



Wheat plant selection for high yields entailed improvement of leaf anatomical and biochemical traits including tolerance to non-optimal temperature conditions

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Abstract

Assessment of photosynthetic traits and temperature tolerance was performed on field-grown modern genotype (MG), and the local landrace (LR) of wheat (*Triticum aestivum* L.) as well as the wild relative species (*Aegilops cylindrica* Host.). The comparison was based on measurements of the gas exchange (A/c_i , light and temperature response curves), slow and fast chlorophyll fluorescence kinetics, and some growth and leaf parameters. In MG, we observed the highest CO_2 assimilation rate (A_{CO_2}), electron transport rate (J_{max}) and maximum carboxylation rate (V_{Cmax}). The *Aegilops* leaves had substantially lower values of all photosynthetic parameters; this fact correlated with its lower biomass production. The mesophyll conductance was almost the same in *Aegilops* and MG, despite the significant differences in leaf phenotype. In contrary, in LR with a higher dry mass per leaf area, the half mesophyll conductance (g_m) values indicated more limited CO_2 diffusion. In *Aegilops*, we found much lower carboxylation capacity; this can be attributed mainly to thin leaves and lower Rubisco activity. The difference in CO_2 assimilation rate between MG and others was diminished because of its higher mitochondrial respiration activity indicating more intense metabolism. Assessment of temperature response showed lower temperature optimum and a narrow ecological valence (i.e., the range determining the tolerance limits of a species to an environmental factor) in *Aegilops*. In addition, analysis of photosynthetic thermostability identified the LR as the most sensitive. Our results support the idea that the selection for high yields was accompanied by the increase of photosynthetic productivity through unintentional improvement of leaf anatomical and biochemical traits including tolerance to non-optimal temperature conditions.

Keywords Wheat · Landrace · *Aegilops* · Photosynthesis · Mesophyll conductance · Heat stress

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Abbreviations

A_{CO_2}	CO_2 assimilation rate
c_a	Reference CO_2 concentration
c_i	Intercellular CO_2 concentration
F_0	Basal fluorescence
F_v/F_m	Maximum quantum yield of photosystem II photochemistry
g_m	Mesophyll conductance
J_{max}	Electron transport rate
LMA	Dry mass per leaf area
LR	Local landrace
MG	Modern genotype
PPFD	Photosynthetic photon flux density
PSII	Photosystem II
RH	Relative air humidity
(V_{cmax})	Maximum carboxylation rate
VPD	Vapor pressure deficit
WR	Wild relative

Introduction

Photosynthesis is the key process necessary for plant production. Therefore, many crop scientists have believed that enhancing photosynthesis at the level of the single leaf would increase yields (Makino 2011). Several studies have used a series of wheats to examine the changes in photosynthetic capacity that have occurred with domestication and the increase in ploidy. Environmental stress may decrease the rate of photosynthesis not only because of detrimental effects on cell biochemistry, but also because of changes in the diffusion of carbon dioxide (CO_2) from the atmosphere to the site of carboxylation (Flexas et al. 2008; Warren 2008). Under high CO_2 levels, the rate of diffusion of CO_2 into the cell increases due to a high concentration gradient; consequently, the uptake of CO_2 through stomata and stomatal conductance increases (Reynolds et al. 2010). Even under normal conditions, the slow diffusion of CO_2 to the site of carboxylation in the chloroplast can significantly limit photosynthesis (Flexas et al. 2008; Warren 2008; Zhu et al. 2010). The diffusion of CO_2 between intercellular airspaces and the Rubisco enzyme is usually described by a parameter called internal or mesophyll conductance (g_m). Different species or genotypes show substantial variation in g_m , and the limiting effects of g_m on photosynthesis are close to that of stomatal conductance (Flexas et al. 2008; Warren 2008; Barbour et al. 2010).

In wheat evolution two major periods were recognized. The first period represents a thousand years of coincidental improvement before the nineteenth century from wild wheat ancestors to local landraces (LRs), well adapted, but generally low-yielding. The systematic breeding starting

later led to gradual improvement of key traits, resulting in release of recent high-yielding modern genotypes (MGs). The history of wheat cultivation is very old. About 10,000 BP, hunter-gatherers began to cultivate wild emmer. Sub-conscious selection gradually created a cultivated emmer (*T. dicoccum*, $2n=4x=28$, genome AuAuBB) that spontaneously hybridized with another goat grass (*Ae. tauschii*, $2n=2x=14$, genome DD) around 9000 BP to produce an early spelt (*T. spelta*, $2n=6x=42$, genome AuAuBBDD) and in next period also another wheat taxa (Kihara 1944; McFadden and Sears 1946; Kerber 1964; Kislev 1980; Dvorak et al. 1998; Matsuoka and Nasuda 2004).

As a modern bread wheat (*Triticum aestivum* L.) cultivars, we denote the semidwarf cultivars carrying Rht1 and Rht2 genes varieties. Release of these genotypes after 1965 led to significant increase of wheat production worldwide, causing that most of cultivated varieties have partly similar genetic background (Smale et al. 2002). By introduction of these new, modern varieties, the harvest index was improved and higher yield potential of new genotypes was obtained. In addition, the breeding was focused on traits such as resistance to pathogens, technological quality of grains, etc. However, the conventional selection practiced by the majority of breeders has not considered directly the physiological or biochemical traits and certainly not the photosynthetic traits. Anyway, it is well known that the yield-based breeding, even in the period before the green revolution, led to the unintentional improvement of many traits.

On the other hand, there are hundreds of thousands of landraces, the local cultivars within the *T. aestivum* L. species not belonging to the group of MGs that constitute the wheats of the world. Most of them are not cultivated in present for agricultural use and they are concentrated in genebanks, which protect them as a heritage. They represent genetic resources of enormous diversity, potentially useful as donors of important traits (Hoisington et al. 1999).

In addition to *Triticum* accessions, the second group of resources useful in wheat breeding represents wild relatives (WRs). The closest relative of wheat growing in Central Europe is the tetraploid jointed goatgrass (*Aegilops cylindrica* Host.). As in the case of other polyploid *Triticeae*, *Ae. cylindrica* is an amphiploidy (genome CCDD), resulting from hybridization between the diploids *Ae. caudata* (CC) and I (DD). There are numerous local forms in Central, but mostly and South Europe and in the Middle East (Guadagnuolo et al. 2001).

Recently, the advanced technical tools for rapid measurements and diagnostics of plants are available. The gas exchange systems enable to perform exact measurements even in field conditions. Moreover, the more precise photosynthetic models were developed, based mainly on the Farquhar–von Caemmerer–Berry Model (Farquhar et al. 1980), which enable to identify the main limitations of

CO₂ assimilation (Long and Bernacchi 2003; Flexas et al. 2004). Recently, the gas exchange measurements are supported by continuous measurements of chlorophyll fluorescence that provide quite a precise estimation of electron transport rate (Flexas et al. 2007; Zivcak et al. 2013). Moreover, chlorophyll fluorescence represents a unique tool for diagnostics of plant health status, photosynthetic performance as well as effects of plant stress on plants (Zivcak et al. 2008; Brestic et al. 2016; Kalaji et al. 2017) and assessment of plant stress tolerance (Li et al. 2014; Kalaji et al. 2016), for review on measuring stress effects on crops, see Brestic and Zivcak (2013).

The aim of our experiments was to apply modern technical and methodical approaches to identify differences in photosynthetic performance, temperature tolerance, and limitations of photosynthetic assimilation (particularly to limitations by mesophyll conductance) in plant material representing different stages of wheat improvement. The MG represents the result of recent breeding programs; it is characterized by low height, favorable canopy architecture and high yields. The LR represents the results of the long period of domestication and intuitive, unconscious selection in local conditions, recently available only in gene banks. Moreover, a secondary WR of genus *Aegilops* was used, which represents the material coming from wild populations, being similar to the status before beginning of wheat cultivation in the past.

Materials and methods

Experimental setup

Experiments with field grown (field trials, Genebank of PPRC-RIPP in Piešťany, Slovak Republic) modern wheat variety ‘Astella’, LR ‘Diosecka’ (both *T. aestivum* L.) and its WR species *Ae. cylindrica* Host. were realized in the regular growing season. The seeds were sown in autumn directly to the soil manually into experimental plots with the area 1.5 m². Previous crop was field peas (*Pisum sativum* spp. *arvense*). In the autumn, plants were fertilized with 330 kg ha⁻¹ NPK (15-15-15), 150 kg ha⁻¹ AMOFOS fertilizer (12% N, 52% P₂O₃) and 120 kg ha⁻¹ potassium chloride (60% K₂O). Spring fertilization was realized in the dose of 110 kg ha⁻¹ of ammonium nitrate with limestone (27% N). Also, the autumn and spring spray against weeds were realized in optimal doses. Average temperature in the monitored period was 17.1 °C; the average soil temperature was 18.3 °C with an average daily sum of rainfalls 3.3 mm; soil humidity 32.5%; air humidity 44.8%.

Analyses of growth and leaf traits

The growth and leaf traits were analyzed regularly during the season. Ten plants of each genotype were taken, measured, and analyzed in laboratory. Only the results recorded after anthesis showing the maximum height, dry mass level and traits of fully developed flag leaves are shown in this paper. Dry mass and leaf area were analyzed for leaves and stems individually, the flag leaf of the main stem was measured separately. The average maximum height, total plant dry mass, flag leaf area, and dry mass per leaf area (LMA) are presented. LMA was calculated as the ratio of flag leaf dry weight and flag leaf area (g m⁻²) according to Radford (1967) and Hunt (1978).

Gas exchange measurements

Measurements of photosynthetic gas exchange were realized with utilization of the open infrared gas analyzer (CIRAS-2; PP-Systems, Hitchin, UK). The net CO₂ assimilation rate (A_{CO_2}), stomatal conductance (g_s), and intercellular CO₂ concentration (c_i) were estimated. All parameters were measured on the flag leaf 3–12 days after anthesis. The measurements were done in laboratory conditions, immediately after cubic intact block of soil with plants was dug out with care, minimizing disturbance to the main part of the root system up to 30 cm depth. The plants in the central position within the block were measured. To avoid the influence of plant removal on the results of gas exchange measurements, only the results obtained on the plants able to fully open the stomata were used for analyses.

Light photosynthetic response curves were measured at leaf temperature set on 25 °C and reference CO₂ concentration (c_a) set to 380 μmol mol⁻¹ (ppm) of CO₂. Photosynthetic photon flux density (PPFD) during light curve was 50, 100, 150, 200, 300, 400, 600, 800, 1000, and 1200 μmol (photons) m⁻² s⁻¹.

Temperature photosynthetic response curves were measured at reference CO₂ concentration (c_a) set to 380 μmol mol⁻¹ (ppm) of CO₂, PPFD was 1000 μmol (photons) m² s⁻¹. Leaf temperature was controlled by leaf chamber, measured by IR sensor and the photosynthetic rates were measured at 10, 15, 20, 25, 30, 35, and 40 °C after 10 min at each temperature.

Air humidity was set to approximate value 60% of RH. In a range of 10–25 °C, air humidity was maintained between 55 and 65% (VPD 0.35–1.0 kPa). At high temperature, the air humidity in the chamber was decreasing; therefore, the air entering the system was humidified. Nevertheless, the relative air humidity at high temperature levels was decreasing below the set value of 60%; for example, at 40 °C the RH in measuring chamber was ~45% (VPD 3.3 kPa). Anyway, as the tested plants were acclimated to high temperature and

low air humidity in field conditions, the high VPD at high temperature level was not causing significant stomata closure within the time intervals of the measurements at high temperature levels.

A/c_i photosynthetic response curves were measured at leaf temperature set on 25 °C and PPFD 1000 μmol (photons) $\text{m}^{-2} \text{s}^{-1}$. Reference CO_2 concentration (c_a) was set to 50, 100, 150, 200, 250, 300, 380, 500, 800, 1000, 1500, and 1800 $\mu\text{mol mol}^{-1}$ (ppm) during A/c_i photosynthesis response curve. Relative air humidity was ~60% (VPD ~0.9 kPa).

To analyze photosynthetic limitations and capacities, the data were analyzed using the A/c_i Curve Fitting 10.0 utility available at <http://landflux.org/Tools.php>. This utility uses the curve-fitting equations described by Ethier and Livingston (2004), based on the kinetic parameters of Farquhar et al. (1980) and Bernacchi et al. (2001). From the model, following parameters were analyzed: ($V_{C_{\text{max}}}$)—maximum

carboxylation rate; J_{max} — CO_2 -saturated electron transport rate of the thylakoids reactions which ultimately supply the necessary energy in the form of ATP and NADPH for the regeneration of RuBP; k_C —carboxylation efficiency equal to the change in assimilation with respect to a change in chloroplast CO_2 ($k_C = dA_{\text{CO}_2}/dc_c$); g_m —leaf mesophyll (internal) conductance between intercellular spaces (substomatal cavity and the chloroplast); $g_{m,t}$ —total mesophyll conductance, i.e., the total conductance between intercellular spaces and chloroplast, ($r_{m,t} = r_m + r_c$; $r_{m,t} = 1/g_{m,t}$; $r_m = 1/g_m$; $r_c = 1/k_C$; $k_C = dA_{\text{CO}_2}/dc_c$).

Test of PSII thermostability

The test of PSII thermostability was realized according to Brestic et al. (2012). The heat treatment was performed in darkness; cuttings from the middle part of the leaves of app. 50 mm length were placed into small sealable polyethylene bags. The bags were closed and then, they were completely submersed into the water in water bath with precisely controlled water temperature for 30 min. The time of exposure was found as sufficient after previous analyses using different exposure times. After 30 min of exposure, the bag was pulled from the bath, kept still in darkness, and after cooling to laboratory temperature (5–10 min) the sample was measured. In a single temperature experiment, the temperature level of 42 °C was used.

Chlorophyll fluorescence measurements

The Chl *a* fluorescence measurements were carried out using Handy-PEA (Hansatech Instruments Ltd, UK); the leaf samples were illuminated with continuous red light (the wavelength in peak 650 nm; the spectral line half-width 22 nm). The light was provided by an array of three

light-emitting diodes. The light pulse intensity used was 3500 $\mu\text{mol m}^{-2} \text{s}^{-1}$; duration of the light pulse was 1 s. The fluorescence signal was recorded with maximum frequency 105 points s^{-1} (each 10 μs) within 0–0.3 ms, after that the frequency of recording gradually decreased collecting all together 118 points within 1 s. Measurements on leaf segments were performed in the middle part of a leaf blade, out of the main leaf vein, after 30 min of dark adaptation, using leaf clips.

Statistical analyses

The majority of reported data represent the weighted mean \pm standard error. Statistical analysis was performed using analysis of variance (ANOVA) followed by the post hoc Tukey HSD test using software Statistica version 9.0 (Statsoft Inc., Tulsa, Oklahoma, USA). The numbers of repetitions used in gas exchange and growth analyses were six to ten. In the PSII thermostability, usually 20 leave samples of each genotype were measured before and after heat treatment.

Results and discussion

As expected, species and genotypes differed in growth and leaf parameters (Fig. 1). Compared to modern variety, the landrace (LR) was much taller, but it had the same plant dry mass and the same flag leaf area, but substantially higher LMA, indicating thicker leaves or leaves with a greater density. In contrary, *Aegilops*, the WR species, was characterized by the smallest plants, small and thin leaves (low LMA) and substantially lower dry mass production compared to both wheat cultivars.

The gas exchange measurements showed significantly higher steady-state net assimilation rate (Fig. 2a). Interestingly, the lower photosynthetic rate in LR or WR compared to MG was not associated with lower intercellular CO_2 concentration (c_i), but we observed the opposite trend (Fig. 2b). Moreover, similar mean values of stomatal conductance (g_s) were observed in all three genotypes (Fig. 2c). The unequal A_{CO_2}/c_i ratio indicates differences in efficiency by which the photosynthetic apparatus uses the CO_2 entering the leaf (Fig. 2d). These results suggest that the differences in CO_2 assimilation were not caused by stomatal limitations of CO_2 diffusion. The highest mitochondrial respiration per leaf area measured in darkness (Fig. 2e) was found in modern variety, significantly lower was in LR and in *Aegilops*. However, when the respiration was recalculated per dry mass unit (Fig. 2f), we observed similar values in the LR and the *Aegilops*, but significantly higher dark respiration in a modern variety.

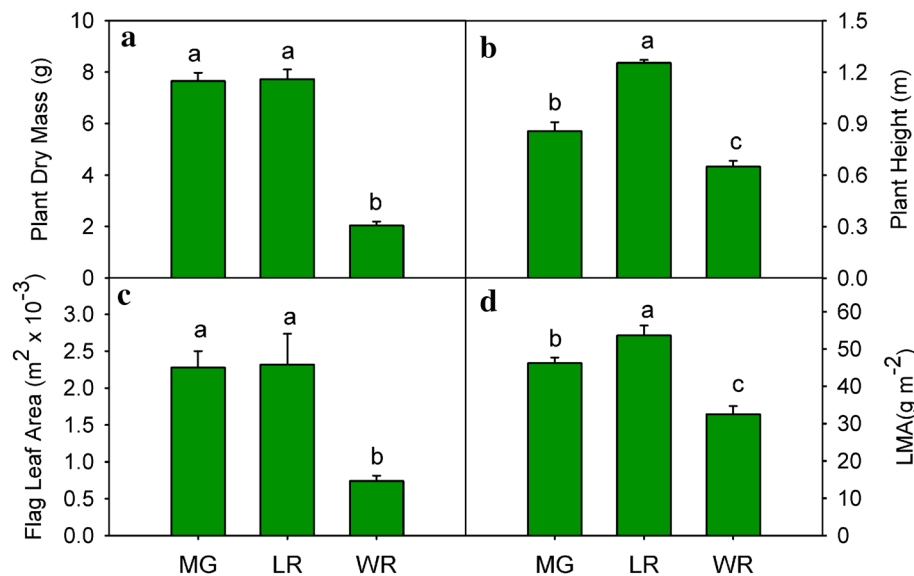


Fig. 1 Comparison of selected growth parameters measured in a modern genotype (MG), landrace (LR), and in a wild relative (WR). The measurements were done after anthesis when the maximum plant height was achieved. **a** The average aboveground dry mass of plants; **b** average plant height; **c** leaf area of a flag (upper) leaf; **d** the dry mass of flag leaf per unit of leaf area (the dry mass per leaf area, LMA). The columns represent weighted mean values ($n=10$).

The error bars represent standard error of mean. Small letters above the bars (*a*, *b*, *c*) indicate significance of mean values of individual parameters, where bars with the different letters indicate significant difference of parameter values at $P=0.05$, but bars with the same letters indicate no significant difference of parameter values among MG, LR, and WR (by ANOVA and post hoc Tukey HSD test)

In general, the results presented in our study confirm that the modern, high-yielding genotype of wheat was better in all traits related to the leaf photosynthesis compared to old LR or wild ancestor. Despite the photosynthesis had never been the direct and conscious selection criterion, the photosynthetic improvement was obviously a necessary by-product of a long-term selection aimed at yield increase. The relationship between the photosynthetic performance and yield is not straightforward, with an accumulation capacity of the sink being extremely important (Peet and Kramer 1980; Borrás et al. 2004); anyway, the studies analyzing releases of crop genotypes through several decades show that yield increases were obtained through increases in harvest index until the 1980s, after which the increase in biomass accumulation and photosynthesis became more important. This indicates a transition from sink to source limitation in two of our major C3 crops (Shearman et al. 2005; Hubbart et al. 2007). Many other research findings strongly support the hypothesis that a sustained increase in leaf photosynthesis can lead to increase in total production of biomass that would be necessary to gain further increase in crop yield (Long et al. 2006; Parry et al. 2007; Zhu et al. 2010; Evans 2013). The scientific dogma about generally oversized capacity of crop photosynthetic apparatus, broadly accepted in 1980s and 1990s, was broken up after the publication of results of series experiments with increased CO₂ in different conditions, documenting a significant increase

of grain yield because of increase of leaf photosynthesis (Drake et al. 1997; Mitchell et al. 1999; Bender et al. 1999; Ainsworth et al. 2002; Ainsworth and Long 2005). Moreover, analysis of photosynthetic rate in Australian bread wheat genotypes with different date of release indicated that the selection for higher grain yield led to unconscious selection for higher photosynthetic rate (Watanabe et al. 1994). Similarly, our results comparing the photosynthetic and the leaf parameters of high-yielding wheat (*T. aestivum* L.) genotype with a WR species of the same group used as a gene donor in wheat breeding (*Ae. cylindrica* Host.) or LR supports this idea (Fig. 1). These data indicate that the WR species of the same group as wheat, having almost the same vegetation period and canopy development as the observed genotype of winter wheat, is much less productive, it has smaller and thicker leaves. Moreover, the leaves of *Aegilops* demonstrated significantly lower metabolic activity, as shown by the rate of mitochondrial respiration per dry mass unit (Fig. 2).

Further analysis of A/c_i curve by Farquhar–von Caemmerer–Berry model (Farquhar et al. 1980) adjusted by Ethier and Livingston (2004) enabled to uncover the individual limitations of CO₂ assimilation process (Fig. 3). Whereas the trend of the maximum carboxylation rate (V_{Cmax}) was very similar to CO₂ assimilation (Fig. 3a), in case of the maximum electron transport rate (J_{max}) we did not observe

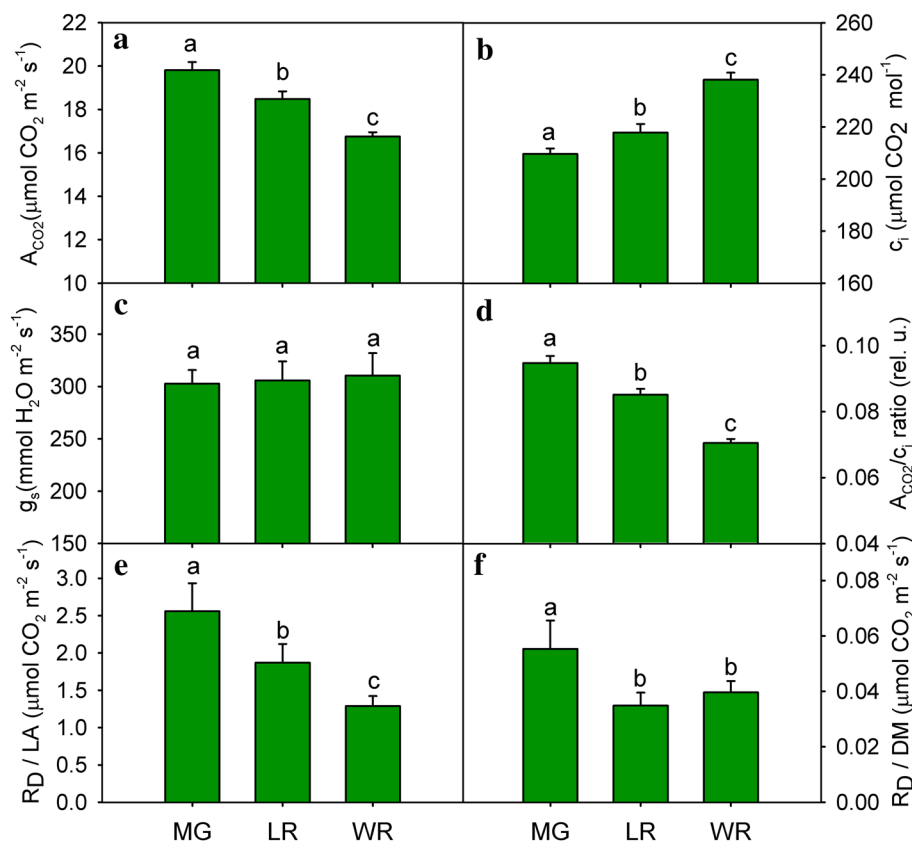


Fig. 2 Photosynthetic parameters obtained from gas exchange measurements in steady state realized in a modern variety (MG), landrace (LR) and in wild relative species (WR). The measurements were done on flag leaf during anthesis and the short time after anthesis. **a** The average CO_2 assimilation rate (A_{CO_2}) measured at 380 ppm of CO_2 ; **b** intercellular CO_2 concentration (c_i); **c** leaf stomatal conductance (g_s); **d** CO_2 assimilation rate to intercellular CO_2 concentration ratio (A_{CO_2}/c_i); **e** dark respiration (absolute value of A_{CO_2} measured in the dark) per leaf area unit; **f** dark respiration (absolute value of A_{CO_2} measured in the dark) recalculated per dry mass unit. The conditions

in leaf chamber were set as follows: leaf temperature 25 °C, PPFD 1000 $\mu\text{mol (photons) m}^{-2} \text{ s}^{-1}$, reference CO_2 concentration 380 ppm, RH ~60%. The columns represent weighted mean values ($n=10$); the error bars represent standard error of mean. Small letters above the bars (*a, b, c*) indicate significance of mean values of individual parameters, where bars with the different letters indicate significant difference of parameter values at $P=0.05$, but bars with the same letters indicate no significant difference of parameter values among MG, LR, and WR (by ANOVA and post hoc Tukey HSD test)

significant differences between the LR and the modern wheat variety (Fig. 3b). Anyway, in *Aegilops*, J_{max} was low. The estimation of internal (mesophyll) conductance (Fig. 3c) suggests substantially lower g_m values in LR compared to the modern variety and *Aegilops*. It is probably due to the different leaf properties, also shown by values of high LMA in LR (Fig. 1d). The total internal conductance $g_{m,t}$ (Fig. 3d) was considerably higher in a modern variety compared to LR and *Aegilops* (Fig. 3d); in the case of LR, the decrease was caused mainly by low mesophyll conductance as well as by moderately lower k_C . In *Aegilops*, the dominant component of total internal resistance was low k_C , as the mesophyll conductance of thin and soft leaves was rather high.

Although the results of our experiments, compared to previously mentioned studies, have not such a high potential to provide conclusions with a general validity, the measurements brought several interesting details uncovering the

specific mechanisms leading to photosynthetic improvements in MGs. Although the higher leaf thickness in wheat compared to *Aegilops* seems to be extremely important, the comparison of MG with a LR shows that the higher LMA does not necessarily lead to higher photosynthesis. The estimates of mesophyll conductance confirmed our expectations that the mesophyll limitation in thin and soft *Aegilops* leaves will be very low. On the other hand, the mesophyll conductance was significantly lower in leaves of LR with thick and hard leaf. The mesophyll conductance represents the conductance for CO_2 flowing from the intercellular airspaces to the carboxylation site in the chloroplasts, which includes barriers consisting of the cell wall, plasma membrane, chloroplast envelope, and stromal thylakoids; both in air and liquid phases (Evans et al. 2009). In previous years, it was shown that g_m plays a crucial role in the regulation of photosynthesis, representing up to 40% of the CO_2 diffusional

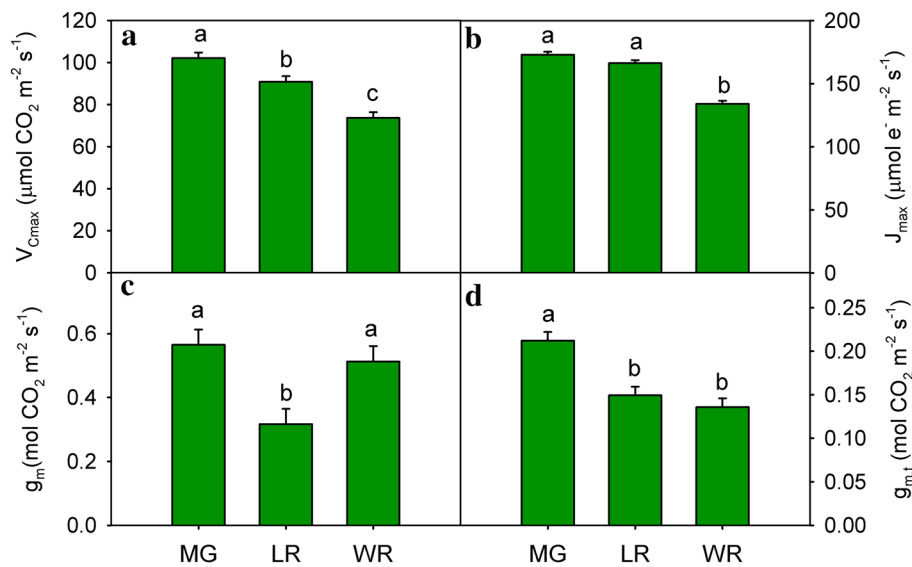


Fig. 3 Model-derived parameters characterizing photosynthetic limitations obtained using data from analyses of A/c_i -curve within gas exchange measurements realized in a modern variety (MG), landrace (LR), and in a wild relative (WR). The measurements were done on flag leaf during anthesis and the short time after anthesis. **a** Maximum rate of carboxylation (V_{cmax}); **b** maximum light driven electron flux (J_{max}); **c** mesophyll (internal) conductance of leaf (g_m); **d**

total conductance of leaf ($g_{m,t}$). The columns represent weighted mean values ($n=6-8$); the error bars represent standard error of mean. Small letters above the bars (*a*, *b*, *c*) indicate significance of mean values of individual parameters, where bars with the different letters indicate significant difference of parameter values at $P=0.05$, but bars with the same letters indicate no significant difference of parameter values among MG, LR, and WR (by ANOVA and post hoc Tukey HSD test)

limitations (Warren 2008). Especially, an increase of mesophyll limitations was observed in non-optimum conditions, such as drought stress (Flexas et al. 2004; Olsovska et al. 2016), high temperature (Bernacchi et al. 2002; Allakhverdiev and Murata 2004; Allakhverdiev et al. 2008), and some other environmental factors limiting the photosynthesis (Allakhverdiev et al. 1997; Bernacchi et al. 2002; Nishiyama et al. 2006; Mohanty et al. 2007; Murata et al. 2007; Flexas et al. 2008; Allakhverdiev 2011). In our experiment (Fig. 3), we used the estimation of mesophyll conductance using the model of Ethier and Livingston (2004), which is based on analysis of the shape of A/c_i curve. Similar to other methods, this model has also some limitations (Pons et al. 1999), especially when it is used in non-optimum conditions. Therefore, the results represent only the rough estimate and possible subtle variances cannot be reliably recognized. However, in our case, we found relatively high differences among observed samples. When mesophyll conductance is high enough [$>0.6 \text{ mol (CO}_2\text{) m}^{-2} \text{ s}^{-1}$], it can be assumed as “infinite” as the mesophyll limitation is relatively low compared to other factors limiting CO_2 assimilation. In wheat LR, the values significantly $<0.6 \text{ mol (CO}_2\text{) m}^{-2} \text{ s}^{-1}$, indicating an important mesophyll limitation even in optimum conditions. Logically, the differences in leaf anatomy can be expected as the main reason of the different g_m . However, the studies performed on species differing significantly with a leaf thickness have shown the opposite trends: the higher

thickness, the lower mesophyll limitation. This was mainly due to the higher surface area of the mesophyll cells (Hanba et al. 1999). Moreover, it was shown that the direct effect of leaf thickness is relatively low, especially in herbaceous plants (Flexas et al. 2012). Kaminski et al. (1990) made the comparison of anatomical parameters and photosynthetic rates per unit leaf area on fully expanded flag leaves of *Triticum* and *Aegilops* species at light saturation. They found that wheats with thinner leaves, lower LMA, and chlorophyll per unit area had the higher photosynthetic rate compared to genotypes with more thick and dense leaves. The surface area of the mesophyll cells per unit volume of mesophyll tissue was similar in all ploidy levels, but the internal (i.e., mesophyll) conductance can be suggested to play the major role in reaching the higher photosynthetic rate in genotypes with thin leaves. Thus, they observed the similar trend to our results, i.e., increase of mesophyll limitation with leaf thickness, which was evident when comparing MG with *Aegilops*. In our case, despite high g_m , the *Aegilops* had lower photosynthesis due to metabolic limitations. More surprising are relatively high differences between the values of g_m indicated in a modern wheat genotype and values in old LR. They can be explained either by unequal thickness of cell walls or in the surface areas of chloroplasts exposed to intercellular air species (Niinemets et al. 2009). A lower dark respiration per dry mass unit in LR compared to MG gives a reason to believe that there is a much higher contribution of

extracellular structures (e.g., due to thicker or lignified cell walls) in leaves of LR compared to MG, which may limit the CO₂ exchange between the gas and liquid phases. This hypothesis, however, needs further investigation.

In addition to diffusion limitations, the catalytic efficiency or the photosynthetic enzyme (especially of Rubisco) plays a major role in reaching high carboxylation efficiency (Parry et al. 2007), which is reflected also in crop breeding programs (Reynolds et al. 2011). Zhu et al. (2010) indicated that the increase of catalytic efficiency would increase the rate of photosynthetic fixation without need of additional Rubisco. This would be beneficial, especially in high light conditions, where the Rubisco enzymatic activity limits the photosynthetic rate. In contrary, the increase in specificity factor would increase net CO₂ uptake in low light conditions, where the electron transport rate limits the photosynthesis; the electron transport chain would be directed away from photorespiration into photosynthesis. Unfortunately, it was shown that increase in specificity factor leads to decrease in catalytic activity of Rubisco (Bainbridge et al. 1995). Therefore, there are conflicting consequences at the level of the canopy. The increased specificity factor would increase light-limited photosynthesis, while the associated decrease in catalytic efficiency would lower the light-saturated rate of photosynthesis (Zhu et al. 2004). The analyses of A/c_i curve and light response curves (Fig. 2) enabled to identify a higher carboxylation efficiency and lower light compensation point of CO₂ assimilation (not shown here) in MG compared to LR and wild species. It indicates that during the selection, the priority was given at an efficiency of photosynthetic in high light at the expense of efficiency at light limiting conditions. A higher photosynthetic performance of modern wheat variety flag leaf went along with a higher level of mitochondrial respiration (Fig. 2). It can be considered as a logical consequence of higher metabolic (mainly nitrogen) demand of high-yielding variety.

Based on the temperature response curve of CO₂ assimilation rates (Fig. 4), the *Aegilops* was found to be more sensitive to high temperature, with a thermal optimum at lower level as compared to wheats. Responses of wheat genotypes were similar. Test of thermostability at PSII level assessed using rapid fluorescence kinetics record (Fig. 5) showed LR to be more susceptible to heat impairment as shown on significant increase of the basal fluorescence (F_0) and the maximum quantum yield of PSII (F_v/F_m). In the fluorescent raw curves, there is an evident local maximum in time of 0.3 ms followed by a subsequent decrease of fluorescence, which is denoted as a K-step. The visual appearance of this step is an indicator of serious damage at the level of oxygen evolving complex (OEC) within photosystem II. The most pronounced K-step increase followed by fluorescent decrease was found in LR indicating the heat susceptibility of this

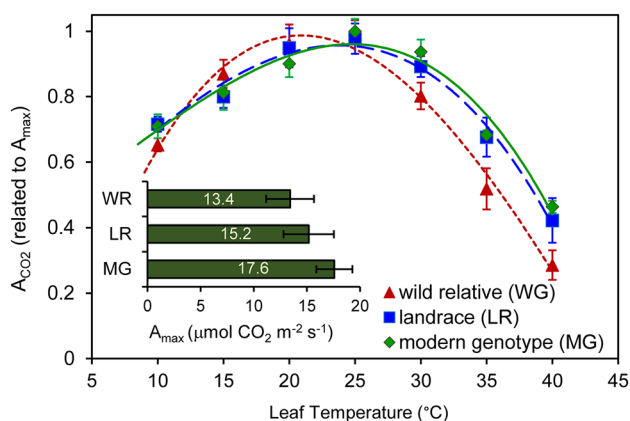


Fig. 4 The temperature response curve of CO₂ assimilation rate measured in leaves of the modern variety (MG), landrace (LR), and in a wild relative (WR). The points represent the relative values of CO₂ assimilation (measured values divided by the maximum assimilation, A_{max}). The values of maximum assimilation rates (A_{max}) recorded in temperature response curves for each genotype are shown on the small plot within the graph

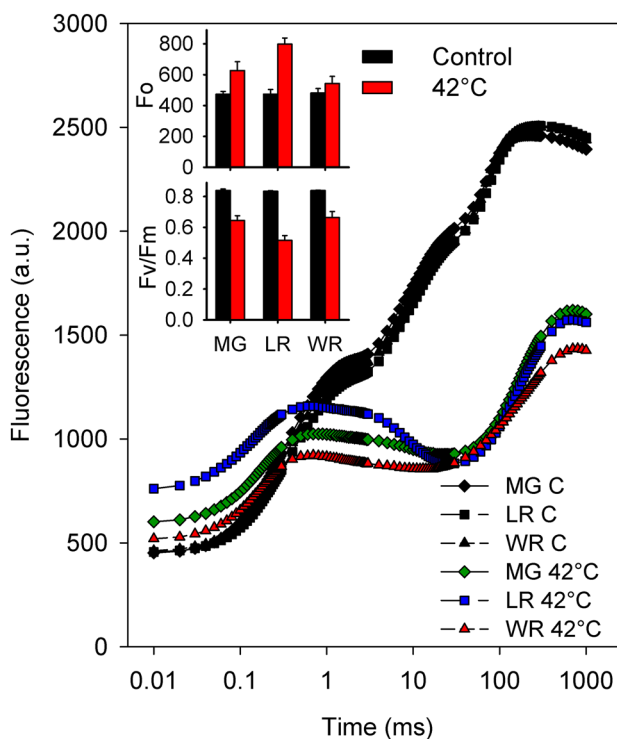


Fig. 5 The results of PSII thermostability test at 42 °C in leaves of the modern variety (MG), local landrace (LR), and wild relative *Aegilops* (WR). The polyphasic curves represent the fast chlorophyll a fluorescence kinetics plotted on the logarithmic time scale (average curve for each genotype). The small bar plots inside the graph show the values of basal fluorescence F_0 and maximum quantum yield of PSII photochemistry (F_v/F_m) before and after heat treatment

genotype. In contrary, the *Aegilops* was found to be the most resistant to heat injury of PSII, as shown by the values of F_o , F_v/F_m , and the fluorescence curve shape.

Very interesting is also the difference between wheats and *Aegilops* in optimum temperature for photosynthesis (Fig. 4). The decrease of photosynthesis at elevated temperature is associated mostly with Rubisco deactivation, which represents a regulatory feedback from one of the processes contributing to the RuBP regeneration capacity (Kubien and Sage 2008). Galmes et al. (2015) found that the optimum temperature (T_{opt}) for Rubisco catalytic activity correlated with specificity factor $S_{c/o}$ for land plants. Despite the lower temperature optima, the *Aegilops* expressed a higher level of PSII thermostability (Fig. 5). Knight and Ackerly (2002) found that photosynthetic thermostolerance is significantly different between genera and species, highly plastic, and this plasticity causes that plants adapted to warmer environments, when measured in the field, have higher temperature tolerance compared to those adapted to moderate conditions. In general, the relationship between temperature optimum for CO₂ assimilation and resistance of PSII to high temperature is complicated, especially when comparing different ecological groups. For example, alpine species with low temperature optima express a higher PSII thermostability compared to many herbs living in the moderate zone (Neuner and Pramsohler 2006). On the other hand, when comparing plants belonging to similar groups, e.g., field crops, the species with higher temperature optima have usually a higher critical temperature. For example, the damage of PSII in barley occurred at temperature by 6 °C lower compared to maize (Havaux et al. 1990). In addition, the differences in PSII thermostability were also found in the genotypes of the same species. For example, in collections of wheat grown in the same field trials, it was found that the PSII thermostability and plasticity were higher in genotypes coming from warmer environments (Brestic et al. 2012). Acclimation capacity to heat stress is associated also with recovery from heat-induced damage (Takahashi et al. 2004), which was shown also in wheat (Kreslavski et al. 2009). As the accession of *Ae. cylindrica* used in this study originates from a warm and dry location, the high PSII thermostability, but also a high temperature optimum can be expected. Therefore, the lower temperature optimum compared to wheat (Fig. 4) seems to be not a consequence of adaptations to low temperature, but the result of the overall setup of CO₂ assimilation pathway, which is, however, not optimal for the needs of high-yielding wheat genotypes, especially taking into an account the gradual elevation of temperature observed worldwide and needs associated with an increase of photosynthetic production in Climate Change conditions.

Conclusions

In summary, our results indicated several differences in photosynthetic traits between MG, LR, and WR species at the different levels, such as leaf anatomy, morphology and biochemistry, CO₂ diffusion, carboxylation efficiency, light use efficiency, and temperature optima. The better photosynthetic performance of modern wheat variety suggests that the long-term wheat selection and breeding towards high yield and optimum phenotype were associated with selection towards optimization of photosynthetic processes. Compared to wild wheat relative species *Aegilops*, we found a higher robustness of the leaf photosynthetic apparatus in wheat genotypes, leading to higher photosynthetic capacity. Moreover, in wheat genotypes, we have identified a wider range determining the optimum temperature limits compared to *Aegilops*, which may contribute to higher photosynthetic productivity across the growing season. On the other way, we found that a lower photosynthetic capacity observed in more robust leaves of old wheat LR was associated with a significant mesophyll limitation of CO₂ fixation. Thus, it must be taken into an account that the use of wild ancestors and LRs in breeding may have some pleiotropic effects related to introgression of undesirable photosynthetic traits resulting in lower photosynthetic productivity.

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Author contributions MZ, MB, and SIA wrote the paper. MZ conducted the statistical analyses and analyses of photosynthetic parameters. PH provided unique biological material and led the field experiments. SM, KK, XY, and XL contributed to experimental design and interpretation of results, and helped to draft the manuscript. Neither the manuscript nor any part of its content has been published or submitted for publication elsewhere. All authors read and approved the final manuscript.

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