

**EFFECT OF WATER DEFICIT ON GAS EXCHANGE  
PARAMETERS, PRODUCTIVITY AND GRAIN  
WHOLESOMENESS OF SPRING WHEAT**

***Jacek Olszewski, Agnieszka Pszczółkowska,  
Monika Makowska, Tomasz Kulik, Adam Okorski***

Department of Plant Diagnostics and Pathophysiology  
University of Warmia and Mazury in Olsztyn

**Key words:** water deficit, spring wheat, photosynthesis, transpiration, stomatal conductance, intercellular CO<sub>2</sub> concentration, grain wholesomeness, BIO-PCR.

**Abstract**

The rate of photosynthesis and transpiration, intercellular CO<sub>2</sub> concentration and stomatal conductance in spring wheat plants were determined in an experiment conducted during the years 2004–2005. The severity of fungal infection of wheat kernels was estimated by a traditional method and a molecular BIO-PCR technique with the use of universal and SCAR primers. It was found that water deficit decreased thousand grain weight, grain weight per plant and the values of gas exchange parameters (including photosynthesis, transpiration, stomatal conductance, intercellular CO<sub>2</sub> concentration), in particular photosynthesis. The values of biometric characters did not decrease. The rate of wheat grain colonization by fungal pathogens was slightly higher under water stress conditions.

**WPLYW DEFICYTU WODNEGO NA WSKAŹNIKI WYMIANY GAZOWEJ,  
PRODUKCYJNOŚĆ I ZDROWOTNOŚĆ ZIARNA PSZENICY JAREJ**

***Jacek Olszewski, Agnieszka Pszczółkowska, Monika Makowska, Tomasz Kulik,  
Adam Okorski***

Katedra Diagnostyki i Patofizjologii Roślin  
Uniwersytet Warmińsko-Mazurski w Olsztynie

**Słowa kluczowe:** niedobór wody, pszenica jara, fotosynteza, transpiracja, przewodność szparkowa, międzykomórkowe stężenie CO<sub>2</sub>, zdrowotność ziarna, BIO-PCR.

**Abstrakt**

W latach 2004–2005 przeprowadzono eksperyment badawczy, w którym mierzono intensywność fotosyntezy i transpiracji, międzykomórkowe stężenie CO<sub>2</sub> oraz przewodność szparkową pszenicy jarej. Określono ponadto zasiedlenie grzybami ziarniaków – metodą tradycyjną oraz molekularną BIO-PCR z wykorzystaniem primerów uniwersalnych oraz typu SCAR. Wykazano, że deficyt wody spowodował obniżenie MTZ, masy ziarna z rośliny oraz wskaźników wymiany gazowej (fotosynteza, transpiracja, przewodność szparkowa, międzykomórkowe stężenie CO<sub>2</sub>), a zwłaszcza fotosyntezy. Nie stwierdzono natomiast spadku wartości cech biometrycznych. Zanotowano także nieco wyższe zasiedlenie ziarna pszenicy jarej patogenami grzybowymi w obiektach z niedoborem wody.

**Introduction**

One of the factors that determine the growth, development and yield of crops is adequate water supply, which is essential to all life processes. Water deficit disturbs metabolic reactions, leads to changes in the chemical composition of seeds as well as to considerable yield loss and quality deterioration (KACPERSKA 1991, GRZESIUK and GÓRECKI 1994, OZTURK and AYOLIN 2004). Water deficit is caused by a substantial water shortage in the soil, atmospheric drought, and the excess of transpiration over absorption (BOCZEK and SZLENDAK 1992, FORDOŃSKI et al. 1994). Moisture deficiency is manifested in plant wilting already when water levels decrease from 75–90% (considered optimal) to 55–70% (GRZESIUK et al. 1999). Long-term drought may damage photosystem II (PS II) structure, which in turn reduces the rate of photosynthesis. Plants respond to water stress by closing their stomata to prevent water loss, which hinders CO<sub>2</sub> assimilation. The adverse changes in the photosynthesis process lead to considerable yield loss. On the other hand, plants grown under water deficit conditions have an ability to alter their metabolism so as to save water and minimize the negative effects of its shortage.

Among the biotic factors affecting the photosynthesis process, an important role is played by various diseases, 80% of which are caused by fungal pathogens. At an advanced stage of a fungal disease, the rate of photosynthesis may be reduced by as much as 75%. This results, among others, from a decrease in leaf surface area caused by damage to the green organs of a plant, plant growth inhibition or the occurrence of extensive necrotic lesions. Moreover, organelle destruction in infected plants leads to disturbances in water relations.

In view of the above, a study was undertaken to determine the effect of water deficit on the morphological characters, gas exchange parameters and grain wholesomeness of spring wheat.

## Materials and Methods

A two-factorial pot experiment was conducted in six replications in the greenhouse of the University of Warmia and Mazury in Olsztyn, during the years 2004–2005. The experimental factors included spring wheat cv. Nawra, and two levels of soil moisture content:

- optimal (60–70% of capillary water capacity),
- deficient (30–35% of capillary water capacity).

Scope of the study:

- determination of gas exchange parameters (the rate of photosynthesis and transpiration, stomatal conductance and intercellular CO<sub>2</sub> concentration), with the use of a LI-COR 6400 portable gas analyzer;
- mycological evaluation of wheat grain by a traditional method and molecular techniques (BIO-PCR, SCAR-PCR), preceded by DNA isolation;
- determination of selected biometric characters of wheat plants.

Gas exchange parameters were measured five times, at several-day intervals, using a LI-COR 6400 gas analyzer (Portable Photosynthesis System, DMP AG S.A. LTD, at a constant CO<sub>2</sub> concentration of 400 ppm and illumination of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The source of photons was a LED Light Source lamp, emitting wide-spectrum light at a peak wavelength of between 670 nm and 465 nm. Measurements were performed on the flag leaf of spring wheat plants, at selected development stages. Mean values for each stage are given in the paper.

In order to determine the health status of spring wheat grain by a traditional method (artificial cultures), 100 kernels were selected randomly of each treatment. The kernels were rinsed under running water for 15 to 20 minutes and surface disinfected with 70% ethyl alcohol and 1% sodium hypochlorite to remove impurities, and next rinsed three times in sterile distilled water. Then the kernels were placed in Petri dishes with a PDA solid medium. The dishes were stored in a thermostat at 20 to 23°C for 7 to 10 days, and next mycelium hyphae were transferred to PDA slants. Finally fungal cultures were identified to genus and species based on their morphological characters observed under an optical microscope, as described in the monographs by ELLIS (1971), GILMAN (1957) and KWAŚNA et al. (1991).

Mycelium hyphae (i.e. live pathogen inoculum with potential infectious properties) obtained from kernels cultured on a PDA medium were isolated in separate Petri dishes for BIO-PCR analysis. After 2 to 3 days mycelium pieces were taken with a scalpel, placed in porcelain mortars and ground in liquid nitrogen. DNA was isolated by the CTAB method (NICHOLSON et al. 1996). Polymerase chain reaction (PCR) was performed using primers known from literature (PARRY and NICHOLSON 1996, HUE et al. 1999).

Experimental results were processed statistically based on a multiple range test involving mean values in homogenous groups, at a significance level of  $\alpha = 0.01$ , with the use of STATISTICA ver. 6.0 software.

## Results and Discussion

The results of the present study, obtained both in the first and second year of the experimental period, revealed a decrease in the values of the tested biometric characters of spring wheat plants under conditions of reduced capillary water capacity, in comparison with the control treatment (Table 1). However, significant differences were observed only with respect to grain weight per plant and thousand grain weight.

Table 1  
Selected biometric characters of spring wheat cv. Nawra under water stress conditions (mean values of 2004–2005)

Cultivar	Water capacity of the soil (%)	Plant height (cm)	Number of spikes per plant	Number of grains per spike	Thousand grain weight (g)	Grain weight per plant (g)
Nawra	60–70%	49.60 <sup>A</sup>	2.90 <sup>A</sup>	11.87 <sup>A</sup>	38.7 <sup>B</sup>	1.29 <sup>B</sup>
	30–35%	45.84 <sup>A</sup>	2.26 <sup>A</sup>	12.64 <sup>A</sup>	30.9 <sup>A</sup>	0.77 <sup>A</sup>

Homogeneous groups A, AB, B – according to Fisher's LSD test

KOCOŃ and SULEK (2004) also noted an average yield decline of around 30% under moisture deficiency conditions. Such a decrease results from the plant's response to water stress involving a decrease in the rate of photosynthesis and growth (LU and ZHANG 1998, STARCK 2002).

Crop yield is largely dependent on the photosynthesis process as well as on the transport and distribution of assimilates (AUSTIN et al. 1977, NALBORCZYK 1989, STARCK et al. 1995). The rate of photosynthesis may be limited by almost all adverse environmental factors (STARCK 1995). One of such factors is water shortage in the soil, related to weather conditions.

It was found that water deficit over the period from ear formation to grain filling in the tested cereal species caused a significant decrease in the rate of photosynthesis, particularly noticeable in the first year of the experiment (Table 2). As regards transpiration and intercellular CO<sub>2</sub> concentration, a similar response was observed later, i.e. from the flowering stage to grain filling. Stomatal conductance remained at a comparable level. In the second year the values of all parameters of gas exchange were similar, but the intensity of changes was slightly lower (Table 3).

Table 2  
Gas exchange parameters in spring wheat under water stress conditions in 2004

Cultivar	Capillary water capacity	Photosynthesis ( $\mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$ )			Transpiration ( $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ )			Intercellular $\text{CO}_2$ concentration ( $\mu\text{mol CO}_2\text{mol}^{-1}$ )			Stomatal conductance ( $\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$ )		
		I	II	III	I	II	III	I	II	III	I	II	III
Nawra	60–70%	12.5 <sup>B</sup>	9.4 <sup>B</sup>	6.3 <sup>B</sup>	3.9 <sup>A</sup>	2.2 <sup>B</sup>	1.1 <sup>B</sup>	337 <sup>A</sup>	254 <sup>B</sup>	192 <sup>B</sup>	0.33 <sup>A</sup>	0.19 <sup>A</sup>	0.08 <sup>A</sup>
	30–35%	5.8 <sup>A</sup>	7.1 <sup>A</sup>	4.9 <sup>A</sup>	3.2 <sup>A</sup>	1.3 <sup>A</sup>	0.4 <sup>A</sup>	303 <sup>A</sup>	212 <sup>A</sup>	114 <sup>A</sup>	0.20 <sup>A</sup>	0.14 <sup>A</sup>	0.05 <sup>A</sup>

I – measurement of gas exchange parameters at the ear formation stage;

II – measurement of gas exchange parameters at the flowering stage;

III – measurement of gas exchange parameters at the grain filling stage.

Table 3  
Gas exchange parameters in spring wheat under water stress conditions in 2005

Cultivar	Capillary water capacity	Photosynthesis ( $\mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$ )			Transpiration ( $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ )			Intercellular $\text{CO}_2$ concentration ( $\mu\text{mol CO}_2\text{mol}^{-1}$ )			Stomatal conductance ( $\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$ )		
		I	II	III	I	II	III	I	II	III	I	II	III
Nawra	60–70%	12.5 <sup>B</sup>	8.3 <sup>A</sup>	8.4 <sup>B</sup>	2.4 <sup>A</sup>	1.8 <sup>A</sup>	3.0 <sup>B</sup>	320 <sup>B</sup>	173 <sup>A</sup>	139 <sup>B</sup>	0.38 <sup>A</sup>	0.06 <sup>A</sup>	0.08 <sup>A</sup>
	30–35%	9.6 <sup>A</sup>	7.7 <sup>A</sup>	5.6 <sup>A</sup>	1.9 <sup>A</sup>	1.6 <sup>A</sup>	1.1 <sup>A</sup>	262 <sup>A</sup>	155 <sup>A</sup>	107 <sup>A</sup>	0.15 <sup>A</sup>	0.06 <sup>A</sup>	0.01 <sup>A</sup>

I, II, III – explanations as in Table 2.

The present results are consistent with the findings of PSZCZÓŁKOWSKA et al. (2003) who demonstrated that the rate of photosynthesis decreased in response to water shortage in the soil. A similar trend was observed by OLSZEWSKI et al. (2007) who studied the response of winter wheat to moisture deficiency. Photosynthesis, especially in the flag leaf, is particularly important during kernel formation, when bottom leaves begin to wilt (INOUE et al. 2004), because the rate of this process affects yield height.

Microscopic mycological analyses of wheat grain, performed in 2004, showed the presence of *Chaetomium* spp. only in the water-deficient treatment (Table 4). In 2005 the rate of fungal infection was substantially higher. Fungal isolates were found in both the control and water-deficient treatment. The dominant species was *Penicillium* ssp. The proportion of potentially pathogenic fungi of the genus *Fusarium* was also high. The occurrence of *Fusarium graminearum*, *Fusarium poae* and *Fusarium proliferatum* was confirmed. *Fusarium proliferatum* is a common species in southern Europe (Spain). OLSZEWSKI et al. (2007) reported the presence of this pathogen under greenhouse conditions, which could be related to high temperature levels during the experiment. In our study the number of fungal isolates was slightly

higher in the water-deficient treatment. According to FORDOŃSKI et al. (1994), water stress decreases plant resistance, thus contributing to increased disease incidence.

Table 4  
Number of fungal isolates in spring wheat grain cv. Nawra under water stress conditions in the years 2004–2005

Fungal species	Control	Water stress
	60–70% capillary water capacity	30–35% capillary water capacity
2004		
<i>Chaetomium</i> spp.		1
<b>Total</b>		<b>1</b>
2005		
<i>Colletotrichum graminearum</i>		1
<i>Fusarium graminearum</i>		1
<i>Fusarium poae</i> (Peck) Wollenw.	4	3
<i>Fusarium proliferatum</i>	3	3
<i>Mucor</i> spp.		1
<i>Penicillium</i> spp.	23	27
<b>Total</b>	<b>30</b>	<b>36</b>

60–70% capillary water capacity – control  
30–35% capillary water capacity – water stress

The results of microscopic examinations were partly confirmed by BIO-PCR analyses with the use of SCAR primers (Figure 1). The PCR products indicated the presence of fungi of the genus *Fusarium*. Further analyses revealed the occurrence of *Fusarium poae* in the second year of the study (Figure 2), as indicated by a PCR product of 220 bp. Similar results were obtained by PARRY and NICHOLSON (1996).

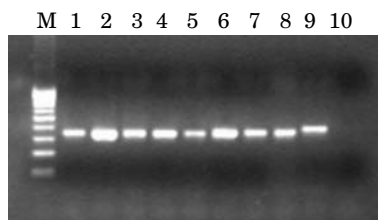


Fig. 1. PCR product obtained from spring wheat grain with the use of species-specific primers (PL58SL/PL28SL) for *Fusarium* spp. under water stress conditions in 2005. M – molecular weight standard 1–5 spring wheat cv. Nawra, control treatment, 6–10 – spring wheat cv. Nawra, water stress

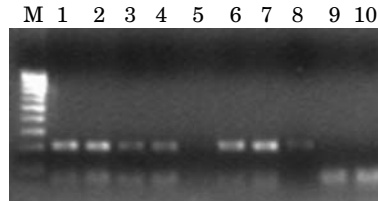


Fig. 2. PCR product obtained from spring wheat grain with the use of species-specific primers (Fp82R/Fp82R) for *Fusarium poae* under water stress conditions in 2005. M – molecular weight standard 1–5 spring wheat cv. Nawra, control treatment, 6–10 – spring wheat cv. Nawra, water stress

SCAR primers do not amplify plant DNA, but they permit pathogen identification directly in the host tissue, with no need for pure culture isolation. They also allow to detect infection at an early stage, and to recognize tissue damage prior to the onset of disease symptoms (CHEŁKOWSKI and WITKOWSKA 1999). The BIO-PCR technique enables to confirm the presence of live pathogen inoculum with potential infectious properties.

It should be stressed that PCR analyses may be an effective tool for presymptomatic diagnostics in plants, applied prior to the appearance of the signs and symptoms of a disease. According to reference data, in some cases pathogens had been identified before disease symptoms became apparent (TURNER et al. 1998).

## Conclusions

1. Water deficit did not decrease the values of the investigated biometric characters of spring wheat plants, but it contributed to a drop in thousand grain weight and grain weight per plant.

2. Water stress resulted in a decrease in the values of gas exchange parameters, particularly in the rate of photosynthesis in the leaves of spring wheat.

3. Members of the genus *Penicillium* and toxin-producing fungi of the genus *Fusarium* were identified in wheat kernels. The rate of wheat grain colonization by fungal pathogens was slightly higher under water stress conditions.

4. The use of the BIO-PCR technique with species-specific SCAR primers permitted the detection of *Fusarium poae* in wheat grain.

## References

- AUSTIN R.B., ERICH J.A., FORD M.A., BLACKWELL R.D. 1977. *The fate of the dry matter, carbohydrates and <sup>14</sup>C lost from the leaves and stems of the wheat during grain filling*. Ann. Bot., 41: 1309–1321.
- BOCZEK J., SZLENDAK E. 1992. *Wpływ stresów roślinnych na porażenie roślin przez szkodniki*. Postępy Nauk Rolniczych. PAN Wydział Nauk Rolniczych i Leśnych, 2(237): 1–17.
- CHEŁKOWSKI J., WITKOWSKA I. 1999. *Identyfikacja patogenów grzybowych zbóż i badanie ich różnorodności genetycznej za pomocą łańcuchowej reakcji polimerazy (PCR)*. Postępy Nauk Rolniczych, 4: 49–60.
- ELLIS M.B. 1971. *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute Kew, Surrey, England.
- FORDOŃSKI G., GÓRECKI R.J., BIENIASZEWSKI T., MAJCHRZAK B. 1994. *Wpływ tiuramu na kiełkowanie, wigor nasion i zdrowotność siewek roślin strączkowych w warunkach stresu chłodnowodnego*. Mat. konf. Uszlachetnianie materiałów nasiennych. PAN, ART Olsztyn, 1994: 81–88.
- GILMAN J.C. 1957. *A manual of soil fungi*. The Iowa State University, Ames USA.
- GRZESIUK S., GÓRECKI R. J. 1994. *Fizjologia plonów*. ART. Olsztyn.
- GRZESIUK S., KOCZOWSKA I., GÓRECKI R.J. 1999. *Fizjologiczne podstawy odporności roślin na choroby*. Wyd. II, ART Olsztyn.
- HUE F.X., HUERRE M., ROUFFAULT M.A., BIEVRE C. 1999. *Specific Detection of Fusarium species in blood and tissues by PCR Technique*. J. Clin. Microbiol., 37(8): 2434–2438.
- INOUE T., INANAGA S., SUGIMOTO Y., AN P., ENEYI A.E. 2004. *Effect of drought on ear and flag leaf photosynthesis of two wheat cultivars differing in drought resistance*. Photosynthetica, 42(4): 559–565.
- KACPEKSKA A. 1991. *Odporność roślin na stresowe abiotyczne czynniki środowiska i metody jej oceny*. Post. Nauk Rol., 91(1–2): 21–32.
- KOCOŃ A., SULEK A. 2004. *Wpływ nawożenia azotem na plon i niektóre parametry jakościowe ziarna pszenicy jarej rosnącej w warunkach niedoboru wody w podłożu*. Ann. Univ. Mariae Curie-Skłodowska, E Agric. LIX(1): 471–478.
- KWAŚNA H., CHEŁKOWSKI J., ZAJKOWSKI P. 1991. *Grzyby*. W: *Flora polska*, t. XXII. PAN Instytut Botaniki Warszawa-Kraków.
- LU C.M., ZHANG J.H. 1998. *Effects of water stress on photosynthesis, chlorophyll fluorescence and photoinhibition in wheat plants*. Austr. J. Plant. Physiol., 25: 883–892.
- NALBORCZYK E. 1989. *Fizjologiczne podstawy produktywności roślin*. Biul. IHAR, 171–172: 133–134.
- NICHOLSON P., LEES A.K., MAURIN N., PARRY D.W., REZANOOR H.N. 1996. *Development of PCR assay to identify and quantify Microdochium nivale var. nivale and Microdochium nivale var. majus in wheat*. Physiol. Mol. Plant Pathol., 48: 257–271.
- OLSZEWSKI J., PSZCZÓLKOWSKA A., KULIK T., FORDOŃSKI G., PŁODZIEN K., OKORSKI A., WASIELEWSKA J. 2007. *Wpływ deficytu wodnego na wskaźniki wymiany gazowej, produktywność i zdrowotność ziarna pszenicy ozimej*. Acta Sci. Pol., Agricul., 6(4): 33–42.
- OZTURK A., AYOLIN F. 2004. *Effect of water stress at various growth stages on some quality characteristics of winter wheat*. J. Agron. Crop Sci., 190: 93–99.
- PARRY D.W., NICHOLSON P. 1996. *Development of a PCR assay to detect Fusarium poae in wheat*. Plant Pathology, 45: 383–391.
- PSZCZÓLKOWSKA A., OLSZEWSKI J., FORDOŃSKI G., PŁODZIEN K. 2003. *Wpływ stresu wodnego i mineralnego na zdrowotność nasion wybranych odmian grochu siewnego i łubinu żółtego*. Acta Sci. Pol. Agric., 2(1): 101–113.
- STARCK Z. 1995. *Współzależność pomiędzy fotosyntezą i dystrybucją asymilatów a tolerancją roślin na niekorzystne warunki środowiska*. Post. Nauk Rol., 3: 19–35.
- STARCK Z. 2002. *Mechanizmy integracji procesów fotosyntezy i dystrybucji biomasy w niekorzystnych warunkach środowiska*. Zesz. Prob. Post. Nauk Rol., 481: 111–123.
- STARCK Z., CHOLUJ D., NIEMYSKA B. 1995. *Fizjologiczne reakcje roślin na niekorzystne czynniki środowiska*. Wyd. SGGW, Warszawa.
- TURNER A.S., LEES A.K., REZANOOR H.N., NICHOLSON P. 1998. *Refinement of PCR – detection of Fusarium avenaceum and evidence from DNA marker studies for phenetic relatedness to Fusarium tricinctum*. Plant Pathology, 47: 278–288.