

## **THE BIOLOGICAL ACTIVITY OF WHEAT, RYE AND TRITICALE VARIETIES HARVESTED IN FOUR CONSECUTIVE YEARS**

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**Abstract.** The effect of the four consecutive crop years including precipitation on the selected biological activities in the three cereal grains genus each two varieties Lama and Puma-wheat, Motto and Amilo-rye, Presto and Vero-triticale was the aim of this study. In the analysed cereals total protein content, extractable protein content, amylolytic activity and anti-amylolytic and antitryptic activity against enzymes from mammalian and insect origin were estimated. Precipitation statistically significantly affected only endogenous amylolytic activity and inhibitory activities against hog pancreas  $\alpha$ -amylase of studied cereals. The analysis of variance has shown statistically significant influence of variety, genus, and the year of harvest as well as interaction between them on the inhibitory activities against *S. granarius* and *T. confusum*  $\alpha$ -amylase and antitryptic activity of cereal seeds studied. However, precipitation did not statistically significantly affect inhibitor activities against all the studied  $\alpha$ -amylase insects.

**Key words:** wheat, rye and triticale seeds, precipitation, extractable proteins, biological activities

### **INTRODUCTION**

Cereal seeds contain biologically active proteins [Muralikrishna and Nirmala 2005, Svensson et al. 2004] which are mainly soluble in water and belong to albumins [Deponte et al. 1976]. Among them are amylases and inhibitors of different enzymes. Biological activities found in cereal grain play an important function from the biochemical, physiological, nutritional and technological point of view [Muralikrishna and Nirmala 2005, Piasecka-Kwiatkowska and Warchalewski 2000 a, b]. Cereal amylases play a significant role in seed germination and maturation as well as starch digestion in organism resulting in the formation of sugars that can be utilized for metabolic activities [Muralikrishna and Nirmala 2005]. When the action of the amylases is decreased by

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inhibitors, nutrition of the organism is impaired causing shortness in energy [Warchalewski 1983]. Therefore, the physiological functions of these inhibitors are as follows: the role in controlling endogenous alpha-amylase activity, possible regulation effect of starch metabolism and regulation of the osmotic pressure via association and dissociation of the inhibitors in response to the varying conditions during the different developmental stages of wheat seeds [Warchalewski 1983, Svensson et al. 2004]. In addition, alpha-amylase inhibitors occur in many plants as part of the natural defence mechanism against insect pests [Carlini et al. 2002, Franco et al. 2002, Oppert et al. 2004]. They are particularly abundant in cereals [Iulek et al. 2000, Svensson et al. 2004] and legumes [Bonavides et al. 2007]. Some results indicate that  $\alpha$ -amylase inhibitors in cereal seeds could be involved in the resistance of grain to post harvest insect infestation [Oppert et al. 2004, Warchalewski and Nawrot 1993]. However, extremely high active insect alpha-amylase inhibitors in the case of *S. granarius*, *T. confusum* and *E. kuehniella* appear to have a limited influence on the developmental parameters studied, although some reduction of insects population might be expected [Warchalewski et al. 2002]. On the other hand, amylase inhibitors are of great interest as potentially important tools of natural and engineered resistance against insect pests in transgenic plants [Carlini et al. 2002].

Wheat, rye and triticale differ in appearance, taste, structure and also in nutritional properties. These properties strongly depended on both the genotype (genus and variety) and agro-environmental conditions (soil, climate, tillering and fertilisation). Whereas the genetically determined influence of the variety (genotype) is concerned with the structure as well as with the quantity of proteins, the influence of growing conditions is based only on quantitative effects [Wieser and Seilmeier 1998]. Only limited results are available for changes in biological activities among the varieties of cereal genus affected by agro-environmental conditions [Warchalewski et al. 2003]. Therefore, the object of this study was to determine the effect of four consecutive crop years including precipitation on selected biological activities in of wheat, rye and triticale grain.

## MATERIAL AND METHODS

The study was carried out on six winter varieties of cereal seeds grown for four consecutive years on experimental plots of the Breeding Station DANKO, Choryń Poland. For the experiment three cereal genus each of two varieties were selected: Lama and Puma (wheat), Motto and Amilo (rye), Presto and Vero (triticale).

Fertilization conditions during all four years were slightly different for wheat comparing to rye and triticale (wheat: 100 kg/ha N, 85 kg/ha P<sub>2</sub>O<sub>5</sub>, 125 kg/ha K<sub>2</sub>O, rye and triticale 75 kg/ha N, 86 kg/ha P<sub>2</sub>O<sub>5</sub>, 125 kg/ha K<sub>2</sub>O).

Total precipitation (rainfall) in consecutive years of harvest was as follow: 448, 575, 407 and 578 mm in the first, second, third and fourth year crops respectively.

None of the studied cereal grain has shown any visible sprouting in all four year crops. Grain moisture content was determined according to AACC Method 44-19/1982. Crude protein content of grain was determined according to ICC standard No 105/1-1980 with the use of Digestion System 20 and Kjeltac System 1003, Distilling Unit-Tecator AB, Sweden and calculating index K for: wheat 5.70, triticale 5.83 and rye

6.25. The falling number of grain ground into whole flour was determined according to the ICC Standard No 107/1 – 1995.

To obtain biologically active proteins from cereal grains three steps of water extraction in three independent runs were used as reported earlier [Warchalewski et al. 1997].

The following were determined in the extracts:

- Extractable protein content according to the Lowry method [Lowry et al. 1951].
- Amylolytic activity (combined alpha- and beta-amylase) according to Bernfeld method [1955] as modified by Warchalewski and Tkachuk [1978].
- Inhibitory activities of cereal grain extractable proteins against exogenous  $\alpha$ -amylases of human saliva, hog pancreas, *Sitophilus granarius* L. – imago, *Tribolium confusum* Duv. – larva, *Ephestia kuehniella* Zell. – young larva were measured according to the modified Bernfeld method as described by Warchalewski et al. [1989].
- Inhibitory activity against bovine pancreas trypsin according to Nomoto and Narahashi method [1959] as modified by Warchalewski and Skupin [1973].

All biochemical determinations were conducted in duplicate. Statistical interdependence among years of harvest, genus and varieties were estimated by one-, two- and three-way analysis of variance (ANOVA) test at the 5% level ( $\alpha = 0.05$ ) of significance of all parameters evaluated. In addition correlation coefficients were calculated between statistically significant dependent parameters at the 5% level ( $\alpha = 0.05$ ) of significance.

## RESULTS AND DISCUSSION

The basic characteristics of the studied cereal grain has been shown in Table 1. The fertilization doses given in four consecutive years of harvest were only slightly different and depended on the requirements of cereal genus and soil type. The doses of nitrogen were higher by 25 kg/ha in the case of wheat varieties comparing to rye and triticale while phosphorus and potassium fertilization were almost the same for cereals grown in all four year. Wieser and Seilmeier [1998] reported that quantities of wheat albumins and globulins were scarcely influenced by different nitrogen fertilization, whereas those of gluten proteins (gliadins and glutenins) were strongly influenced. During the four year growing period conditions, monthly rain precipitation could influence biological activities studied. Therefore, the rainfall during the four year growing period was recorded. Detailed monthly precipitation was given in Table 2. According to Kent and Evers [1994] an annual rainfall of 229-762 mm which fall more in spring than in summer, suits the best grain quality. In the case of our study precipitation (March to June) was as follow: 197.1, 269.2, 157.3 and 209.9 mm in four consecutive years of harvest respectively. However, the last two months (June, July) before harvest seems to be crucial for kernels filling. The highest total rain precipitation of 281.5 mm was noted in June/July just before 4 year crop was collected (Table 2). As can be seen in Table 1, in this year wheat, rye and triticale grain showed higher level of crude protein content and lower values of falling number (Table 1). On the other hand, the extremely low rain precipitation 7.8 mm in July of the second year of growing (Table 2) induced in wheat varieties the lowermost total crude protein and the highermost extractable protein contents (Table 1 and 3). The highest 269.2 mm rain precipitation recorded in spring time

Table 1. The basic characteristics of cereal grains studied  
 Tabela 1. Charakterystyka podstawowa badanego ziarna

Year crop Rok zbioru	Cereal variety Odmiana zboża	Moisture content Wilgotność %	Crude protein content % d.wt. Białko surowe % s.s.	Falling number Liczba opadania s
Wheat – Pszenica				
1	Lama	12.8	11.26	264
	Puma	13.2	12.60	334
2	Lama	12.4	10.94	334
	Puma	12.6	9.98	353
3	Lama	12.4	13.6	384
	Puma	12.6	15.56	386
4	Lama	12.4	14.48	282
	Puma	12.6	13.75	284
Rye – Żyto				
1	Motto	12.6	10.48	216
	Amilo	13.1	12.02	284
2	Motto	12.7	10.36	331
	Amilo	12.2	10.21	353
3	Motto	12.7	9.79	276
	Amilo	12.8	9.89	326
4	Motto	12.6	13.90	152
	Amilo	13.1	10.23	289
Triticale – Pszenżyto				
1	Presto	12.9	14.08	68
	Vero	12.8	13.28	88
2	Presto	13.1	10.20	242
	Vero	12.6	10.96	292
3	Presto	12.5	11.06	151
	Vero	12.7	10.85	242
4	Presto	12.5	13.40	62
	Vero	12.6	16.02	62

of second year of cereal grain grown resulted in statistically significant increase of biological activities studied irrespective of the cereal genotype (Table 3). The different extractable proteins content, mostly albumins, ranging from 1670 (wheat) to 2685 mg/100 g d.wt. (rye) were found in cereal grain studied during four years (Table 3). These water extractable proteins are known to be rich in biologically active proteins [Deponete et al. 1976, Warchalewski et al. 1989, 1997]. The highest extractable proteins content was noted in all year crops of rye varieties. On the contrary the lowest, approximately 18%,

Table 2. Monthly precipitation during the four analysed years  
Tabela 2. Opady w poszczególnych miesiącach w czterech badanych latach

Year srop Rok zbioru	Monthly precipitation, mm – Opady miesięczne, mm												Year precipitation Opady roczne mm
	VIII	IX	X	XI	XII	I	II	III	IV	V	VI	VII	
1	48.4	15.3	21.0	31.8	47.2	11.7	31.4	14.3	40.5	22.2	119.8	44.3	448
2	61.5	57.3	38.4	61.2	47.8	17.9	13.5	21.0	68.8	57.1	122.3	7.8	575
3	31.5	18.0	26.1	42.2	38.9	23.8	33.4	78.7	19.6	38.1	20.9	35.9	407
4	31.9	18.2	27.1	31.9	35.4	54.3	35.1	34.7	6.8	20.6	147.8	133.7	578

Table 3. Extractable protein content as well as biological activities of cereal grains studied  
Tabela 3. Zawartość białka rozpuszczalnego w badanych ziarnie i jego aktywności biologiczne

Year crop Rok zbioru	Cereal variety Odmia- na zbóż	Extractable protein content mg/100 g d.wt. Białko wyekstra- howane mg/100 g s.s.	Amylolytic activity UAA/ 100 g d.wt.	Inhibitory activity against – Aktywność inhibitorowa wobec						bovine pancreas trypsin UAP×10 <sup>4</sup> / 100 g d.wt. trypsiny trzustki wołowej JAP×10 <sup>4</sup> / 100 g s.s.
				$\alpha$ -amylase, UAA×10 <sup>3</sup> /100 g d.wt. $\alpha$ -amylazy, JAA×10 <sup>3</sup> /100 g s.s.		hog pancreas trzustki wieprzo- wej		human saliva śliny ludzkiej		
1	2	3	4	5	6	7	8	9	10	
Wheat – Pszenica										
1	Lama	1 935	56 900	618	735	0.789	387	1 486	4 740	
	Puma	1 845	45 699	484	941	0.573	328	1 396	469	
2	Lama	1 980	62 276	914	1 183	0.753	228	1 237	3 457	
	Puma	1 935	53 763	484	1 290	0.717	312	1 362	716	
3	Lama	1 670	37 634	495	1 048	0.789	211	1 380	3 417	
	Puma	1 680	34 946	430	1 102	0.753	220	1 326	938	
4	Lama	1 980	53 763	473	1 281	0.502	254	1 299	2 894	
	Puma	1 800	49 283	409	1 048	0.538	251	1 371	430	
Rye – Żyto										
1	Motto	2 400	32 258	233	179	0	242	430	8 444	
	Amilo	2 655	45 699	366	385	0	271	806	9 333	
2	Motto	2 580	45 699	387	538	0.215	115	735	7 605	
	Amilo	2 685	60 036	237	860	0.215	158	753	8 888	
3	Motto	2 060	24 194	222	995	0.215	113	780	5 827	
	Amilo	2 235	22 401	301	860	0.215	165	842	5 437	

Table 3 – cont.

1	2	3	4	5	6	7	8	9	10
4	Motto	2 445	33 154	312	645	0.215	120	869	5 975
	Amilo	2 050	30 466	215	565	0.215	165	887	5 644
Triticale – Pszenżyto									
1	Presto	2 190	56 452	226	367	0.215	258	681	6 568
	Vero	2 525	60 036	358	484	0.566	301	769	5 333
2	Presto	1 785	72 581	290	663	0.430	194	645	5 728
	Vero	2 275	86 022	301	842	0.609	210	645	4 642
3	Presto	1 740	53 763	258	780	0.466	161	914	2 558
	Vero	1 830	48 387	226	896	0.645	122	968	2 553
4	Presto	2 020	45 699	333	959	0.717	143	950	3 071
	Vero	2 400	53 763	233	636	0.609	142	950	1 637

of extractable protein content was washed out from wheat varieties comparing to average value determined from all remaining cereal samples. Warchalewski et al. [1998] suggested that albumins in rye and triticale are noticeable fractions and are significantly higher than in wheat. The results presented in Table 3 support this suggestion. The extractable proteins investigated have shown some differences in biological activities among the studied cereal seeds (Table 3).

Amylolytic activities were different and depended on genus, varieties and year crop. The highest amylolytic activity was found in triticale Vero variety collected in the second year of harvest (86022 UAA/100 g d.wt.), while the lowest in rye Amilo variety in the third year crop (22401 UAA/100 g d.wt.). All the analysed cereal seeds collected in the third year, except Presto variety, showed the lowest endogenous amylolytic activity, average 30%, when compared to cereals from remaining crops. This year the lowest rainfall from April to July was recorded (Table 2). Correlation coefficient between measured amylolytic activity of the studied cereals and recorded precipitation was statistically significant ( $\alpha = 0.05$ ,  $r = 0.411$ ).

The highest average amylolytic activity (four years crops) had both triticale varieties, whereas wheat varieties 17% lower. Also other authors [Ceglińska et al. 1998] indicate that triticale varieties had higher amylolytic activity compared to wheat varieties. The amylolytic activity of rye varieties was approximately 33% lower compared to the other genus studied.

Table 3 presents results of inhibitory activities of extractable proteins. The highest inhibition activity of  $\alpha$ -amylases from mammalian sources were found in extractable proteins from wheat varieties. Average of 70% higher against  $\alpha$ -amylases from human saliva and 50% higher against  $\alpha$ -amylases from hog pancreas comparing to the other cereal genus studied. The lowest inhibition activity against  $\alpha$ -amylases from mammalian sources showed proteins extracted from both rye varieties. On the other hand, rye varieties have distinguishable highest level of inhibitory activity against bovine trypsin (Table 3). Similar tendencies were observed for wheat, rye and triticale species reported recently within different lines [Warchalewski et al. 1998].

As can be seen in Table 4 in the four consecutive crop years only the year of harvest in relation to variety did not statistically significantly affect inhibition activity against human saliva  $\alpha$ -amylase. On the contrary in the case of inhibition activity against hog pancreas  $\alpha$ -amylase in the studied cereal seeds all sources of changes including also year of harvest versus variety were statistically significant (Table 4). In addition the recorded monthly precipitation particularly in January and March statistically significantly affected activities against hog pancreas  $\alpha$ -amylase  $r = -0.413$ ,  $r = -0.500$  respectively.

Table 4. Analysis of variance different inhibitory activities found in extractable proteins of cereal grains studied

Tabela 4. Analiza wariancji różnych aktywności inhibitorowych białka wyekstrahowanego z badanego ziarna

Source of change Źródło zmienności	Year of harvest Rok zbioru	Genus Gatunek	Variety Odmiana	Year of harvest / genus Rok zbioru / gatunek	Year of harvest / variety Rok zbioru / odmiana	Year of harvest / genus/ variety Rok zbioru / gatunek / odmiana
1	2	3	4	5	6	7
Degrees of freedom Stopnie swobody	3	2	1	6	3	6
Inhibition activity of human saliva $\alpha$ -amylase Aktywność inhibitorowa wobec $\alpha$ -amylazy śliny ludzkiej						
Coefficient F Współczynnik F	28.40	672.45	14.53	16.52	2.61	6.88
Significance F Istotność F	0	0	0	0	0.06	0
Inhibition activity of hog pancreas $\alpha$ -amylase Aktywność inhibitorowa wobec $\alpha$ -amylazy trzustki wieprzowej						
Coefficient F Współczynnik F	378.51	436.50	34.69	10.70	10.80	15.74
Significance F Istotność F	0	0	0	0	0	0
Inhibition activity of <i>Sitophilus granarius</i> $\alpha$ -amylase Aktywność inhibitorowa wobec $\alpha$ -amylazy <i>Sitophilus granarius</i>						
Coefficient F Współczynnik F	36.63	387.43	47.25	14.62	34.35	14.93
Significance F Istotność F	0	0	0	0	0	0
Inhibition activity of <i>Tribolium confusum</i> $\alpha$ -amylase Aktywność inhibitorowa wobec $\alpha$ -amylazy <i>Tribolium confusum</i>						
Coefficient F Współczynnik F	207.59	420.42	10.88	20.12	49.39	6.50
Significance F Istotność F	0	0	0	0	0	0

Table 4 – cont.

	1	2	3	4	5	6	7
Inhibition activity of <i>Ephestia kuehniella</i> $\alpha$ -amylase Aktywność inhibitorowa wobec $\alpha$ -amylazy <i>Ephestia kuehniella</i>							
Coefficient F Współczynnik F		1.12	39.53	0.27	1.05	0.04	0.66
Significance F Istotność F		0.35	0	0.61	0.40	0.99	0.69
Inhibition activity of bovine pancreas trypsin Aktywność inhibitorowa wobec trypsyny trzustki wołowej							
Coefficient F Współczynnik F		740	1 997	831	143	73	80
Significance F Istotność F		0	0	0	0	0	0

Inhibitory activities against insects  $\alpha$ -amylases studied were different and depended on genus and cereal varieties (Table 4). In extractable proteins of all the studied cereal seeds inhibitory activity against *T. confusum*  $\alpha$ -amylase was approximately 60% higher comparing to this activity measured against *S. granarius*  $\alpha$ -amylase (Table 3). In the case of inhibitory activity against *E. kuehniella*  $\alpha$ -amylase this activity was 1000 times lower comparing to remaining insects  $\alpha$ -amylases (Table 3) and didn't depend on variety and year of harvest versus genus and variety (Table 4). Similar results were reported earlier by Warchalewski and Nawrot [1993]. The highest inhibitory activity against the studied insects  $\alpha$ -amylase were noticed in wheat varieties irrespective of the year crop (Table 3). The analysis of variance presented in Table 4 showed a statistically significant influence of all the sources of change on the inhibitory activities against *S. granarius* and *T. confusum*  $\alpha$ -amylase of the cereal seeds studied. On the other hand, it should be pointed out that the recorded precipitation did not affect statistically significantly all the inhibitors activities studied against insects  $\alpha$ -amylase.

It is believed, that plant seed  $\alpha$ -amylase inhibitors may play a natural protective role against insect infestation [Opert et al. 2004, Sivakumar et al. 2006]. According to Gatehouse et al. [1986] and Warchalewski et al. [2002] some insects like *Tribolium confusum* are able to detoxify these inhibitors by increasing production of their own  $\alpha$ -amylase. Therefore, extractable proteins from cereal grains may have some influence on the reduction of insects populations by extending their development time during storage time of cereals.

## CONCLUSIONS

1. The highest average extractable proteins content was noted in each years crop of rye varieties, while the lowermost in the wheat varieties studied.
2. Amylolytic activities were different and depended on cereals genus, variety and year crop. The highest amylolytic activity was found in triticale varieties whereas the lowest in the rye varieties studied.



3. Correlation coefficient between endogenous amylolytic activity of the cereal seeds investigated and recorded precipitation was statistically significant at the level  $\alpha = 0.05$ ,  $r = 0.411$ .

4. The highest inhibition of  $\alpha$ -amylases from mammalian sources was found in extractable proteins from wheat varieties, while the lowest in rye varieties irrespectively of the year of harvest.

5. The year of harvest, genus, variety and year of harvest versus genus or variety as well as precipitation influenced statistically significantly inhibitory activity against hog pancreas  $\alpha$ -amylase of all the studied seeds.

6. Extractable proteins of all studied cereal seeds show the highest inhibitory activity against *T. confusum*  $\alpha$ -amylase and the lowest against *E. kuehniella*  $\alpha$ -amylase.

7. Among all the studied cereal seeds in wheat varieties found the highest inhibitory activities against  $\alpha$ -amylases of *T. confusum*, *S. granarius* and *E. kuehniella*.

8. The analysis of variance showed statistically significant influence of variety, genus, and year of harvest as well as interaction between them on the inhibitory activities against *S. granarius*, *T. confusum*  $\alpha$ -amylase and antytryptic activity of the cereal seeds studied.

9. Precipitation did not statistically significantly affect inhibitors activities studied against insects  $\alpha$ -amylase.

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## **AKTYWNOŚĆ BIOLOGICZNA ZIARNA ODMIAN PSZENICY, ŻYTA I PSZENŻYTA POCHODZĄCEGO Z CZTERECH LAT ZBIORÓW**

**Streszczenie.** Celem pracy było określenie wpływu czterech lat zbioru, z uwzględnieniem opadów, na wybrane aktywności biologiczne trzech gatunków zbóż. Do badań wybrano po dwie odmiany pszenicy – Lama i Puma, żyta – Motto i Amilo oraz pszenżyta – Presto i Vero. W ziarnie oznaczono całkowitą zawartość białka, zawartość białka rozpuszczalnego oraz określono jego aktywność amyloliczną, antyamyloliczną i antytrypsynową wobec enzymów trawiennych ssaków oraz owadów. Spośród badanych aktywności wielkość opadów w poszczególnych latach statystycznie istotnie wpływała tylko na aktywność endogennych amylaz i aktywność hamującą działanie  $\alpha$ -amylaz trzustyki wieprzowej. Analiza wariancji wykazała statystycznie istotny wpływ zarówno odmiany, gatunku zbóż i roku zbioru, jak i interakcji pomiędzy nimi na aktywność hamującą działanie  $\alpha$ -amylaz *S. granarius* i *T. confusum* oraz aktywność antytrypsynową. Nie stwierdzono natomiast statystycznie istotnych związków pomiędzy wielkością opadów a aktywnością inhibitorową ziarna zbóż wobec wszystkich badanych  $\alpha$ -amylaz owadzych.

**Słowa kluczowe:** ziarno pszenicy, ziarno żyta, ziarno pszenżyta

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