Gas exchange and *Triticum* sp. with different ploidy in relation to irradiance

V. Hejnák, H. Hniličková, F. Hnilička, J. Andr

Department of Botany and Plant Physiology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic

ABSTRACT

Different species of *Triticum* were grown during a greenhouse experiment, including *T. monococcum* L., *T. dicoccum* Schrank, *T. durum* Desf., *T. spelta* L. and *T. aestivum* cv. Vánek. The goal was to establish the influence of irradiance on the parameters of photosynthetic performance in relation to their ploidy. Photosynthetic rate (P_n), transpiration (E) and stomatal conductance (g_s) were measured at irradiance ranging from 217–1305 µmol/m^2/s. In all monitored species, saturation irradiance for photosynthesis at the level of 609 µmol/m^2/s was reached. The highest average P_n was measured in the diploid *T. monococcum* (32.5 µmol CO_2/m^2/s) while the lowest P_n occurred in the hexaploid *T. spelta* (22.0 µmol CO_2/m^2/s). The P_n in hexaploid *T. aestivum* (29.6 µmol CO_2/m^2/s) was comparable with the tetraploid *T. durum*. Similarly, E also decreased with the increase of ploidy. The highest g_s was measured in *T. durum* (1.03 mol CO_2/m^2/s) and *T. aestivum* (0.99 mol CO_2/m^2/s). In all monitored species a close linear dependency was recorded between P_n and g_s. Species with lower ploidy reach maximum values of P_n with lower g_s. Hexaploid *T. aestivum* and tetraploid *T. durum*, require higher g_s in order to achieve higher P_n and yet they do not reach the P_n values of species with lower ploidy.

Keywords: wheat; photosynthetically active radiation; polyploid; phenotypic plasticity; genotypes; photosynthesis-irradiance curves

Species of the genus *Triticum* primarily exist in three ploidy levels – diploid, tetraploid and hexaploid. Polyploids are also associated with greater phenotypic plasticity, which may increase their range of ecological tolerance (Otto and Whitton 2000, Ramsey 2011). As a result of genome polyploidization, new species have been created, which may show different adaptive responses to the changing environment (Wendel 2000). In the process of genetic improvement of bread wheat, photosynthetic rate per unit leaf area has decreased significantly from the net photosynthetic rate (P_n) of the crop's diploid ancestors (donors of the A, B, and D genomes that make up hexaploid bread wheat) (Huang et al. 2007).

In the case of *Triticum* sp., the most discussed factors causing changes in photosynthetic rate during the evolution of this genus were higher leaf area, as well as a large number of stoma. Vyas et al. (2007) and Hull-Sanders et al. (2009) state that the photosynthesis plays a fundamental role in plant fitness and is a prime example of how polyploids can differ dramatically from their diploid progenitors. According to Austin et al. (1980) and LeCain et al. (1989) the photosynthesis rate correlated strongly negatively with leaf area, leaf width and the mean plan area per mesophyll cell and correlated positively with stomatal frequency and number of veins per mm of leaf width. The diploid wheat species had the highest stomatal frequency and the lowest stomatal length and width. The hexaploid species had a lower stomatal frequency than the tetraploid species. Diploid and hexaploid wheat have significantly higher water use efficiency, compared to tetraploid wheat (Khazaei et al. 2010).

Li et al. (2008) state that as ploidy increased, the stomatal length, width, perimeter and area were found to increase. The stomatal density was found to decrease in A, B, D genomes, while no differences were found in stomatal indices among ploidy levels, indicating that the stomata became larger, but were still fewer, during the evolution progress. High correlation was observed between photosynthetic rate and stomatal conductance.

It is known that photosynthetic rate is also influenced by the genotype, water availability, growth phase, mineral nutrition, etc. (Shinozaki and Dennis 2003, Apel and Hirt 2004). The factor given by the very essence of photosynthetic reaction is light radiance. Marshall and Biscoe (1980) expressed the mutual relation between photosynthesis and radiance in the case of wheat using a non-rectangular hyperbole. The maximum effectiveness of use of light radiance is reached during lower light intensity levels. At full sun, plants may suffer from absorbing excessive amounts of light energy. This results in a decrease of the process of transforming light energy into chemical bonds and the inception of photoinhibition (Muller et al. 2001, Ort 2001). According to Austin et al. (1986), the levels of some diploid species of wheat have higher rates of light-saturated photosynthesis than hexaploid bread wheat.

The goal of the experiment was to evaluate the influence of different radiance on photosynthetic performance and establish the relationship between photosynthetic rate and stomatal conductance in selected monitored samples of the *Triticum* species with different ploidy.

MATERIAL AND METHODS

Plant material, growth and experimental conditions. Samples of *Triticum* sp. with different ploidy were selected for monitoring: *T. monococcum* L., *T. dicoccum* Schrank, *T. durum* Desf., *T. spelta* L., *T. aestivum* L. cv. Vánek (Gene Bank of the Crop Research Institute in Prague-Ruzyně). The experiments took place during 2010–2014 in the greenhouse of the Czech University of Life Sciences in Prague.

They were conducted in semi-controlled conditions (natural light conditions, air temperature 23 ± 2°C/16 ± 2°C day/night, relative air humidity 65% min and 85% max). The juvenile plants were grown in containers with the volume of 2 dm^3 in siliceous sand with a grain size of 1–2 mm. Fifteen plants were grown in each container. Each species was grown in four repetitions. The plants were watered twice a week with a complete nutritious solution (Knop's solution) in the amount of 300 mL. The remaining days of the week plants were watered with distilled water according to gravimetric determination of the water loss in every individual container. Water content was maintained by watering at the level of 70% of soil water capacity.

Leaf gas exchange measurements. The net photosynthetic rate (P_n), the rate of transpiration (E) and stomatal conductance (g_s) were measured on the second leaf from the top in juvenile plants in a development stage of 13–15 DC (Zadoks scale; Zadoks et al. 1974), using the LCpro+ portable gas exchange system (ADC BioScientific Ltd., Hoddesdon, UK). Gas exchange was measured from 8:00 A.M. to 11:30 A.M., Central European Time.

Irradiance was 217, 348, 609, 870, 1087 and 1305 $\mu mol/m^2/s$ of photosynthetically active radiation (PAR), the temperature in the measurement chamber was 21°C, the CO_2 concentration was 420 ± 35 vpm ($\mu mol/mol$), the air flow rate was 205 ± 30 $\mu mol/s$ and the duration of the measurement for each sample was a 15 min interval after the establishment of steady-state conditions inside the measurement chamber. The measurements of these parameters were done repeatedly on three leaves randomly taken from four plants.

Statistical analysis. A statistical evaluation of the experiment was made using the analysis of variance (ANOVA) and the values obtained were compared in further detail, using the Tukey's test at the significance level $P < 0.05$. Statistical analyses were performed using Statistica 9.0 CZ for MS Windows software (Tulsa, USA).

RESULTS AND DISCUSSION

When leaves are exposed to increased irradiance, the rate of absorbing CO_2 increases, at first in proportion to the amount of irradiance, then more slowly up to the maximum value of its absorption. The dependency of pure photosynthesis on irradiance may be expressed by the saturation curve. When reaching irradiance saturation (I_s), the photosynthetic reaction is saturated by light

and the photosynthesis either further increases only slightly or not at all (Larcher 2003).

Figure 1a shows saturation curves for the individual test species. The saturation irradiance at the level of 609 μmol/m²/s was reached in all species of *Triticum* sp. The differences in P_n for this irradiance and the subsequent higher values of irradiance were statistically insignificant in all test species. *T. monococcum* had the highest P_n upon reaching its saturation irradiance (34.3–37.6 μmol CO_2/m²/s). The lowest P_n at saturation irradiance was measured in *T. spelta* (23.1–25.5 μmol CO_2/m²/s). There are several reports in the literature indicating that the flag leaves of some diploid ancestor species have a P_{max} up to 40% greater than those of modern wheat cultivars (Austin et al. 1982, 1986).

Austin (1990) and Kaminski et al. (1990) state that lower values of P_{max} were reached in hexaploid species than in tetraploid species. Our results show that P_n at saturation irradiance in the case of hexaploid *T. aestivum* (31.1–33.9 μmol CO_2/m²/s)

reached comparable values of the tetraploid *T. durum* (30.0–32.8 μmol CO_2/m²/s).

Ye and Yu (2008) state for *T. aestivum* the value of P_{max} 22.8 μmol CO_2/m²/s (at 25°C and concentration of CO_2 350 μmol/mol). The photoinhibiting effect of light irradiance that would result in the decrease of P_n was not recorded in any of the monitored species in case of higher values of irradiance. As stated by Krause (1988) and Osmond (1994), photoinhibition has been defined as a light-dependent decrease in photosynthetic efficiency that may or may not be associated with a decrease in P_{max} as a result of the absorption of excess light energy.

According to Shimazaki et al. (2007) the opening of stomata is influenced by the mutual coordination of light signalling, light-energy conversion, membrane ion transport and metabolic activity in guard cells. With the increasing irradiance at constant temperature and humidity, the stomatal conductance increased in all test species. In cases
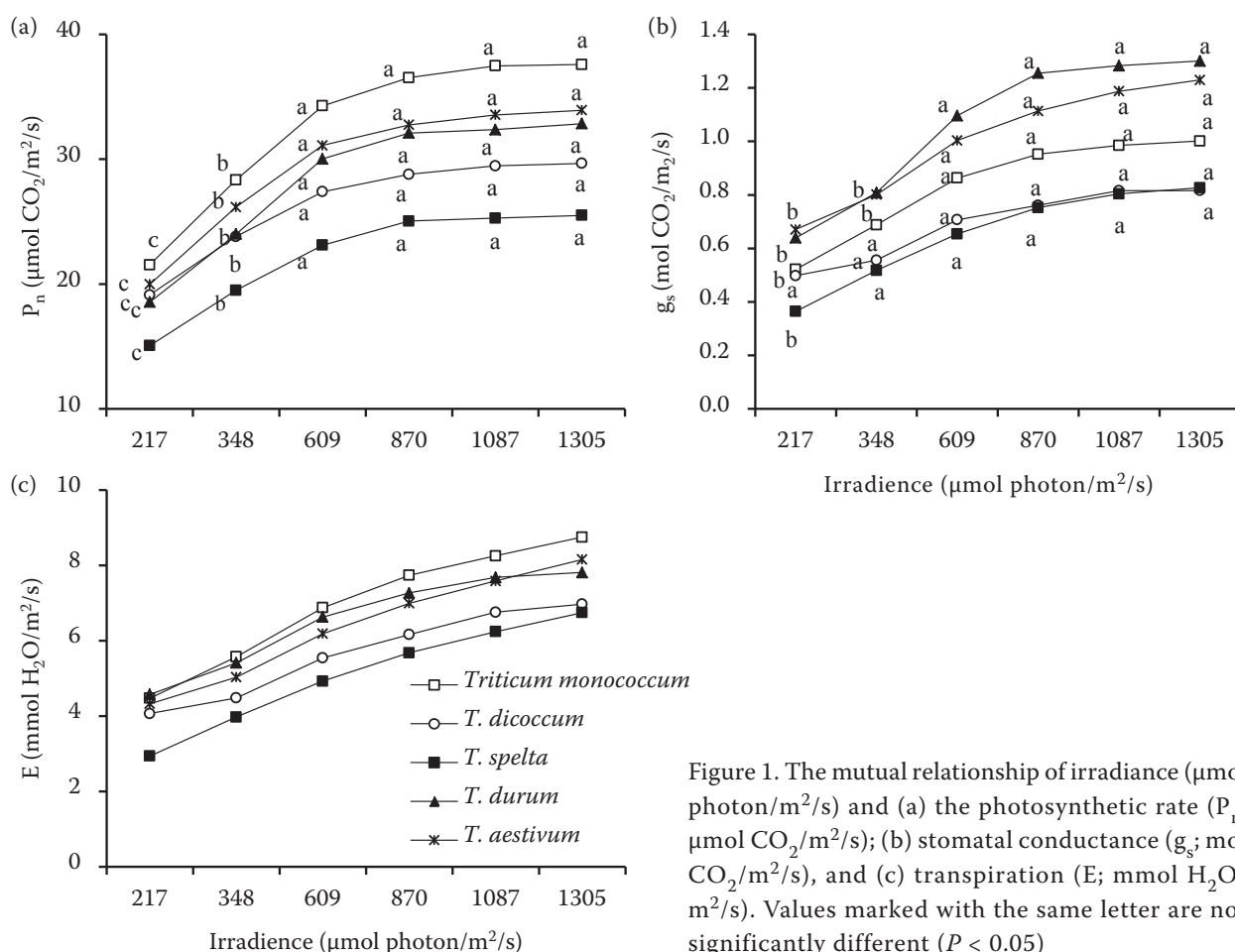

Figure 1. The mutual relationship of irradiance (μmol photon/m²/s) and (a) the photosynthetic rate (P_n; μmol CO_2/m²/s); (b) stomatal conductance (g_s; mol CO_2/m²/s), and (c) transpiration (E; mmol H_2O/m²/s). Values marked with the same letter are not significantly different ($P < 0.05$)

Table 1. Photosynthetic rate (P_n; μmol CO_2/m^2/s), transpiration (E; mmol H_2O/m^2/s), stomatal conductance (g_s; mol CO_2/m^2/s). The stated values are the average ± standard error

Ploidy	Genome		P_n	E	g_s
2n = 2x = 14	AA	*Triticum monococcum* L.	32.5 ± 0.67[a]	6.9 ± 0.67[ab]	0.83 ± 0.026[b]
2n = 4x = 28	AABB	*Triticum dicoccum* Schrank	26.0 ± 0.56[c]	5.6 ± 0.20[c]	0.69 ± 0.024[c]
2n = 4x = 28	AABB	*Triticum durum* Desf.	28.3 ± 0.66[b]	6.4 ± 0.19[b]	1.03 ± 0.046[a]
2n = 6x = 42	AABBDD	*Triticum spelta* L.	22.0 ± 0.50[d]	5.0 ± 0.18[c]	0.64 ± 0.028[c]
2n = 6x = 42	AABBDD	*Triticum aestivum* L.	29.6 ± 0.54[b]	6.4 ± 0.17[b]	0.99 ± 0.034[a]

Values within a column marked with the same letter are not significantly different ($P < 0.05$)

of *T. monococcum* and *T. spelta,* no significant changes in g_s were measured at the level of irradiance 348–1305 μmol/m^2/s and in *T. durum* and *T. aestivum* at the level of irradiance 609–1305 μmol/m^2/s. In *T. dicoccum* the increase of g_s in relation to irradiance was not significant (Figure 1b).

As stated in many studies, the density of stomata decreases with the increase of ploidy level in *Triticum* sp., while the width and length of the stomatal opening increase (McFadden and Sears 1984, Evans and Seemann 1984). Despite this Li et al. (2008) state that there is no mutual relation between stomatal characteristics and stomatal conductance.

The average of the measured values of physiological parameters are shown in Table 1. The highest P_n was measured in *T. monococcum* (32.5 μmol CO_2/m^2/s), while the lowest was in *T. spelta* (22.0 μmol CO_2/m^2/s). As is apparent from the results, the evolutionary trend toward a decrease of P_n in relation to ploidy level was confirmed. With the exception of *T. aestivum* (29.6 μmol CO_2/m^2/s), where a number of authors state lower values of P_n in their experiments compared to diploid and tetraploid species (Austin et al. 1982, Johnson et al. 1987, Li et al. 2008).

Contrary to that, Wang and Zhang (2015) indicate that with increasing ploidy level, the P_n did not significantly change. Wang et al. (2008) state that the cultivars bred in 1990s had higher P_n than cultivars bred in the 1950s through 1970s. The higher P_n in the case of *T. aestivum*, despite being a hexaploid species, may be explained by the influence of the genotype of the given cultivar Vánek. Many studies focus on the subject of influence of parent-species genome of parent species on individual physiological parameters. Yet, according to Watanabe et al. (1997), the pho-

tosynthesis of polyploids was not dependent on P_n of the donor genomes.

Similar results were measured in regard to transpiration rate, where the highest E was measured in *T. monococcum* (6.9 mmol H_2O/m^2/s) while the lowest occurred in *T. spelta* (5.0 mmol H_2O/m^2/s) and *T. dicoccum* (5.6 mmol H_2O/m^2/s) (Table 1 and Figure 1c).

The measured values of g_s also show differences between individual species. *T. durum* (1.03 mol CO_2/m^2/s) and *T. aestivum* (0.99 mol CO_2/m^2/s) had the highest g_s. To the contrary, the lowest values were measured in *T. dicoccum* (0.69 mol CO_2/m^2/s) and *T. spelta* (0.64 mol CO_2/m^2/s) (Table 1). The acquired results do not correspond with the opinion that hexaploid species with larger stoma have lower g_s, while diploid species with large amounts of small stomata have higher g_s (Li et al. 2008). To the contrary, Wang and Zhang (2015) state that with increased ploidy level, the E and g_s increase.

Many studies focus on the mutual relationship between P_n and g_s (Farquhar and Sharkey 1982, Jarvis and Davies 1998) and the modelling of the course of this dependency (Yu et al. 2004, Ye and Yu 2008). As stated by Li et al. (2008), there is a close dependency between P_n and g_s. In the case of all species, a close linear dependency was recorded between P_n and g_s (Figure 2 and Table 2). In a number of physiological and ecological studies, the linear dependency between P_n and g_s is described according to the Ball-Berry model (Ball et al. 1987).

Other models concerning relationships between P_n and g_s, while simulating different ecological conditions, are also based on this model. As apparent from Figure 2, the types of wheat with lower ploidy level (*T. monococcum)* reach maximum values of P_n with lower g_s. To the contrary, species with
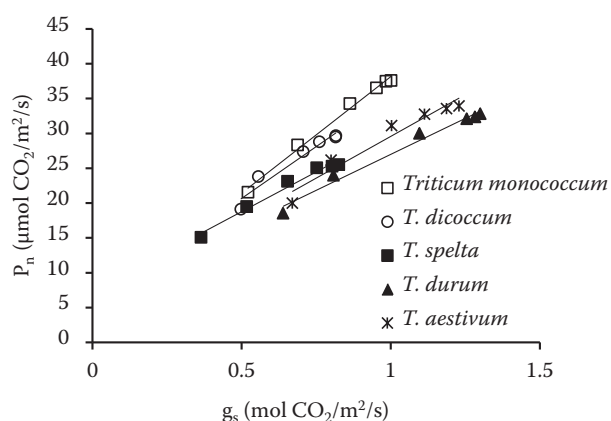
Figure 2. Mutual relationship of photosynthetic rate (P_n; µmol CO_2/m²/s) and stomatal conductance (g_s; mol CO_2/m²/s) in monitored species

Table 2. The characteristic of the course of the linear dependency of the photosynthetic rate (P_n) and stomatal conductance (g_s) in monitored species

	$P_n - g_s$
Triticum monococcum L.	$y = 33.411x + 4.7201$; $r^2 = 0.99$; $P < 0.05$
T. dicoccum Schrank	$y = 29.693x + 5.8195$; $r^2 = 0.93$; $P < 0.05$
T. durum Desf.	$y = 20.628x + 6.3738$; $r^2 = 0.98$; $P < 0.05$
T. spelta L.	$y = 22.77x + 7.3829$; $r^2 = 0.97$; $P < 0.05$
T. aestivum L.	$y = 23.896x + 5.666$; $r^2 = 0.94$; $P < 0.05$

higher ploidy levels (*T. aestivum*), including the tetraploid *T. durum*, require higher g_s in order to reach maximum P_n and yet still fail to reach such high values of P_n as species with lower ploidy. Species *T. dicoccum* and *T. spelta* require only low values of g_s in order to reach their maximum P_n.

As is apparent from the obtained results, the parameters for gas exchange are different in the individual monitored specimens of the *Triticum* sp. Higher values of P_n and E were measured in species with lower ploidy (diploid and tetraploid). The experiment failed to prove a lower P_n in the hexaploid species *T. aestivum.* Maximum P_n was measured in all monitored *Triticum* species during the saturation irradiance at the level of 609 µmol/m²/s while, in all species, a close dependency between photosynthesis and stomatal conductance was proven.

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Corresponding author:

Ing. Helena Hniličková, Ph.D., Česká zemědělská univerzita v Praze, Fakulta agrobiologie, potravinových a přírodních zdrojů, Katedra botaniky a fyziologie rostlin, Kamýcká 129, 165 21 Praha 6 – Suchdol, Česká republika; e-mail: hnilickova@af.czu.cz