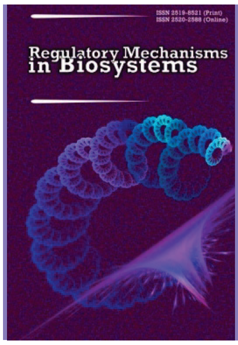

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Effects of drought on photosynthetic induction in leaves of different wheat genotypes under dark-to-light transition

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Adjustment of photosynthetic processes to an increase in irradiance constrains the CO_2 assimilation and photosynthetic carbon gain compared to that which would be obtained if photosynthesis reached its terminal value instantaneously. Acceleration of photosynthesis induction under field conditions of fluctuating light opens up new perspectives for increasing yields. However, there is little information on response of photosynthetic processes to changes in the light under drought conditions. In a pot experiment, we have studied the peculiarities of response of CO_2- and H_2O-gas exchange parameters in flag leaves of 3 winter wheat genotypes to a transition from dark to bright light under drought conditions, to reveal the features of drought effect on photosynthetic induction processes. The plants were exposed to a 7-day drought (30% FC) during the flowering stage. After that, the watering of the treated plants was restored to the control level (70% FC), which was maintained until the end of the growing season. Induction curves of CO_2 assimilation and transpiration of the flag leaves were recorded after keeping them in the darkness for 30 minutes, then the light was turned on. It was revealed that drought impacts the photosynthetic apparatus by reducing its maximum functional intensity as well as by diminishing its ability to respond to changing light conditions. Specifically, drought slows the rate of gas exchange increase during transitions from dark to light. High-yielding wheat genotypes, which had higher assimilation rates and stomatal conductance under optimal watering, showed increased sensitivity to drought. The reduction in the CO_2 assimilation rate in wheat leaves under drought was primarily due to damage to the photosynthetic apparatus in mesophyll cells, rather than inhibition of stomatal conductance. This conclusion is supported by C_i value calculations, which were highest at the lowest CO_2 assimilation rate at the beginning of light exposure and lowest at the highest CO_2 assimilation rate when reaching a steady-state plateau. The stronger impact of the drought on the biochemical components of the photosynthetic apparatus than on stomata is also suggested by a decrease in instantaneous water use efficiency (WUEi) during photosynthesis. The genotypic differences in the effects of drought on the dynamics of photosynthetic induction parameters during dark-to-light transitions in wheat leaves suggest the potential of these traits for evaluating breeding material. This could enhance the ecological plasticity of new wheat varieties.

Keywords: *Triticum aestivum* L.; photosynthesis; transpiration; photosynthetic induction; drought, grain productivity.

Introduction

Wheat is the leading source of food calories for the world population. After a significant improvement in global wheat yields during the Green Revolution, attributed to an increase in the harvest index, yield growth slowed sharply at the beginning of this century (Long & Ort, 2010; Ray et al., 2012, 2013; Morgun et al., 2022; Chourghal et al., 2023). The harvest index of modern cultivars is now about 60% of the total aboveground biomass and is close to the biological limit (Zhu et al., 2010). Therefore, new innovative approaches are needed to further improve the genetic potential of wheat yield (Reynolds et al., 2011; Long et al., 2015).

The flag leaf of wheat is considered to supply the greatest part of the carbohydrates that accumulate in the grain during its filling (Carmo-Silva et al., 2017). The proportion of assimilates translocated from the flag leaf to the spike has been found to have increased over the past 50 years with the increasing harvest index (Sanchez-Bragado et al., 2014). The photosynthetic efficiency of wheat, like that of all leading crops, is below its theoretical potential, although it has been slightly improved by breeding. Therefore, photosynthesis is currently considered an important area for further improvement of yield genetic potential (Parry et al., 2011).

It should be noted that, until recently, measurements, analysis, and approaches to improve CO_2 assimilation by leaves have focused mainly on photosynthetic rates under steady-state saturating light. However under field conditions in crops, leaves are subjected to significant fluctuations in light throughout the day due to intermittent cloud cover, dynamic self-shading caused by leaf movement, and changes in the angle of the exposure to the sun (Slattery et al., 2018; Tanaka et al., 2019; Wang et al., 2020). Although the flag leaves settle in the upper layers of canopy, the light regime they experience is also unstable due to intermittent cloudiness and occasional shading by other flag leaves or ears due to wind (Taylor & Long, 2017; Wang et al., 2020).

Adjustment of CO_2 assimilation to changes in irradiance is known to take a certain time to recover efficiency in both sun-to-shade and shade-to-sun transitions. In response to increased irradiance, photosynthesis does not reach its final value instantly, but with a certain delay (Deans et al., 2019a). In a dynamic sense, photosynthetic limitation at the leaf level can be defined as a reduction in total photosynthetic performance compared to that which would be obtained if photosynthesis reached its terminal value instantaneously. Because of the slow adjustment to changes in irradiance caused by both biochemical and diffusion factors, leaves and canopies, in general, perform at a lower efficiency than under steady-state conditions. According to some estimates, in wheat losses of assimilated carbon due to unstable light conditions in the field can be about 20% compared to theoretically possible under stable light conditions (Taylor & Long, 2017). It has also been found that although stomatal conductance and CO_2 assimilation rate are often correlated, studies in a light-dynamic environment have shown a discrepancy between these parameters, which may lead to a decrease in the efficiency of these physiological processes (Faralli et al., 2019a). Stomata slow-opening reactions limit assimilation, and their slow closing reduces the efficiency of water use. Significant genotypic variations in stomatal opening time and time to steady state CO_2 assimilation has been reported (Salter et al., 2019; Taylor et al., 2020). Therefore, the attention of researchers is being attracted to the processes of photosynthesis induction under changing light conditions (Murchie et al., 2018; Slattery et al., 2018; Tanaka et al., 2019), the improvement of which opens up new perspectives for increasing yields.

Photosynthetic induction is the process by which a leaf begins to assimilate CO_2 when transitioning from darkness (or low light) to high light, and is characterized by a delay in photosynthetic efficiency (Acevedo-Siaca et al., 2021). At the biochemical level, limitations of photosynthesis rate induction at such transitions are caused by four main processes: activation of Rubisco by its activase, photoactivation of enzymes involved in the regeneration of ribulose bisphosphate, accumulation of intermediate products of carbon metabolism (Mott & Woodrow, 2000; Slattery et al., 2018). Immediately after an increase in light intensity, photosynthesis is limited by the accumulation of Calvin cycle intermediates, and the slow induction of light-activated photosynthetic enzymes (Mott & Woodrow, 2000; Taylor et al., 2022). Under normal conditions, the formation of the ribulose bisphosphate pool restricts photosynthesis for approximately 60 seconds after the increase in light, whereas full light activation of Rubisco usually takes 8–10 minutes and is the main cause of delayed induction at this stage (Mott & Woodrow, 2000; Soleh, 2016). At the leaf level, photosynthesis is limited also by the entry of CO_2 into the intercellular spaces due to the slow stomata opening. While stomata in some monocot species respond quite quickly to increasing light, in most cases, it takes a long time for them to get fully opened, potentially creating a significant restriction on assimilation (Vico et al., 2011; McAusland et al., 2016; Deans et al., 2019b; Zhang et al., 2019). Mesophyll conductance itself, according to some data, has little effect on CO_2 assimilation under light changes compared to stomatal conductance (Sakoda et al., 2021).

Based on experimental data and theoretical assumptions, many researchers propose increasing the speed of photosynthetic induction under variable light conditions as a promising approach to improve the photosynthetic performance of agricultural crops (Kaiser et al., 2015; Kromdijk et al., 2016; McAusland et al., 2016; Taylor & Long, 2017; Kaiser et al., 2018; Lawson & Vialet-Chabrand, 2018; Slattery et al., 2018). Transgenic manipulations to accelerate the adaptation to transitions from sun to shade and vice versa have already shown significant benefits for improving plant productivity in the field (Kromdijk et al., 2016; Faralli et al., 2019b; Long et al., 2022). For example, overexpression of the gene encoding Rubisco-activase in rice led to an acceleration in the photosynthesis induction rate, increased yield, and improved nitrogen use efficiency (Yoon et al., 2020).

Therefore, the study of the peculiarities of leaf gas exchange induction under variable light conditions opens up new opportunities for improving photosynthesis and sustainability in terms of water use and crop yield (Kaiser et al., 2018; Lawson & Vialet-Chabrand, 2018; Deans et al., 2019a, 2019b; Faralli et al., 2019b; Eyland et al., 2021; Long et al., 2022). The obtained results are discussed from the perspective of using different features to develop ideotypes for specific growing environments. It is suggested that intraspecific variation in the rate of stomatal reactions may be a potential target for breeding to optimize wheat physiological responses to dynamic field conditions, and to create cultivars that maximize photosynthesis and minimize non-productive water losses in a variable light environment (Vialet-Chabrand et al., 2017; Slattery et al., 2018; Wang et al., 2020).

However, the vast majority of works dealing with these problems do not take into account the impact on plants of such a widespread stress factor as drought, the relevance of which is increasing due to global climate changes. It is known that drought suppresses stomatal conductance and CO_2 assimilation in wheat, the genotypes of which differ significantly in the effects of water shortage on these physiological parameters (Morgun et al., 2019b; Lakhneko et al., 2023). But there is scarce information on how coherent CO_2 assimilation and stomatal conductance respond to changes in light intensity under drought conditions. It was reported that the reduced availability of water caused asymmetric reactions of stomatal opening and closing, exacerbated uncoupling between A and stomatal conductance under dynamic light conditions (Faralli et al., 2019a).

The aim of our work was to study the peculiarities of the dynamics of CO_2- and H_2O-gas exchange parameters during dark-to-light transition in flag leaf of wheat plants of different genotypes under optimal and insufficient water supply and to highlight the features of drought effect on induction processes.

Materials and methods

The research was carried out on plants of winter bread wheat (*Triticum aestivum* L.) of the varieties Yednist and Podilska Nyva, and the selection line UK 95/17, which were grown outdoors in pots with 10 kg of fertilized soil, in natural light and temperature conditions under transparent polyethylene film roof. Initially, the number of plants in a pot was 20 and during the experiment it was thinned to 14 due to harvesting plants for measuring biochemical indices. Fertilizers were added in equal quantities ($N_{80}P_{80}K_{80}$ + $N_{80}P_{80}K_{80}$ mg/kg of soil) when the pots were filled with soil and in the middle of the stem elongation stage (BBCH 35).

For the control plants, soil moisture was maintained at the level of 70% of field capacity (FC) throughout all growing season. At the beginning of flowering stage, watering of one half of plants was stopped, reducing the soil moisture for 3 days to the level of 30% FC, which was maintained for the next 7 days. After that, watering of the drought-treated plants was restored to the control level (70% FC), which was maintained until the end of the growing season. The soil moisture in the pots was monitored gravimetrically twice a day.

Determination of relative water content, photosynthetic pigment content, gas exchange rate, and chloroplast antioxidant enzyme activity in the flag leaves of both control and treated plants was carried out on the seventh day of growth at 30% FC. Components of grain productivity in plants were determined after reaching full grain maturity by weighing air-dry materials. The relative water content in leaves was determined according to the standard method (González & González-Vilar, 2007). To determine the dry weight, the samples were fixed at 105 °C for 30 min and dried to a constant weight at 65 °C. The total chlorophyll (*a* + *b*) content in leaves was measured spectrophotometrically (Wellburn, 1994) after extraction from fresh material with dimethyl sulfoxide (DMSO) and calculated based on dry weight.

The photosynthesis and dark respiration rates were measured under controlled conditions using an open gas exchange system with an infrared gas analyzer, GIAM-5M (RF), operated on a differential scheme, according to standard methods (Bush et al., 2024). The middle parts of the intact flag leaves of the main shoot (2 in parallel) were placed in a temperature-controlled chamber (25 °C) and illuminated with a TA-1150 W LED spotlight with color temperature of 5200 K. The illumination at the chamber level was 1500 μmol/(m^2 • s) of photosynthetically active radiation. Conditioned atmospheric air (humidity of 9.5–10.0 mbar) was blown through the chamber at a rate of 1 L/min. The transpiration rate was recorded under controlled conditions with gas analyzer EGM-5 (PP Systems, USA), and was calculated based on the difference in air humidity at the inlet and outlet of the leaf chamber. The same device was also used to record the CO_2 concentration in the air at the entry to the leaf chamber. Other gas exchange parameters (stomatal conductance, CO_2 concentration in intercellular spaces) were calculated according to (Bush et al., 2024).

Induction curves of photosynthesis and transpiration flag leaves were recorded according to the following protocol. After placing the leaves in the chamber, they were kept in the darkness for 30 minutes. At the end of this period, the dark respiration rate was recorded, and the light was turned on (immediately at full power). The CO_2 and H_2O gas exchange rates were recorded with intervals of 10 min over a period of 50 min.

Determination of chloroplast antioxidant enzymes activity – superoxide dismutase (SOD) and ascorbate peroxidase (APX) – was carried out as described in the previous paper (Morgun et al., 2019a).

Repetition of the experiment – 5 pots per treatment, repetition of water deficit determinations 5 times, analytical repetition of chlorophyll content and antioxidant enzymes activity determinations for combined sample of leaves of 5 separate plants – 4 times, determinations of indices of gas exchange – 4 times. The components of grain productivity for each treatment were estimated as an average of 20 plants. The data were statistically analyzed using ANOVA and criterion of significant differences of Tukey's test for average values. The results are expressed as mean and standard error (x ± SE). Differences between the data were considered significant at $P < 0.05$.

Results

A one-week drought at 30% FC resulted in a significant decrease in relative water content (RWC) in flag leaves of wheat plants of all genotypes studied (Fig. 1). In the plants of Yednist variety the RWC in leaves decreased by 12.2%, in Podilska Nyva variety – by 13.1%, and the UK 95/17 line by –14.5%, compared to the control. The differences between genotypes were statistically insignificant under both watering regimes.

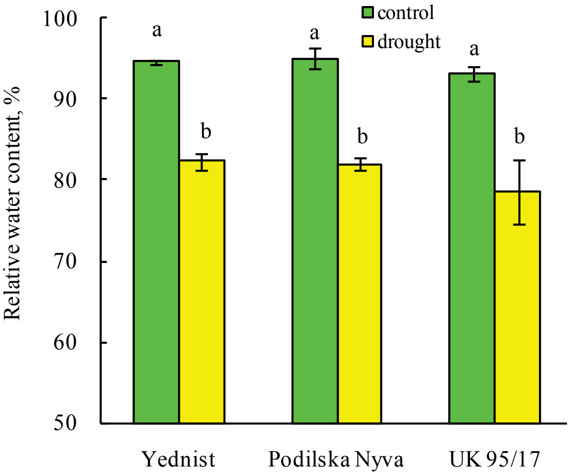

Fig. 1. Effect of soil drought (7 days at 30% FC) on relative water content (RWC) in the flag leaves of winter wheat genotypes: mean values ± SE, n = 5, values designated by the same letters, are statistically insignificant at $P < 0.05$

The chlorophyll content in flag leaves of wheat plants of both varieties on the seventh day of drought decreased by 27.1% compared to the control, and in the UK 95/17 line – by 19.2% (Fig. 2). It should be noted that genotypic differences in this index on response to drought were more pronounced than in RWC. In the control plants of Yednist and Podilska Nyva varieties, the chlorophyll content exceeded that of the UK 95/17 line. The same trend was observed under conditions of drought against the background of a general decrease in chlorophyll content.

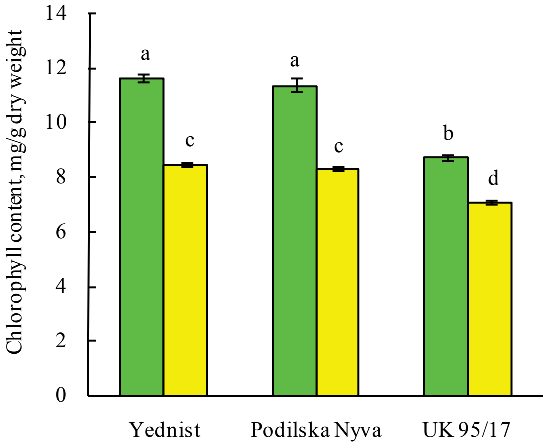

Fig. 2. Effect of soil drought (7 days at 30% FC) on chlorophyll content in the flag leaves of winter wheat genotypes: mean values ± SE, n = 4, values designated by the same letters, are statistically insignificant at $P < 0.05$

Measurements of the CO_2 assimilation induction curves in flag leaves after the transition from darkness to light revealed significant genotypic differences in the dynamics of this process in both control and treated plants (Fig. 3). It should be noted that the value of CO_2 exchange rate, which corresponded to the zero-time point, was negative and reflected the intensity of the dark respiration. Its value was similar in all genotypes at both watering regimes. The CO_2 assimilation in control plants increased sharply, however, differently among genotypes. During the first 10 minutes of illumination, the CO_2 assimilation rate in flag leaves of the control plants of the Podilska Nyva variety increased most rapidly, exceeding that of the UK 95/17 line by 1.4 times and that of the Yednist variety by 2.5 times. Within the next 20 minutes, the net assimilation rate continued to increase, but at a slower pace. Forty minutes after the light was switched on, the plants of all genotypes already showed a clear tendency towards reaching the saturation plateau of the induction curve. After 50 minutes of illumination, the net assimilation rate in control plants reached steady state with the highest level in the UK 95/17 line, which was 14 and 60 % higher than in the varieties Podilska Nyva and Yednist, respectively.

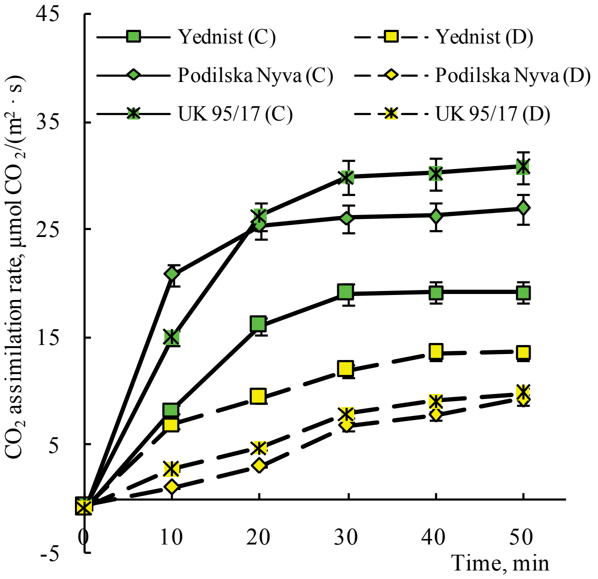

Fig. 3. Induction curves of net CO_2 assimilation rate on dark-to-light transition in the flag leaves of winter wheat genotypes under optimal soil moisture (C) and drought (D) (7 days at 30% FC): mean values ± SE, n = 4

The plants exposed to a seven-day drought showed differences from the control ones both in terms of the dynamics of induction curves and the steady-state level of CO_2 assimilation activity (Fig. 3). The speed of CO_2 assimilation increase after switching on the light in treated plants was noticeably slower than in control plants except for the Yednist variety that displayed negligible dissimilarity for the first 10 minutes. A plateau (or a tendency to it) was noted only at the end of the measured period, and the final values of the net CO_2 assimilation rate were significantly lower than in the control. Thus, this index was 29.0%, 65.7%, and 68.0% lower than in control plants for the varieties Yednist, Podilska Nyva, and the line UK 95/17, respectively.

Under drought conditions, the genotypes' range in the level of photosynthesis was different than under optimal water supply. The steady state CO_2 assimilation activity was the highest in plants of the Yednist variety. The Podilska Nyva variety had lower speed of increase in CO_2 assimilation rate at the beginning of the induction curve than the line UK 95/17, but the final values of photosynthetic activity practically did not differ in these genotypes. It should be noted that during the first 10 minutes after switching on the light, the net assimilation rate in the leaves of Yednist variety plants rose much faster than in other genotypes, showing the advantage of the Yednist variety under drought stress.

For a more detailed analysis of the dynamics of CO_2 assimilation induction in wheat leaves, we calculated two parameters for control and treated plants of each genotype: (1) the time taken for the rate of CO_2 assimilation by the leaf to reach 50% of saturation, and (2) the percentage of the CO_2 assimilation rate achieved 30 minutes after the light was switched on, relative to the saturation level. Among the control plants, according to the first parameter, the Podilska Nyva variety stood out; the net assimilation rate in its leaves reached 50% of saturation twice as fast as in other genotypes (5.5 minutes versus 10.5–11.5 minutes). For the second parameter, the differences between the plants of all studied genotypes were less pronounced. Thirty minutes after the light was switched on, the CO_2 assimilation rate in their leaves reached 88–98% of the maximum.

Drought led to a significant differentiation among wheat genotypes for both parameters. Under stress condition, the shortest time required to reach 50% of the maximum photosynthesis rate was observed in plants of the Yednist variety (11.8 minutes). Following in order of increasing time (i.e., delaying CO_2 assimilation induction) were the Podilska Nyva variety (23.3 minutes) and the UK 95/17 line with the longest time (25.2 minutes). Thirty minutes after the light was switched on, the maximum increase in the CO_2 assimilation rate was observed in the plants of the Yednist variety (87.7% of the maximum under drought). This parameter was significantly lower in the Podilska Nyva variety (74.1%) and the UK 95/17 line (61.4%).

Another important trait of leaf gas exchange is the transpiration rate. The transpiration induction curves after switching on the light were somewhat similar to those for photosynthesis, with the exception that they started with values above zero (Fig. 4). The similarity consisted in the gradual increase of this index during 30 minutes from the moment of switching on the light, reaching the plateau of saturation at the end of the measuring period. Among the control plants, the highest transpiration was observed in the Podilska Nyva variety (especially the first 20 minutes) and the UK 95/17 line (after 30 minutes), and at the end of the measurement period they were equal. In plants of the Yednist variety, the final level of transpiration was significantly lower (by 1.6 times); similarly, as it was for CO_2 assimilation.

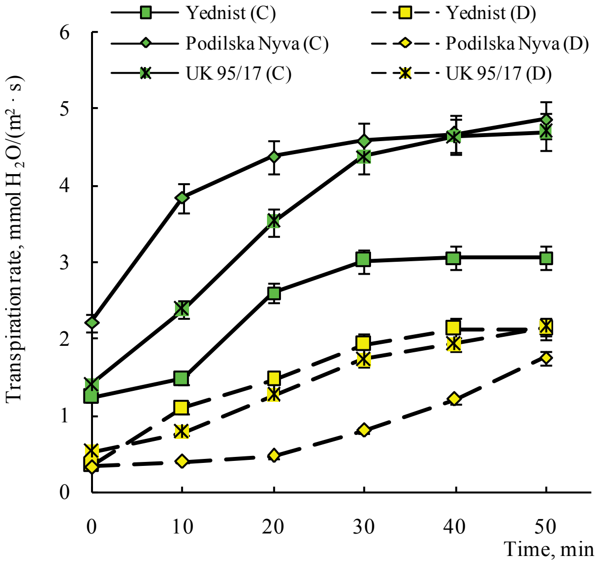

Fig. 4. Induction curves of transpiration rate on dark-to-light transition in the flag leaves of winter wheat genotypes under optimal soil moisture (C) and drought (D) (7 days at 30% FC): mean values ± SE, n = 4

The seven-day soil drought led to a significant decrease in transpiration rate of the flag leaves of all genotypes, albeit to varying degrees (Fig. 4). The final value (50 minutes after switching on the light) of its rate in stressed plant of the Yednist variety was 69.8% of the control, Podilska Nyva – 36.2%, and UK 95/17 line – 45.7% of the control. It should be noted that in the Podilska Nyva variety, the transpiration rate practically did not change during the first 20 min of illumination, and only then did it begin to increase gradually until the end of the light period, which appeared to be insufficient to reach the saturation plateau for this variety. Furthermore, the values of transpiration rate in the UK 95/17 line over light induction period were much higher than those in the Podilska Nyva variety in contrast to the photosynthesis induction curves. As observed in the case of photosynthesis (Fig. 3), the transpiration rate in the Yednist variety increased more rapidly during the first 10 minutes compared to other genotypes. In general, 50 minutes after the start of the light, the transpiration rate in stressed plants was the highest in the Yednist variety and the UK 95/17 line, and slightly lower in the Podilska Nyva variety. According to the trend observed in the dynamics of transpiration in plants of the Podilska Nyva variety, it could be assumed that under an extended light period, this index would be equal to the Yednist variety and the UK 95/17 line.

Using CO_2 and H_2O gas exchange data measured during the period of photosynthesis induction in control and drought-treated plants, we built relationships between the transpiration and photosynthesis rates and calculated the value of the instantaneous water use efficiency in photosynthesis (WUEi), which reflects the integrative ratio of stomatal and non-stomatal limitations under changing light intensity. It was revealed that these relationships are well approximated by linear equations (Fig. 5). The coefficient at the argument of the function quantitatively characterizes how many μmol of CO_2 are fixed by a leaf per 1 mmol of evaporated H_2O.

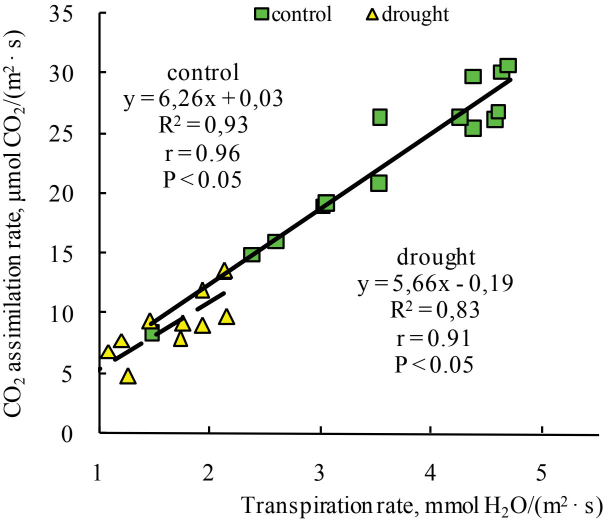

Fig. 5. Relationships between transpiration and net CO_2 assimilation rates in the flag leaves of winter wheat genotypes under optimal soil moisture and drought (7 days at 30% FC)

It can be seen from the obtained equations that WUEi was significantly lower in drought-treated plants than in control plants. In treated plants, this index was 5.66 μmol CO_2/mmol H_2O, while in control plants, it was 6.26 μmol CO_2/mmol H_2O. That is, to fix 1 mol of CO_2, 177 and 160 mol of H_2O had to be used, respectively (or to fix 1 g of CO_2 – 72.4 and 65.6 g of H_2O, respectively).

Calculations of stomatal conductance revealed that it was significantly lower in drought-treated than in control plants (Fig. 6). Among the latter, according to this index, plants of the Podilska Nyva variety and the UK 95/17 line stood out. The flag leaves' stomatal conductance in these two genotypes under the conditions of sufficient water supply, 30 min after switching on the light was 2.4 times higher than that of the Yednist variety and continued to increase, while in the last variety, it already began to reach the plateau of saturation. In drought-treated plants, these genotypic differences were leveled off. The difference in stomatal conductance between the Yednist variety and the UK 95/17 line was insignificant. In drought-treated plants of the Podilska Nyva variety, the stomatal conductance was lower and practically did not change during the first 20 min of illumination, and only then did its gradual increase begin. At the end of the light period this index was almost the same in all genotypes.

It is worth noting that a distinct delay (lag period) in the stomatal conductance increase during the first 10 or even 20 minutes after switching on the light was inherent to varying degrees in both control and treated plants of all varieties. In control plants, this lag was more prominent in the Yednist variety and the UK 95/17 line. In drought-treated plants, it was more visible in the Podilska Nyva variety and the UK 95/17 line, and practically absent in the Yednist variety.

In order to assess the role of stomatal and non-stomatal factors in limiting CO_2 assimilation in control and stressed plants during the dark-to-light transition, we calculated the concentration of CO_2 in the leaf intercellular spaces (C_i), which shows the balance between the rate of CO_2 diffusion via stomata and the rate of CO_2 consumption in the carboxylation reaction (including effects of CO_2 transfer into the chloroplast) (Deans et al., 2019a). We did not consider the zero point, which corresponds to darkness here, since no photosynthetic assimilation of CO_2 occurs at that time. It was revealed that the C_i value in well-watered plants varied insignificantly during photosynthesis induction, with little difference between genotypes studied (Fig. 7). In drought-treated plants, the C_i value after 10 minutes of light significantly exceeded that of the control. Subsequently, this was followed by a decrease in C_i and after approximately 30 minutes, this parameter stabilized at a level lower (in the Podilska Nyva variety) or higher (in the UK 95/17 line) than the control values. The final values of Ci in the leaves of control and treated plants of the Yednist variety differed insignificantly.

The obtained data indicate coupled increase in stomatal conductance CO_2 assimilation rate in leaves of control plants. Therefore, no clear relationships between the values of C_i and CO_2 assimilation rate measured during the dark-to-light transition in these plants were observed (Fig. 7). However, higher values of C_i after 10 minutes of light exposure in drought-treated plants showed that biochemical processes at the initial steps of CO_2 assimilation induction were inhibited by drought more strongly and limited photosynthesis more than stomatal conductance did. This resulted in an inverse relationship between the values of C_i and the CO_2 assimilation rate in drought-treated plants.

Determination in flag leaves of the activities of the main antioxidant enzymes of chloroplasts – SOD and APX – revealed that they were significantly higher in drought-treated than in control plants (Fig. 8). There was almost no difference in SOD activity between the control plants of the Yednist variety and the UK 95/17 line, with slightly lower activity observed in the Podilska Nyva variety (Fig. 8a). Under drought conditions, SOD activity increased the most in plants of the Yednist variety (by 2.4 times), followed by the Podilska Nyva variety (by 2.2 times), and the UK 95/17 line (by 1.6 times), compared to the control.

At the same time, the control plants of the Yednist variety exceeded the APX activity of the other two genotypes, which differed insignificantly from each other (Fig. 8b). Under drought conditions, APX activity increased compared to control the most in plants of the UK 95/17 line (by 1.9 times) and the Podilska Nyva variety (by 1.8 times), and the least – in the Yednist variety (by 1.6 times).

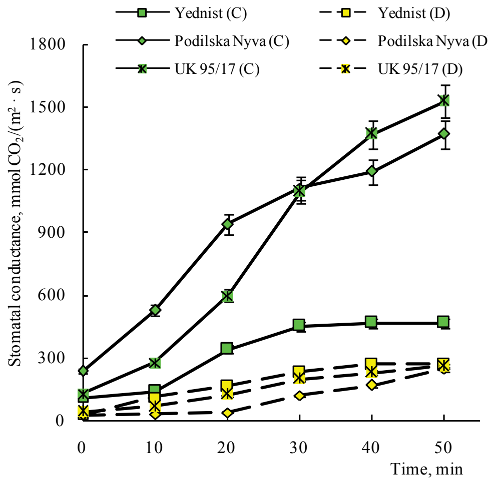

Fig. 6. Induction curves of stomatal conductance on dark-to-light transition in the flag leaves of winter wheat genotypes under optimal soil moisture (C) and drought (D) (7 days at 30% FC): mean values ± SE, n = 4

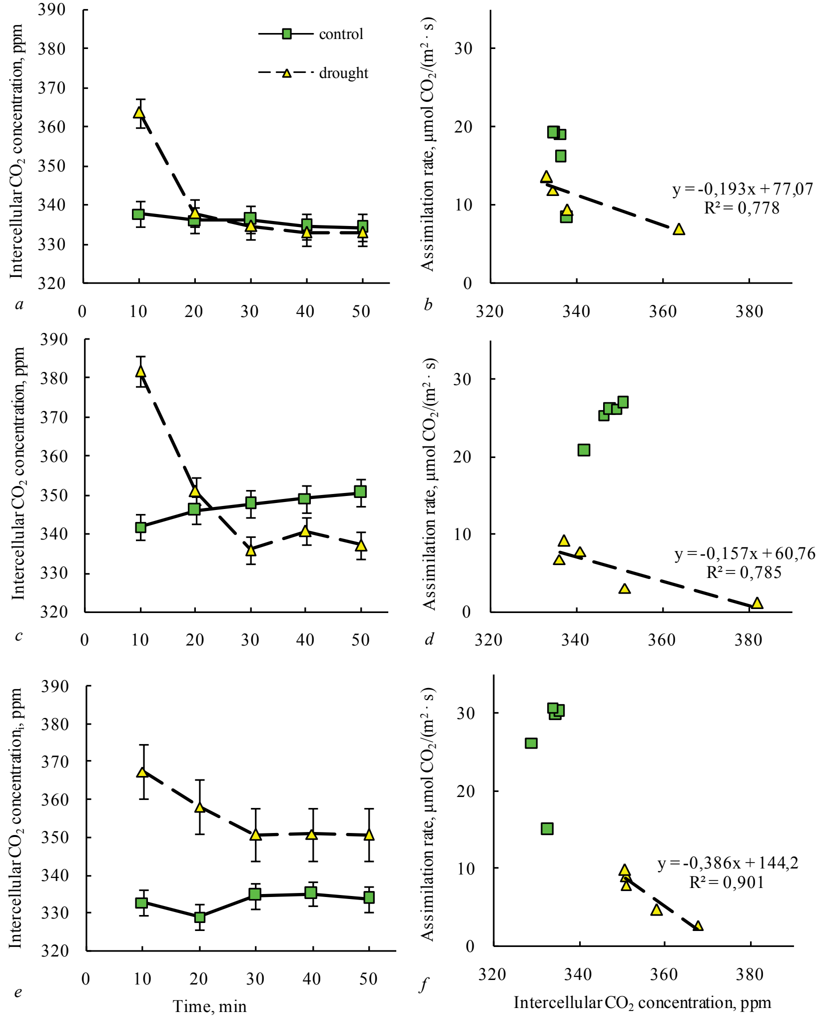

Fig. 7. Intercellular CO_2 concentration (C_i) dynamics (*a*, *c*, *e*), and relationships between C_i and net CO_2 assimilation rate (*b*, *d*, *f*) in the flag leaves of winter wheat genotypes under optimal soil moisture and drought (7 days at 30% FC) during the photosynthesis light induction: *a*, *b* – Yednist, *c*, *d* – Podilska Nyva, *e*, *f* – UK 95/17; mean values ± SE, n = 4

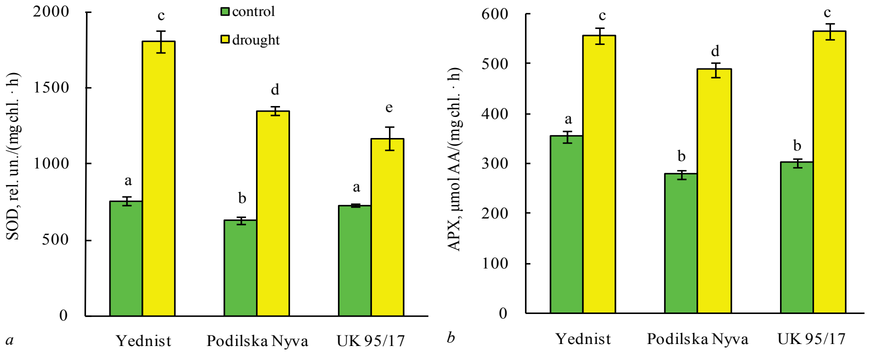

Fig. 8. Effect of soil drought (7 days at 30% FC) on superoxide dismutase (SOD) (*a*) and ascorbate peroxidase (APX) (*b*) activities in the flag leaves of winter wheat genotypes: mean values ± SE, n = 4, values designated by the same letters, are statistically insignificant at P < 0.05; AA – ascorbic acid

The analysis of wheat plant productivity components after reaching full grain maturity revealed that a one-week soil drought during the flowering period significantly reduced both the total weight of the above-ground part and grain productivity in all genotypes, albeit to varying degrees (Table 1). In the control, the highest grain productivity of both the main shoot and the whole plant was observed in the UK 95/17 line and the Podilska Nyva variety. At the same time, there was practically no difference between them in the grain weight from the main shoot, but for the whole plant grain weight was higher in UK 95/17 due to a larger number of grains through higher number of productive shoots. It should be noted that the advantage of these genotypes in grain productivity over the Yednist variety was caused precisely by the high grain number in the ear.

Among drought-treated plants, the lowest degree of the decrease in main shoot and the whole plant grain productivity was observed in the Yednist variety (by 25.5% and 29.5% respectively, compared to the control, Table 1). In the Podilska Nyva variety, the losses in grain yield were somewhat higher (by 26.8% and 37.5%, respectively), and the drought had the strongest effect on the UK 95/17 line plants grain productivity, which almost halved. Plants of the Podilska Nyva variety were almost not inferior to the Yednist variety by the relative decrease in the main shoot productivity under the influence of drought. However, at the level of the whole plant, the first variety lost to the second due to the strongest decrease in the grain number. At the same time, in the plants of the Podilska Nyva and Yednist varieties, the 1000 grains weight under the influence of drought practically did not change, while in the UK 95/17 line it significantly decreased compared to the control.

The harvest index (HI) in the control plants was quite high – within (0.52–0.57). Under the influence of drought, this index decreased by 12.9%, 13.9%, and 18.8% in Yednist and Podilska Nyva varieties, and the UK 95/17 line, respectively. It is interesting that the whole plant grain weight in drought-treated plants of Yednist variety and UK 95/17 line was almost the same (within 2.43–2.65 g), and only plants of the Podilska Nyva variety significantly exceeded this level (3.05 g). This is explained by the fact that the Yednist variety showed higher resilience of grain productivity under the influence of drought than the UK 95/17 line. In plants of the Podilska Nyva variety, the relative decrease in grain weight was higher than in the Yednist variety, but due to high productivity under optimal conditions, drought-treated plants of this variety had an advantage over others in grain weight. Control plants of the UK 95/17 line generally demonstrated the highest grain productivity among the studied genotypes, but they also showed the strongest decrease in treated plants. As a result, under drought treatment they slightly exceeded the Yednist variety, whose productivity under normal conditions was significantly lower.

Table 1
Effect of drought (7 days at 30% FC at flowering stage) on the components of the main shoot and whole plant productivity of modern winter wheat genotypes (x ± SE; n = 20)

Sample	Genotype	Treatment	Shoot(s) weight, g	Grain weight, g	Grain number, pcs.	1000 grains weight, g	Harvest index (grain weight/shoot weight)	Number of productive shoots, pcs.
Main shoot	Yednist	control	3.31 ± 0.14	1.77 ± 0.09	41.2 ± 1.7	42.9 ± 0.9	0.53 ± 0.01	N/A
		treatment	2.86 ± 0.08	1.32 ± 0.05*	30.1 ± 1.1*	44.3 ± 0.9	0.46 ± 0.01*	N/A
		% of control	86.4	74.5	73.0	103.4	86.3	N/A
	Podilska Nyva	control	4.54 ± 0.2	2.52 ± 0.1	54.0 ± 2.2	46.4 ± 0.9	0.55 ± 0.01	N/A
		treatment	3.75 ± 0.17	1.84 ± 0.10*	38.8 ± 1.6*	47.8 ± 2.0	0.50 ± 0.03	N/A
		% of control	82.7	73.2	71.9	102.9	90.5	N/A
	UK 95/17	control	4.41 ± 0.19	2.51 ± 0.11	53.9 ± 1.5	46.3 ± 1.1	0.57 ± 0.01	N/A
		treatment	2.93 ± 0.10	1.32 ± 0.06*	38.3 ± 1.2*	34.8 ± 1.7*	0.45 ± 0.01*	N/A
		% of control	66.3	52.4	71.0	75.0	79.1	N/A
Whole plant	Yednist	control	6.55 ± 0.32	3.44 ± 0.17	83.5 ± 3.7	41.2 ± 1.0	0.52 ± 0.01	2.42 ± 0.11
		treatment	5.31 ± 0.29*	2.43 ± 0.13*	58.6 ± 4.4*	41.7 ± 0.8	0.46 ± 0.01*	2.25 ± 0.11
		% of control	81.1	70.5	70.2	101.2	87.1	92.9
	Podilska Nyva	control	8.65 ± 0.65	4.89 ± 0.37	109.1 ± 8.6	45.1 ± 0.8	0.57 ± 0.01	2.38 ± 0.17
		treatment	6.28 ± 0.36*	3.05 ± 0.19*	66.2 ± 3.6*	45.6 ± 1.2	0.49 ± 0.02*	2.05 ± 0.11*
		% of control	72.6	62.5	60.6	101.3	86.1	86.0
	UK 95/17	control	9.63 ± 0.47	5.24 ± 0.29	120.6 ± 6.6	43.7 ± 0.9	0.54 ± 0.01	2.90 ± 0.18
		treatment	6.11 ± 0.40*	2.65 ± 0.18*	83.3 ± 5.2*	32.5 ± 1.5*	0.44 ± 0.02*	2.79 ± 0.18
		% of control	63.5	50.6	69.1	74.4	81.2	96.1

Note: * – significant difference compared to the control (P < 0.05); N/A – not applicable.

Discussion

The one-week drought at 30% FC caused a similar decrease in RWC (to 78.6–82.4%) in the flag leaf of all cultivars studied (Fig. 1). According to widely accepted classification, this reduction corresponds to moderate stress (Laxa et al., 2019). However, it resulted in different changes in the parameters of photosynthetic apparatus performance. So, in plants of the Yednist variety the declines in net CO_2 assimilation rate, transpiration and stomatal conductance under drought conditions were much smaller than in other cultivars (Fig. 3, 4 and 5). It can be assumed that the Yednist variety plants have more effective osmoregulation and other stress-protective mechanisms (Zahra et al., 2023) ensuring less damage to photosynthetic apparatus. The drought resulted in a significant reduction in chlorophyll content in the leaves of all genotypes studied. However, this damage to the photosynthetic apparatus can also be considered a protective reaction of acclimation. The drought-induced decrease in CO_2 assimilation leads to a strong reduction in the use of energy and reducing equivalents, which are formed as a result of the functioning of the chloroplast electron transport chain (ETC). This causes over-reduction of ETC components and provokes the formation of toxic reactive oxygen species (ROS) and radicals. Under such conditions, electrons from the ETC can pass directly to oxygen, forming the superoxide anion radical (Dumanović et al., 2021). The latter is detoxified by chloroplast's SOD, forming H_2O_2, which in turn is decomposed by APX into H_2O and O_2. Usually, these processes occur under normal conditions, but due to the above-mentioned reasons, they are significantly intensified under drought, to which plants respond by activating protective mechanisms, in particular by increasing the activity of antioxidant enzymes, as observed in our experiments (Fig. 8). At the same time, hydrogen peroxide in moderate amounts is an important signaling molecule involved in triggering widespread defense signaling systems, including those responsible for stomatal movement (Ehonen et al., 2019).

Based on the above, a decrease in the chlorophyll content in leaves under drought conditions contributes to a decrease in the absorption of light quanta by the light-harvesting antenna, which reduces the risks of overreduction of chloroplasts' ETC components, and therefore the amount of ROS formed. However, the latter cannot be completely prevented, as evidenced by an increase in the activity of antioxidant enzymes. Interestingly, the chlorophyll content in the flag leaf of the plants of the UK 95/17 line under normal conditions was the lowest among the studied genotypes – almost at the level of this index under drought conditions in the Yednist and Podilska Nyva varieties (Fig. 2). This can explain the smallest relative decrease in chlorophyll content under stress compared to other genotypes. Also, this line had the smallest relative increase in SOD activity (Fig. 8), which may indicate less superoxide anion radical formation due to less ETC overload. Recently, the protective role of a decrease in chlorophyll content in preventing photoinhibition and photodamage, and in improving photosystem II (PSII) efficiency, was demonstrated in experiments with tomato plants treated with salicylic acid (Moustakas et al., 2022).

Although the drought-induced changes in RWC and chlorophyll content in the studied genotypes were similar, the reduction in the CO_2 assimilation rate in the treated plants relative to the control ones was differential. The plants of the more productive Podilska Nyva variety and the UK 95/17 line showed a larger decline in photosynthetic activity under drought than the Yednist variety. It is worth noting, however, that the CO_2 assimilation rate and speed of photosynthesis induction in well-watered (control) plants of the Podilska Nyva variety and the UK 95/17 line were 1.4–1.6 times higher than in control plants of the Yednist variety (Fig. 3). This lower level of CO_2 assimilation rate was not related to smaller chlorophyll content or RWC in leaves (Figs. 1 and 2). Under drought conditions, the Yednist variety had a higher CO_2 assimilation rate and speed of photosynthesis induction than both other genotypes, displaying superior drought tolerance.

As was mentioned above, a decrease in the speed and completeness of the photosynthetic apparatus response to a change in light conditions leads to a decline in the amount of assimilated carbon, and therefore plant productivity (Taylor & Long, 2017). Under normal growing conditions, the main shoot and the whole plant grain productivity were the highest in the Podilska Nyva variety and the UK 95/17 line (Table 1). The latter was distinguished by the highest net assimilation rate under saturation plateau (Fig. 3). Control plants of the Podilska Nyva variety were somewhat inferior by this index to the UK 95/17 line, but had the highest speed of the increase in CO_2 assimilation rate during dark-to-light transition. The last feature provides an advantage under fluctuating light conditions, which plants usually experience in the field.

The drought significantly impaired the dynamics of photosynthesis induction in the studied cultivars. The time taken for the rate of CO_2 assimilation to reach 50% of the saturation level in drought-stressed plants increased approximately twofold. This implies an additional reduction in the efficiency of photosynthesis, and the carbon gain under natural field conditions. The plants of the Yednist variety under drought maintained the highest photosynthesis among the studied genotypes and had the best parameters of induction curves. This can explain the smallest decrease in their grain productivity compared to conditions of optimal water supply. Plants of the Podilska Nyva variety and the UK 95/17 line had a significantly lower maximum photosynthetic rate under stress, but the parameters of the induction curve were somewhat better in the first genotype, which, in our opinion, led to a relatively smaller decrease in their grain productivity compared to the plants of the UK 95/17 line (Table 1).

Therefore, our results clearly demonstrate that drought affects the state of the photosynthetic apparatus not only with regard to its maximum functioning activity but also the velocity of response to changes in light conditions, which also affects plant performance. It was revealed that under drought conditions, wheat genotypes differentiate according to the parameters of the CO_2 assimilation induction curves more clearly and otherwise than under normal conditions. The delay in photosynthetic induction under drought was observed in experiments with plants of rice and soybean (Sakoda et al., 2022) and a hybrid Populus species (Tang & Liang, 2000).

It is established that the main factors reducing the intensity of the CO_2 assimilation under drought conditions are a decrease in stomatal conductance, a slowdown in the diffusion of CO_2 from the intercellular spaces of the leaf to the centers of carboxylation (mesophyll conductance), as well as a violation of electron transport and ATP synthesis in chloroplasts, a decrease in activity and a disturbance of the synthesis of Rubisco and other enzymes of the Calvin-Benson cycle and increase in the intensity of photorespiration (Zahra et al., 2023). As it is noted above, all these factors, apparently excluding photorespiration, play a differential role in limiting the CO_2 assimilation during different stages of dark-to-light transition (Mott & Woodrow, 2000; Taylor et al., 2020; Sakoda et al., 2021). It was reported that biochemical factors imposed the main limitation on the dynamics of photosynthesis induction with only slight effects of stomatal conductance in well-watered and drought-stressed plants of a hybrid *Populus* species (Tang & Liang, 2000). However, the major role of stomatal conductance in the speed and completeness of photosynthetic induction was shown in experiments with rice and soybean plants (Sakoda et al., 2022).

The main function of the stomata is to optimize the balance between the entry of CO_2 into the leaf and the loss of water during transpiration, maintaining CO_2 concentration in the intercellular spaces of the leaf (C_i) sufficient for its assimilation in the chloroplasts of the mesophyll cells, and minimizing water losses (Lawson & Vialet-Chabrand, 2018; Nunes et al., 2020). Accordingly, C_i and the water relations of the leaf are the main factors in the regulation of stomatal conductance (Ehonen et al., 2019). In this context, the value of C_i reflects the balance between stomatal and non-stomatal factors of photosynthesis limitation, increasing when the portion of stomatal limitation diminishes and decreasing when it rises.

The drought significantly affected stomatal conductance in our experiment (Fig. 6). However, the strength of the manifestation of this effect differed markedly between genotypes. Thus, in plants of the Yednist variety, stomatal conductance under drought conditions decreased by 1.74 times compared to the control, in the Podilska Nyva variety – by 5.56 times, and in the UK 95/17 line – by 5.87 times. At the same time, the last two genotypes had the highest stomatal conductance under conditions of sufficient water supply. There are reports in the literature that species with higher stomatal conductance under high water availability show greater sensitivity to stomata closure during leaf dehydration, i.e., a higher leaf water potential at which stomatal conductance is reduced by 50% (Henry et al., 2019). Our data not only confirm this regularity, but also demonstrate its manifestation within genotypes of the same species.

Under conditions of optimal watering, the lower stomatal conductance in the Yednist variety, compared to other studied cultivars, corresponded with lower CO_2 assimilation, although this variety had relatively high chlorophyll content and a C_i value practically the same as in the UK 95/17 line. It could be suggested that the low stomatal conductance in the Yednist variety is an adaptive trait contributing to high drought tolerance, but it is probably related to lower grain productivity compared to the other two genotypes.

Analysis of C_i dynamics during photosynthesis induction in well-watered and drought-stressed plants (Fig. 7) shows that drought affected the portion of stomatal limitation of CO_2 assimilation differentially in the studied genotypes. Higher-than-control C_i values in the leaves of drought-treated plants at the beginning of light exposure indicate that the activity of the photosynthetic processes in the chloroplasts of mesophyll cells was inhibited more severely than stomatal motion. Since it is known that the synthesis of energy cofactors (ATP and NADPH) for build-up of intermediates of Calvin-Benson cycle and the activation of Rubisco are major limiting factors of photosynthesis induction during the first 10 minutes of illumination (Mott & Woodrow, 2000; Taylor et al., 2020), the chloroplast electron transport and Rubisco-activase can be suggested as one of the main targets of drought stress impact affecting CO_2 assimilation. The damage to the reaction centers of photosystem II under drought is well documented (Kornyeyev et al., 1998; Sommer et al., 2023). It has also been shown that inhibition of ATP synthesis can restrict CO_2 assimilation under mild and moderate drought stress (Lawlor & Cornic, 2002). The scarcity of energy cofactors can be the main reason for low activity of Rubisco activase and a slowdown in Rubisco activation and therefore the rate of CO_2 assimilation increases not only at the beginning of light exposure but throughout the entire period of photosynthesis induction (Amaral et al., 2024). During the last 20 minutes of photosynthesis induction, the values of C_i in leaves of control and drought-treated plants of the Yednist variety did not differ displaying coherence in stomatal and non-stomatal limitation to CO_2 assimilation. It can be assumed that in the stressed plants of the UK 95/17 line, the final values of C_i were higher than in the control ones due to a stronger negative drought effect on the processes of CO_2 assimilation in mesophyll cells than on stomatal conductance. However, in plants of the Podilska Nyva variety, stomatal conductance in drought-stressed plants was suppressed more than the biochemical processes in mesophyll cells, leading to a decrease in C_i compared to the control.

The analysis of the relationship between C_i and the CO_2 assimilation rate revealed a quite close inverse dependence in treated plants of all genotypes but not in control plants (Fig. 7). In our experiments, this relationship displays clearly the drought-induced increase in the portion of non-stomatal (biochemical) limitation of CO_2 assimilation at the beginning of photosynthesis induction, which was partly diminished at the end. In control plants, such a relationship was absent, because the stomata maintained C_i at an almost constant level under a gradual increase in the CO_2 assimilation throughout the period of photosynthesis induction.

The higher contribution of biochemical factors, compared to stomatal conductance, to drought-induced inhibition of photosynthesis is indicated also by changes in the value of WUEi, calculated from the relationships between transpiration and net CO_2 assimilation rates, in control and treated plants (Fig. 5). The drought-stressed plants had lower WUEi, i.e. fixed less CO_2 for the same amount of evaporated water, compared to control ones. This is an another evidence that the suppression of the leaf photosynthetic apparatus under the drought was caused to a greater extent by the damage to the functioning photosynthesizing mesophyll cells than by a decrease in stomatal conductance. Under field conditions, WUEi depends on light conditions, temperature, air and soil humidity, growth stage, and genotypic characteristics of plants, and can vary widely during the growing season (Lopez et al., 2019). Since in our experiments the measurement of the photosynthesis and transpiration rates was carried out under controlled light and temperature conditions, and the humidity of the air supplied to the chamber, as well as the soil in the pots was also stabilized, the parameter obtained as a result of the discussed relationship analysis can be considered quite representative for wheat.

As was mentioned above, the drought-induced decrease in CO_2 assimilation intensifies the formation of toxic ROS in chloroplasts, which are detoxified by chloroplast SOD and APX (Dumanović et al., 2021). A significant increase in the activity of these enzymes was observed in our experiments (Fig. 8). It is worth noting that functioning SOD and APX in chloroplasts is also considered as one of the pathways for electron transfer in the chloroplast electron transport chain (ETC) – the so-called water-water cycle (WWC) or Asada-Halliwell cycle, which has important regulatory and protective functions (Endo & Asada, 2008). Like the cyclic electron transport, the WWC contributes to protection of photosystems from photodamage as well as increases the rate of ATP synthesis relative to NADP reduction. It is believed that under steady-state light intensity, the activity of the WWC is relatively low even under stressful conditions (Walker et al., 2020). However, the relative activity and physiological significance of the WWC increase under fluctuating light, particularly at the start of transition from darkness or shading to bright light, when the intensity of electron transport exceeds the requirements of CO_2 assimilation (Endo & Asada, 2008; Sun et al., 2020). It can be suggested that the bigger increase in the SOD activity in leaves of the stressed plants of the Yednist variety ensured not only the better antioxidant protection but also a larger capacity of the WWC compared to the other cultivars. The increased, due to WWC, synthesis of ATP contributed probably to the quicker increase in CO_2 assimilation rate during the first 10 minutes in stressed plants of that variety.

Conclusions

The present study revealed that drought affects the state of the photosynthetic apparatus not only by reducing its maximum functional activity but also by worsening its ability to respond to changes in light, particularly by inhibiting the speed of the increase in gas exchange rate under dark-to-light transition, implying reduced carbon gain under fluctuating light conditions, which can be a limiting factor for crop growth and yield in the field. According to the parameters of CO_2 assimilation induction curves, plants of different wheat genotypes under drought conditions are more clearly differentiated and in distinct ways compared to those under optimal watering.

We found that high-yielding wheat genotypes with higher assimilation rate and stomatal conductance under optimal watering had greater sensitivity to drought. The suppression of CO_2 assimilation intensity in wheat leaves under drought conditions was mainly caused by damage to the photosynthetic apparatus in mesophyll cells rather than inhibition of stomatal conductance. This is confirmed by the calculations of C_i values, which displayed the highest magnitude at the lowest CO_2 assimilation rate at the beginning of light exposure and the lowest magnitude at the highest CO_2 assimilation rate when steady-state plateau was reached. Due to the stronger impact of drought on the biochemical components of photosynthetic apparatus than on the stomata, the instantaneous water use efficiency (WUEi) during photosynthesis decreases in stressed plants, i.e. these plants fixed less CO_2 for the same amount of evaporated water than well-watered ones.

The effects of genotypic differences in drought on the dynamics of the parameters of photosynthetic induction under dark-to-light transition in wheat leaves indicate the potential of using these traits to evaluate breeding material to increase the ecological plasticity of new wheat varieties.

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