

## THE EFFECT OF WATER STRESS ON THE GAS EXCHANGE PARAMETERS, PRODUCTIVITY AND SEED HEALTH OF BUCKWHEAT (*Fagopyrum esculentum* MOENCH)

Agnieszka Pszczółkowska, Gabriel Fordoński, Tomasz Kulik,  
Jacek Olszewski, Krystyna Płodzień, Maciej Łojko

Department of Diagnostics and Plant Pathophysiology, University of Warmia and Mazury in Olsztyn, Plac Łódzki 5  
10 – 727 Olsztyn, Poland  
e-mail: agnieszka.pszczolkowska@uwm.edu.pl

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### Abstract

The present pot experiment studied the effect of different soil moisture contents (60 – 70% CWC (capillary water capacity) – control; 30 – 35% CWC – water stress) on buckwheat productivity, the gas exchange parameters and health of buckwheat nuts. It was found that water deficit affected adversely certain biometric features investigated (plant height, number of nuts per cluster) and caused a decrease in seed weight per plant. It was also shown that water stress reduced the values of the investigated gas exchange parameters (photosynthesis rate, transpiration rate, intercellular-space CO<sub>2</sub> concentration, and stomatal conductance) relative to the control treatment. Different soil moisture contents did not have a clear effect on fungal colonization of seeds. The multiplex PCR assays did not enable the detection of the genes responsible for mycotoxin synthesis. Under water deficit conditions, an increase was found in the content of albumin and globulin fractions as well as of glutelin fractions.

**Key words:** buckwheat, gas exchange parameters, health, traditional method, PCR

### INTRODUCTION

In the global healthy food markets, more and more attention is paid to buckwheat. Its seeds are not only a source of many nutrients, but also a source of biologically active compounds not considered to be nutrients (Klepacka and Fornal, 2006). Buckwheat nuts can be a valuable source for the production of both functional food and food additives (Troszyńska et al. 2000). In its composition, the fruit of buckwheat contains non-gluten protein with a balanced amino acid composition and a large amount of raw fat in which unsaturated fatty acids are predominant. Moreover, buckwheat kernels contain

macro- and micronutrients such as: Mg, K, Cu, Zn, Fe, much needed in the diet, as well as group B vitamins and antioxidant compounds (Holasova et al. 2002; Dietrych-Szóstak and Suchecki, 2006). Among the biologically active compounds, polyphenols and inositol deserve special attention (Klepacka and Fornal, 2006).

The valuable chemical composition of buckwheat nuts gives the possibility of using buckwheat as raw material for the production of functional, medicinal and protective food (Kwiatkowski, 2008). Taking into account the above aspects, it seems to be important to ensure optimal conditions for the growth and development of this plant.

Elements such as buckwheat grain yield, yield structure and the chemical composition of seeds are driven by the genotype, weather conditions, agricultural practices and their interrelationships (Podolska, 2006). Water deficit is of essential significance among the abiotic stress factors reducing plant productivity (Skrabka, 1992). Water deficit in plants is caused by the lack of available water in the soil and atmospheric drought often accompanying high temperatures as well as the predominance of the process of transpiration over water absorption (Boczek and Szlendak, 1992; Fordoński et al. 1994). Water deficiency is one of the most important factors reducing crop yields (Starck et al. 1995; Grzesiuk et al. 1999).

The health of buckwheat grain harvested, which is to be used as raw material for processing, is also of essential significance. The literature (Grzesiuk et al. 1999; Irzykowska, 2006; Pszczółkowska, 2008) confirms that cereals are exposed to a number of factors leading to quantitative and qualitative yield losses during the entire growing period. Champel

et al. (2004) also showed that agricultural produce could be contaminated with toxins during the vegetation period in the field as well as during harvest, storage and transport.

Among pathogens, the fungi of the genus *Fusarium*, which represent a large group of the species posing a serious threat to crop plants across the world, deserve special attention. The fungal species of the genus *Fusarium*, when colonising agricultural produce, contaminate it with toxins which have an adverse effect on the health of people and animals consuming such products. Numerous species of the genus *Fusarium* are toxic and produce mycotoxins from the group of trichothecenes, zearalenones and fumonisins (Kwaśna et al. 1991; Schilling et al. 1996; Yu, 2000; Edwards, 2004).

The aim of the present study was to determine the effect of water deficit on the morphological features, gas exchange parameters, health, and protein fraction content in buckwheat nuts.

## MATERIAL AND METHODS

The present pot experiment was carried out in a greenhouse of the University of Warmia and Mazury in Olsztyn in the period 2004 – 2005, in quadruplicate. The study object was buckwheat cv. Kora. Buckwheat was grown in Kick-Brauckmann pots. The pots were filled with typical brown soil with the following nutrient availability:  $P_2O_5$  –  $64.1 \text{ mg} \times 100 \text{ g}^{-1}$  of soil,  $K_2O$  –  $13.0 \text{ mg} \times 100 \text{ g}^{-1}$ ,  $Mg$  –  $19 \text{ mg} \times 100 \text{ g}^{-1}$  and pH in 1N KCl – 6.78. After emergence 10 plants per pot were left. During the growing process, the plants were fed with Hoagland's medium.

The experimental factors were as follows:

Soil moisture:

1. 60-70% of capillary water capacity (control).
2. 30-35% of capillary water capacity from the full flowering stage (water stress).

The plants were watered with distilled water 2-3 times per day, controlling soil moisture with a soil moisture meter in accordance with the methodological assumptions.

The scope of the study covered the following:

1. To determine more important biometric features of the plants and seed weight per plant.
2. To determine the gas exchange parameters using a LI-COR 6400 portable photosynthesis system. The determinations were performed at a constant  $CO_2$  concentration of 400 ppm and a light intensity of  $1000 \mu\text{mol m}^{-2} \times \text{s}^{-1}$ . The source of photons was a LED light source emitting light with the main peak wavelength of 670 nm and the lower peak wavelength of 465 nm. The measurements were made three times (at the following stages: beginning of

flowering, full flowering, nut formation) on the youngest fully developed leaf. The results of the abovementioned measurements were averaged. Each measurement was repeated ten times.

3. To evaluate seed health using the traditional method and PCR after prior DNA isolation.

In order to evaluate seed health using the traditional method, samples of 100 nuts each were randomly selected, then the seeds were surface sterilized with 70% ethanol and 1% sodium hypochlorite as well as they were washed three times in sterile distilled water. The material so prepared was plated on Petri dishes with solidified PDA medium. The dishes with the kernels placed on them were stored in a laboratory oven at a temperature of 20 – 23°C for 7 – 10 days, and subsequently the grown fungal cultures were identified to genus and species based on the morphological features under an optical microscope, using the available monographic studies of Ellis (1971) and Kwaśna et al. (1991).

To perform the PCR and multiplex PCR assays, DNA isolation was first carried out using the column method (Kulik et al. 2007) and next, in order to detect more important pathogens of the genus *Fusarium* (*Fusarium culmorum*, *Fusarium graminearum*, *Fusarium poae*, *Fusarium sporotrichioides*) in the seeds, in the PCR reaction there were used species-specific primers, available in the literature (Hue et al. 1999; Kulik et al. 2004; Kulik, 2008; Pszczółkowska, 2008), and appropriately selected thermal profiles. The potential toxin-producing ability (the ability to produce trichothecenes, NIV and DON chemotypes as well as enniatins) of the species of *Fusarium* spp. was determined, using the specific primers for the genes determining mycotoxin synthesis (Edwards et al. 2001; Chandler et al. 2003; Kulik et al. 2007). The PCR reaction mixture comprised the following: FailSafe™ PCR 2X Premix E, 0.2U polymerases Fail Safe™ Enzyme Mix Only, a primer mix; 5.75  $\mu\text{l}$  of deionized water and 5  $\mu\text{l}$  of template DNA. The samples prepared for the PCR analysis were subjected to cyclical temperature changes in a Mastercycler Gradient thermal cycler (Eppendorf). In each case, the PCR reaction was performed in duplicate.

In order to perform electrophoresis, 1.5% agarose gel was prepared with an addition of ethidium bromide. Electrophoresis was carried out in an electric field with a voltage of 50 V for 1.5 hours. The PCR product size was estimated by comparing it to a molecular weight marker – Step Ladder 50 bp (Sigma-Aldrich, USA).

4. To determine protein fraction content in buckwheat seeds.

A 3g seed sample was ground in an IKA A10 laboratory mill (Labortechnik) in such a manner so

Table 1  
Species-specific primers for the detection of *Fusarium* species and chemotypes in buckwheat seeds

Fungal species, chemotype, gene	Primer	PCR product size	References
<i>Fusarium</i> ssp.	P58SL 5'-AGT ATT CTG GCG GGC ATG CCT GT-3'	339 bp.	HUE F.X., HUERRE M., ROUFFAULT M. A., BIEVRE C. 1999. Specific Detection of <i>Fusarium</i> Species in Blood and Tissues by PCR Technique. <i>Journal of Clinical Microbiology</i> , 37(8): 2434–2438.
	P28SL 5'-ACA AAT TAC AAC TCG GGC CCG AGA-3'		
<i>Fusarium culmorum</i>	igscl1 5'-CGG CTC CCG GGT AGG CAA CTC-3'	340 bp.	PSZCÓŁKOWSKA A. 2008. Diagnostics of fungal pathogens by the PCR and traditional methods and the productivity of winter wheat ( <i>Triticum aestivum</i> L.) as dependent on fungicide protection Rozprawy i monografie, 140, UWM Olsztyn
	igscln2 5'-CAT TCC CTA GGC CCC TTA ACT GG-3'		
<i>Fusarium graminearum</i>	igsgral 5'-TTC AGG GTA GGC TTT CAG TTA GGA-3'	499 bp.	
	igsgra2 5'-GGA GAA GAG GGC TGC AGC GTT GG-3'		
<i>Fusarium poae</i>	poal 5'-CTT GGT AGG GGG GAC AGA CAC GC-3'	203 bp.	KULIK T. 2008. Development of duplex PCR assay for the simultaneous detection of <i>Fusarium poae</i> and <i>F. sporotrichioides</i> from wheat. <i>Journal of Plant Pathology</i> 90(3):441-447.
	poa2 5'-CCA TTC CAC GCT CGA CAG ACC TG-3'		
<i>Fusarium sporotrichioides</i>	fspts2k 5'-CTT GGT GTT GGG ATC TGT GTG CAA-3'	288 bp.	
	P28SL 5'-ACA AAT TAC AAC TCG GGC CCCC AGA-3'		
<i>Fusarium</i> spp.	HATrif 5'-CAG ATG GAG AAC TGG ATG GT-3'	260 bp.	EDWARDS S.G., PRGOZLIEV S.R., HARE M. C., JENKINSON P. 2001. Quantification of trichothecene-producing <i>Fusarium</i> species in harvested grain by competitive PCR to determine efficacies of fungicides against <i>Fusarium</i> head blight of winter wheat. <i>Applied and Environmental Microbiology</i> 67: 1575–1580.
	HATrir 5'-GCA CAA GTG CCA CGT GAC-3'		
<i>Chemotyp-NIV</i>	Tri13/NIVF 5'-CCA AAT CCG AAA ACC GCA G-3'	312 bp.	CHANDLER E., SIMPSON D.R., THOMSETT M.A., NICHOLSON P. 2003. Development of PCR assays to Tri7 and Tri13 trichothecene biosynthetic genes, and characterization of chemotypes of <i>F. graminearum</i> , <i>F. culmorum</i> and <i>F. cerealis</i> . <i>Physiological and Molecular Plant Pathology</i> , 62: 355–367
	Tri13R 5'-TTG AAA GCT CCA ATG TCG TG-3'		
<i>Chemotyp 15-acetyl DON</i>	Tri315F 5'-CTC GCT GAA GTT GGA CGT AA-3'	864 bp.	
	Tri315R 5'-GTC TAT GCT CTC AAC GGA CAA C-3'		
<i>Chemotypes 3-acetyl DON</i>	Tri303F 5'-GAT GGC CGC AAG TGG A-3'	586 bp.	
	Tri303R 5'-GCC GGA CTG CCC TAT TG-3'		
<i>F. avenaceum</i> , <i>F. tricinatum</i> , <i>G. pulicaris</i>	esyal 5'-GGT CTC GAT CCA TCC AAG TC-3'	401 bp.	KULIK T., PSZCÓŁKOWSKA A., FORDONSKI G., OLSZEWSKI J. 2007. PCR approach based on the <i>esy1</i> gene for the detection of potential enniatin-producing <i>Fusarium</i> species. <i>International Journal of Food Microbiology</i> , 116: 319–324
	esy2 5'-GTG AAG AAG GCA GGC TCA AC 3'		
<i>F. poae</i> , <i>F. sporotrichioides</i>	esysp1 5'-GGC CTT GAG CCA TCC AGA TC-3'	273 bp.	
	esysp2 5'-CTC GTT GGT AGC CTG CGA TCG-3'		
<i>F. proliferatum</i>	esypro1 5'-GAT CAA CTC AGT CGC GCA GTA-3'	225 bp.	
	esypro2 5'-TCC TCC TCA CGC TCC TCC A-3'		
<i>Giberella pulicaris</i>	esysam1 5'-TGA TTC TCA ACT CCG TCG TTC A-3'	332 bp.	
	esysam2 5'-CAC AGC CTT CAT GTT CTT GGG-3'		

that all particles could be sieved through a 400 µm mesh sieve (particles smaller than 250 µm accounted for 90%). The samples were degreased with petroleum ether in Soxhlet extractors (16 hours). After evaporation of the solvent, 100 mg portions of powder were weighed and placed in Eppendorf tubes, and then three protein fractions were extracted according to Wieser et al. (1998).

1. Albumins + globulins – triple extraction of 1 cm<sup>3</sup> of the mixture (0.4mol/L NaCl + 0,067 mol/L HKNa-PO<sub>4</sub> (pH 7.6)
2. prolamins – triple extraction of 1cm<sup>3</sup> of the mixture (60% ethanol)
3. glutelins – double extraction of 1cm<sup>3</sup> of the mixture (50% propanol-1 + 2 mol/L urea 0.05 mol/L Tris HCl (pH 7.5) + 1% DTE under nitrogen.

The first two protein fractions were extracted at room temperature using an Eppendorf thermomixer (10-minute extraction). Glutelins were extracted at a temperature of 60°C in the thermomixer. After each extraction, the mixture was centrifuged at 11000 x g. The collected fractions were lyophilized and then dissolved in 2 cm<sup>3</sup> of the respective phase (1-3), cleaned through a Spartan – 3NY filter with a 0.45 µm mesh and transferred to glass vials. The determinations were made using a Hewlett Packard Series 1050 system with the following parameters: column RP-18 Vydac 218TPP54, 5 µm, 250x4.6 mm, pre-column Zorbax 3000SB-C18 4.6x12.5 mm, column temperature 45°C, mobile phase flow rate 1ml/min, injection size 20 µl. The separation was performed using a two-component gradient. The proportion of component A: 0 min 75%, 5 min 65%, 10 min 50%, 17 min 25%, 18 min 15%, 19 min 75% . The first gradient (A) was water with an addition of 0.1% TFA, the second gradient (B) was ACN with an addition of 0.1%TFA. The detection was carried out using a detector manufactured by the same company, and the reading was done at a wavelength of 210 nm.

The results were analysed based on HPLC 3D Chem Station software (Hewlett Packard).

The assays were carried out at the Department of Processing and Chemistry of Plant Raw Materials, Faculty of Food Sciences, University of Warmia and Mazury in Olsztyn.

## RESULTS AND DISCUSSION

Atmospheric conditions during plant growth and development have a decisive influence on productivity. This is confirmed by Podolska (2006) in her study in which she indicates that the lowest buckwheat yields were obtained in the year in which the third decade of May was characterized by a shortage of rainfall and high air temperature, which in effect prolonged buckwheat emergence and inhibited its initial growth. In the same year, the flowering and nut set period also occurred when there was a very small amount of rainfall and high temperature.

Among the investigated biometric features of buckwheat cv. Kora, a significant decrease was found in seed weight per plant, number of nuts per cluster and plant height under water deficit conditions (Table 2). Liszewski (2002) also reports that summer drought does not have a beneficial effect on buckwheat seed set and filling, while Olszewski et al. (2007) showed that water deficit and high temperature in a greenhouse had an adverse impact on some elements of the wheat yield structure and productivity.

Water stress resulted in a reduction of the gas exchange parameters both in the first and second year of the study. A decrease in the rates of photosynthesis and transpiration as well as in intercellular-space CO<sub>2</sub> concentration and stomatal conductance was shown under water deficit conditions relative to the control (Tables 3 and 4). In the domestic and foreign literature, there are no data on the effect of different soil moisture contents on the gas exchange parameters in buckwheat. However, Olszewski et al. (2007, 2008) and Pszczółkowska et al. (2003) indicate the adverse influence of water deficit on the gas exchange parameters in wheat, pea and yellow lupin.

Table 2  
Selected biometric features of buckwheat cv. Kora under water stress conditions (means of 2004 – 2005)

Cultivar	Soil water capacity, %	Plant height, cm	Number of branches	Number of clusters per plant	Number of nuts per cluster	Thousand seed weight (g)	Seed weight per plant, g
Kora	60 – 70 %	136.17 b	2.60 a	23.59 a	6.00 b	29.81 a	4.20 b
	30 – 35 %	118.55 a	2.52 a	24.26 a	3.67 a	27.97 a	2.49 a

Homogeneous groups a, ab, b, according Fisher's LSD test

Table 3  
Gas exchange parameters in buckwheat under water stress conditions in 2004

Cultivar	Capillary water capacity of soil	Photosynthesis ( $\mu\text{molCO}_2 \times \text{m}^{-2} \times \text{s}^{-1}$ )	Transpiration ( $\text{mmolH}_2\text{O} \times \text{m}^{-2} \times \text{s}^{-1}$ )	Intercellular-space $\text{CO}_2$ concentration ( $\mu\text{molCO}_2 \times \text{mol}^{-1}$ )	Stomatal conductance ( $\text{molH}_2\text{O} \times \text{m}^{-2} \times \text{s}^{-1}$ )
Kora	60–70%	15.10 a	4.00 a	269.40 a	0.31 a
	30–35%	6.90 b	1.00 b	153.60 b	0.05 b

Mean values of three measurements (I – measurement of gas exchange parameters at the beginning of flowering; II – measurement of gas exchange parameters at full flowering; III – measurement of gas exchange parameters at the nut formation stage) Homogeneous groups a, ab, b, according Fisher's LSD test

Table 4  
Gas exchange parameters in buckwheat under water stress conditions in 2005

Cultivar	Capillary water capacity of soil	Photosynthesis ( $\mu\text{molCO}_2 \times \text{m}^{-2} \times \text{s}^{-1}$ )	Transpiration ( $\text{mmolH}_2\text{O} \times \text{m}^{-2} \times \text{s}^{-1}$ )	Intercellular-space $\text{CO}_2$ concentration ( $\mu\text{molCO}_2 \times \text{mol}^{-1}$ )	Stomatal conductance ( $\text{molH}_2\text{O} \times \text{m}^{-2} \times \text{s}^{-1}$ )
Kora	60–70%	14.90 a	3.00 a	226.80 a	0.32 a
	30–35%	8.20 a	1.80 b	170.00 a	0.07 b

Mean values of three measurements (I – measurement of gas exchange parameters at the beginning of flowering; II – measurement of gas exchange parameters at full flowering; III – measurement of gas exchange parameters at the nuts formation stage) Homogeneous groups a, ab, b, according Fisher's LSD test

Table 5  
Number of fungal isolates in buckwheat cv. Kora nuts under water stress conditions in 2004

Fungal species	Control	Water stress
	60–70% CWC 60–70% of capillary water capacity	30–35% CWC 30–35% of capillary water capacity
1. <i>Alternaria alternata</i> Keissler Nees	2	1
2. <i>Cladosporium cladosporioides</i> (Fr.) de Wries	6	8
4. <i>Chaetomium</i> spp.	4	4
4. <i>Epicoccum purpureans</i> Link ex Schlecht		1
5. <i>Fusarium poae</i> (Peck) Wollenw.	2	
6. <i>Penicillium</i> spp.	6	5
5. Non-sporulating mycelia	2	4
<b>Total</b>	<b>22</b>	<b>23</b>

CWC – capillary water capacity of soil: 60–70% – control, 30–35% – water stress

Table 6  
Number of fungal isolates in buckwheat cv. Kora nuts under water stress conditions in 2005

Fungal species	Control	Water stress
	60 – 70% CWC 60 – 70% of capillary water capacity	30 – 35 % CWC 30 – 35% of capillary water capacity
1. <i>Alternaria alternata</i> Keissler Nees	1	
2. <i>Cladosporium cladosporioides</i> (Fr.) de Wries		1
<b>Total</b>	<b>1</b>	<b>1</b>

CWC – capillary water capacity of soil: 60 – 70% – control, 30 – 35% – water stress

Fungal colonisation of buckwheat cv. Kora seeds was at a similar level in the first year of the study. 22 fungal isolates were identified in the control seeds and 23 isolates in the water stress treatment (Table 5). Among the pathogenic fungi, the presence of *Fusarium poae* was only found in the control, while among the saprophytic fungi, the species of *Cladosporium cladosporioides* and the fungi of the genus *Penicillium* were predominant. Under water stress conditions, the species of *Cladosporium cladosporioides* and the fungi of the genera *Penicillium* and *Chaetomium* were found in greatest numbers. But the year 2005 was not favourable for the development of the fungi on buckwheat nuts. The presence of only 1 fungal isolate was found in the control treatment and 1 isolate under water deficit conditions. Both identified fungal cultures belonged to the saprophytic species (Table 6). The study of Wachowska and Kwiatkowski (2006) also confirms the presence of hyphal fungi of the genera *Cladosporium*, *Penicillium* and *Aspergillus* on nuts of buckwheat grown under different harvesting options.

Problems associated with the presence of fusariotoxins in cereals and other products of natural origin may occur in Poland (Chęłkowski, 1985). When colonizing agricultural produce, the fungi of the genus *Fusarium* contaminate it with toxins which have an adverse effect on the health of people and animals consuming such products. Many species of the genus *Fusarium* are toxic and produce mycotoxins from the group of trichothecenes, zearalenones, fumonisins, and enniatins (Yu, 2000; Edwards, 2004; Kulik et al. 2007).

In this study, the presence of *Fusarium* spp. was found, using the PCR method, in buckwheat nuts

obtained from the plants with respect to which optimal soil moisture conditions were applied during the growing period in the second year of the study (Tab. 7). The results obtained by applying the specific primers for four fungal species of the genus *Fusarium* showed only the presence of *Fusarium poae* in the water stress-treated seeds in the first year of the study. The pair of poa1/poa2 primers used in the investigations generated a 203-bp product in accordance with the results obtained by Kulik (2008). A negative result was obtained in the multiplex PCR reaction using the specific primers for the genes responsible for the production of mycotoxins from the group of trichothecenes and enniatins. This demonstrates that the buckwheat grain assayed was free from secondary fungal metabolites – mycotoxins – and that it was safe raw material which could be used for functional food production.

As a result of the effect of a pathogen or other environmental stresses on a plant, protein synthesis and accumulation occur. These can be enzymatic, structural, signal proteins, with the properties of metabolic inhibitors, as well as protective proteins with regard to the cell structures, called stress proteins (Kozłowska and Konieczny, 2003).

In the present study, an increase was found in the content of albumin + globulin fractions and glutelin fractions under water deficit conditions (Fig. 1). Konopka et al. (2007) also reports that water stress is one of the stresses which affect changes in amount of protein fractions. Dietrych-Szóstak et al. (2008) showed that the delayed sowing date caused a decrease in buckwheat yield and TSW (thousand seed weight), but an increase in protein content.

Table 7  
 PCR analysis of the response of buckwheat cv. Kora nuts to fungi of the genus *Fusarium* and their toxin-producing ability

Fungal species, chemotype, gene	Primer	PCR product size (bp)	Treatment (soil moisture content)			
			60 – 70% CWC 60 – 70% capillary water capacity		30 – 35% CWC 30 – 35% capillary water capacity	
			2004	2005	2004	2005
<i>Fusarium</i> spp.	P58SL/P28SL	339	-	+	+	-
<i>Fusarium avenaceum</i>	Faf/Faf	920	-	-	-	-
<i>Fusarium culmorum</i>	igscul1/igsculn2	340	-	-	-	-
<i>Fusarium graminearum</i>	igsgra1/igsgra2	499	-	-	-	-
<i>Fusarium poae</i>	igspoa1/igspoa2	203	-	-	+	-
<i>Fusarium sporotrichioides</i>	fspits2k/spo2	696	-	-	-	-
Gen tri5 tri5 gene	HATri13R/HATri13F	260	-	-	-	-
Chemotyp NIV NIV-chemotype	Tri1NIVF/Tri13R	312	-	-	-	-
Chemotyp 15-acetyl DON 15-acetyl DON chemotype	Tri315F/Tri315R	864	-	-	-	-
Chemotyp 3-acetyl DON 15-acetyl DON chemotype	Tri303F/Tri303R	586	-	-	-	-
Gen esyn1 ( <i>F. avenaceum</i> , <i>F. tricinatum</i> , <i>G. pulicaris</i> ) esyn1 gene ( <i>F. avenaceum</i> , <i>F. tricinatum</i> , <i>G. pulicaris</i> )	esya1/esya2	401	-	-	-	-
Gen esyn1 ( <i>F. poae</i> , <i>F. sporotrichioides</i> ) esyn1 gene ( <i>F. poae</i> , <i>F. sporotrichioides</i> )	esypro1/esypro2	273	-	-	-	-
Gen esyn1 ( <i>F. proliferatum</i> ) esyn1 gene esyn1	esypro1/esypro2	225	-	-	-	-
Gen esyn1 ( <i>G. pulicaris</i> ) esyn1 gene ( <i>G. pulicaris</i> )	esysam1/esysam2	332	-	-	-	-

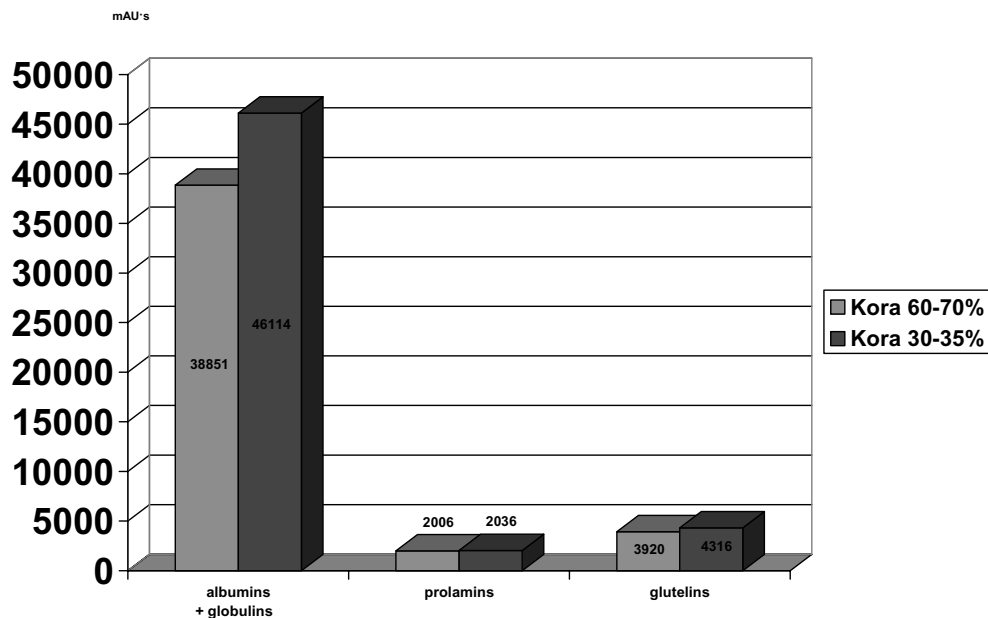


Fig. 1. Protein fraction content in buckwheat nuts obtained from plants grown under different soil moisture conditions (calculated as  $\text{mAU} \times \text{s}$ )

## CONCLUSIONS

1. Water deficit affected adversely some biometric features (plant height, number of nuts per cluster) and caused a decrease in seed weight per plant.
2. Water stress resulted in a significant reduction in the investigated gas exchange parameters (photosynthesis rate, transpiration rate, intercellular-space  $\text{CO}_2$  concentration, and stomatal conductance) relative to the control treatment, in particular in the first year of the study.
3. Different soil moisture contents did not have a clear effect on fungal colonization of seeds.
4. The results of the multiplex PCR reaction did not show the presence of homologs of the genes responsible for mycotoxin production.
5. Under water deficit conditions, an increase was found in the content of albumin and globulin fractions as well as of glutelin fractions.

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**Wpływ stresu wodnego na wskaźniki wymiany gazowej, produktywność i zawartość grzybni (*Fagopyrum esculentum*)**

**Streszczenie**

W przeprowadzonym doświadczeniu wazonom badano wpływ zróżnicowanego uwilgotnienia gleby (60 – 70% kpw – kontrola; 30 – 35% kpw – stres wodny) na plonowanie, wskaźniki wymiany gazowej oraz zdrowotność orzeszków gryki. Stwierdzono, że deficyt wodny wpłynął niekorzystnie na niektóre badane cechy biometryczne (wysokość roślin, liczba

orzeszków w gronie) oraz spowodował zmniejszenie masy orzeszków z rośliny. Wykazano również, że stres wodny obniżył intensywność badanych wskaźników wymiany gazowej (intensywność procesu fotosyntezy, transpiracji, stężenie międzykomórkowe CO<sub>2</sub>, przewodność szparkowa) w stosunku do obiektu kontrolnego. Zróżnicowane uwilgotnienie podłoża nie wpłynęło jednoznacznie na zasiedlenie nasion przez grzyby. Analizy multiplex PCR nie umożliwiły wykrycia genów odpowiedzialnych za syntezę miko toksyn. W warunkach niedoboru wody stwierdzono wzrost zawartości frakcji albuminy i globuliny oraz glutelin.