAKAWA, M. – SASAKI, N. – AIZA, H. – FUKITA, H. – KITAHANA, Y. – TAKAHASHI, N.: Growth and germination inhibitors in rice husks. Phytochemistry, *16*, 1977: 45–58.

KROGMEIER, M. J. – BREMNER, J. M.: Effects of phenolic acids on seed germination and seedling growth in soil. Biol. Fertil. Soils, 8, 1989: 116–122.

KUDRJAVCEV, G. R.: Relation between the germination of sugar beet seeds and their apigenin glucoflavonoids. Dokl. Akad. Nauk SSSR, 23, 1979: 849–851.

LACHMAN, J. – PIVEC, V. – ORSÁK, M.: Polyphenols and enzymic oxidative browning of potatoes regarding their quality. In: Proc. Symp. Chemical Reactions in Foods III, Prague, 25–27 September 1996: 17–20.

LACHMAN, J. – PIVEC, V. – TÁBORSKÝ, J.: Polyfenolické sloučeniny hrachu setého (*Pisum sativum* L.) ve vztahu ke kvalitě osiva (Polyphenolic compounds of pea as related to the seed quality). Zpráva pro projekt GAČR 503/93/0213 "Biologické a fyzikální vlastnosti osiva – agronomický význam" (Report for the project GACR 503/93/0213 "Biological and physical properties of seed – agronomic importance"). 1995. 18 p.

LECAT, S. – CORBINEAU, F. – CÔME, D.: Effects of gibberelic acid on the germination of dormant oat (*Avena sativa* L.) seeds as related to temperature, oxygen and energy metabolism. Seed Sci. Technol., 20, 1992: 421–433.

LENOIR, C. – CORBINEAU, F. – CÔME, D.: Rôle des glumelles dans la dormance des semences d'orge. Physiol. Vég., 21, 1983: 633–643.

LERNER, H. R. – MAYER, A. M. – EVENARI, M.: The nature of germination inhibitors present in dispersal units of *Zygophyllum dumosum* and *Trigonella arabica*. Physiol. Plant., *12*, 1959: 245–250.

MAILLARD, M. N. – SOUM, M. H. – BOIVIN, P. – BERSET, C.: Antioxidant activity of barley and malt: relationship with phenolic content. Lebensm.-Wiss. Technol., 29, 1996: 238–244.

MATHEIS, G.: Polyphenol oxidase and enzymatic browning of potatoes (*Solanum tuberosum*). I. Chem. Mikrobiol. Technol. Lebensm., 11, 1987a: 5–12.

MATHEIS, G.: Polyphenol oxidase and enzymatic browning of potatoes (*Solanum tuberosum*). II. Enzymatic browning and potato constituents. Chem. Mikrobiol. Technol. Lebensm., 11, 1987b: 33–41.

OBERTHUR, L. – BLAKE, T. K. – DYER, W. E. – ULLRICH, S. E.: Genetic analysis of seed dormancy in barley (*Hordeum vulgare* L.). J. Quant. Trait Loci (Electronic Publication), 1995, Avail. URL: http://probl.nalusda.gov:8000/otherdocs/jqtl/jqtl 1995-05/ dormancy.html.

PEREZ-GARCIA, F. – CERESUELA, L. – GONZALES, A. E. – AGUINAGALDE, I.: Flavonoids in seed coats of *Medicago arborea* and *M. strasseri* (*Leguminosae*). Ecophysiological aspects. J. Basic Microbiol., *32*, 1992: 241–248.

RICHARD-RORGET, F. – GAUILLARD, F. – HUGUES, M. – JEAN-MARC, T. – BOIVIN, P. – NICOLAS, J.: Inhibition of horse bean and germinated barley lipoxygenase by some phenolic compounds. J. Food Sci., 60, 1995: 1325–1329.

RIDENOUR, C. F. – XIONG, J. – MACIEL, G. E.: Investigation of germination and aging in Moravian III barley grain by nuclear magnetic resonance. Biophys. J., 70, 1996: 511–531.

SONDHEIMER, E. – TZOU, D. S. – GALSTON, E. C.: Abscisic acid levels and seed dormancy. Plant Physiol., 43, 1968: 1443–1447.

SPRENT, J. I.: Nitrogen fixation. In: PALEG, L. G. – ASPINALL, D. (eds.): The Physiology and Biochemistry of Drought Resistance in Plants. Sidney, Australia, Academic Press 1981: 131–143.

TE KRONY, D. M.: An evaluation of the accelerated ageing test for soybeans. Assoc. Official Seed Analysts Newsletter, 59, 1985 (1): 96.

TE KRONY, D. M.: Accelerated ageing test ISTA. Seed Vigor Testing Handbook. AOSA, 1995. WILLIAMS, P. M. – ROSS, J. D. – BRADBEER, J. W.: Studies in seed dormancy VII. The abscisic acid content of seeds and fruits of *Coryllus avellana* L. Planta, *110*, 1973: 303–310.

WITTY, J. F. – MINCHIN, F. R. – SKOT, L. – SHEEHY, J. E.: Nitrogen fixation and oxygen in legume root nodules. Oxf. Surv. Plant Mol. Cell Biol., 1986 (3): 275–314.

Received for publication on February 13, 1997

LACHMAN, J. – PIVEC, V. – HOSNEDL, V. (Česká zemědělská univerzita, Agronomická fakulta, Praha, Česká republika):

Změny obsahu polyfenolů v obilkách ječmene a semenech hrachu po testu urychleného stárnutí.

Scientia Agric. Bohem., 28, 1997 (1): 17-30.

Polyfenolické látky obsažené v semenech, především v jejich obalových vrstvách, jsou důležitou skupinou sekundárních metabolitů, které hrají významnou roli v obranném systému a jemném mechanismu přístupu kyslíku k embryu, neboť jejich oxidace polyfenoloxidázami redukuje zásobování embrya kyslíkem. Inhibiční účinek se zvyšuje s teplotou, neboť kyslík se stává méně rozpustným a oxidace fenolických sloučenin je intenzivnější. Na třech kultivarech ječmene a třech kultivarech hrachu vždy ze dvou různých lokalit byly sledovány změny v obsahu celkových polyfenolů (TP) a m-dihydroxylových fenolických skupin polyfenolů typu katecholu, resorcinolu a floroglucinolu (CRP) v obilkách ječmene a semenech hrachu po ošetření testem urychleného stárnutí, kdy bylo na semena působeno zvýšenou teplotou a vlhkostí. Celkové polyfenoly byly stanoveny Folin-Ciocalteuovým činidlem a m-dihydroxylové fenolické skupiny polyfenolů typu katecholu, resorcinolu a floroglucinolu p-dimethylaminoskořicovým aldehydem. Obilky ječmene a semena hrachu se značně liší v obsahu polyfenolických látek – 103,88 mg ve 100 g obilek ječmene a 32,55 mg ve 100 g semen hrachu. Zvláště velké rozdíly byly v obsahu polyfenolů typu katecholu, resorcinolu a floroglucinolu, kdy obilky ječmene obsahovaly průměrně 54,99 mg, zatímco semena hrachu obsahovala pouze nepatrné množství (0,72 mg ve 100 g semen). Tyto hodnoty potvrzují vysokou antioxidační účinnost obilek ječmene, která je způsobena především flavan-3-oly a flavan-3,4-dioly (gallokatecholem, (-)-epikatecholem, prokyanidinem a prodelfidinem). Deteorační ošetření zvýšenou vlhkostí a teplotou způsobilo ve všech případech průkazný nárůst obsahu celkových polyfenolů a u ječmene také m-dihydroxylových fenolických skupin CRP. Nárůst obsahu těchto látek byl u ječmene i hrachu srovnatelný – 37,22 % rel. u celkových polyfenolů v obilkách ječmene a 47,71 % rel. v semenech hrachu. Pro polyfenoly typu

katecholu, resorcinolu a floroglucinolu byly odpovídající hodnoty 14,09 % rel. a 10,52 % rel. Jak v semenech hrachu, tak i v obilkách ječmene byly nalezeny meziodrůdové a mezilokalitní rozdíly v obsahu celkových polyfenolů i polyfenolů typu katecholu, resorcinolu a floroglucinolu. Např. u ječmene kultivar "FORUM" obsahoval 96,82 mg celkových polyfenolů/100 g a kultivar "AKCENT" 115 mg celkových polyfenolů/100 g, u kultivarů hrachu "KOMET" byla tato hodnota 31,72 mg/100 g a "LANTRA" 33,56 mg/100 g. Ve srovnání s vlivem podmínek prostředí a lokalit byly meziodrůdové rozdíly nižší. U vzorků ječmene byl průměrný obsah celkových polyfenolů z lokality Krásné údolí 88,44 mg/100 g a z lokality Chrlice 119,32 mg/100 g. Pro polyfenoly typu katecholu, resorcinolu a floroglucinolu byly odpovídající hodnoty 51,08 a 58,90 mg/100 g. Podobně u vzorků hrachu byl průměrný obsah celkových polyfenolů z lokality Chrlice 30,89 mg/100 g a z lokality Staňkov 34,22 mg/100 g. Pro katecholy, resorcinoly a floroglucinoly byly odpovídající hodnoty 0,83 a 0,60 mg ve 100 g semen.

obilky ječmene; semena hrachu; celkové polyfenoly; stanovení s Folin-Ciocalteuovým činidlem; m-dihydroxylové fenolické skupiny; stanovení s p-dimethylaminoskořicovým aldehydem; test urychleného stárnutí; deteorační změny; meziodrůdové rozdíly; vliv lokality

## Contact Address:

Doc. Ing. Jaromír Lachman, CSc., Česká zemědělská univerzita, Agronomická fakulta, Kamýcká 129, 165 21 Praha 6-Suchdol, Česká republika, tel.: 02/24 38 27 20, fax: 02/20 92 03 12