Sensitivity of fast chlorophyll fluorescence parameters to combined heat and drought stress in wheat genotypes

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Abstract: This study aimed to characterise the specific phenotypic responses and the sensitivity of photosynthetic parameters to progressive drought in modern wheat genotypes. In pot experiments, we tested eight wheat genotypes (*Triticum* sp.) that differed in ploidy level and country of origin. Water stress was simulated by the restriction of irrigation, which led to a decreased leaf relative water content of up to 70%. During gradual dehydration, changes in the structure and function of photosystem II (PSII) were analysed using the fluorescence parameters derived from fast fluorescence kinetics (OJIP transient). The results indicated that a group of JIP test-based parameters demonstrated sensitivity to drought, including genotype-specific responses. Severe drought stress led to a decrease in the photochemical efficiency of PSII (F_v/F_m), a reduction in the number of active PSII reaction centers (RC/ABS) and a decrease in parameters, indicating overall photochemical performance at the PSII level (performance indices PI_{abs} and PI_{tot}). These findings demonstrate that the approaches used in our experiments were useful and reliable in monitoring the physiological responses of individual varieties of wheat exposed to stress conditions, and they have application potential as selection criteria in crop breeding. The contribution of the high-temperature effects on the photochemical responses under water deficit conditions is also discussed.

Keywords: photosynthesis; non-invasive methods; stress tolerance; environmental constraints; crop physiology

Photosynthesis is an essential and unique process that occurs in nature, and it involves a number of complex processes related to the conversion of solar radiation and their transformation into the energy of chemical bonds, which takes place in photochemical processes through the fixation of CO_2 and the formation of assimilates. Therefore, the success of cultural species and their genotypes depends on the efficiency of the photosynthetic apparatus (Long et al. 2015, Brestič et al. 2018, Hussain et al. 2021).

During evolutionary processes, plant regulatory mechanisms have undergone adaptation to various climatic changes, such as adaptation to fluctuating irradiation, limited and excess precipitation, or low and high temperatures. These adaptations allow the plants to flourish even under rapidly changing climatic conditions, despite the inhibition of their photosynthetic processes. Therefore, the environment is often perceived as a complex factor that modulates or limits crop growth and production processes (Sharkey 2005, Yeh et al. 2012, Zivcak et al. 2017). Increasing the drought and high-temperature tolerance of crops is one of the critical challenges facing plant research and breeding practices. The production of wheat, as one of the world's strategic crops, is reduced by 10–40% every year due to long-

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term water deficits, and high temperatures are also associated with changes in the environment toward climatic extremes (Farooq et al. 2014).

Drought is the most limiting abiotic factor for most plants, and it causes internal water deficit and stress. Therefore, this factor determines the sensitivity of individual genotypes and the activity of many defence mechanisms or inhibitory processes. Drought intensity and frequency are expected to increase, which will lead to a decline in plant yields and an increase in plant susceptibility to drought (Lu and Zhang 1999). One of the primary direct responses to drought is the closure of stomata, which leads to a reduction in CO2 uptake and an increase in the retention of water content in the leaves. In the case of long-term dehydration, the importance of hydraulic signalling increases. However, despite the regulatory processes, plant tissues become dehydrated, which leads to a reduction in plant performance, including effects observed at the level of photosynthetic electron transport and PSII photochemistry that can be well assessed using chlorophyll fluorescence techniques (Brestič and Živčák 2013). Several authors have suggested that chlorophyll fluorescence can be more efficiently used as a suitable selection criterion for genotype screening and as an indicator of the effects of climate change on crop species (Sharma et al. 2012, Kalaji et al. 2014, Zivcak et al. 2017).

There are several methods of addressing drought stress in crop plants to decrease yield losses, including changes in cultivation technologies and agronomic practices (Wang et al. 2003, Yang et al. 2007, Araus and Caims 2014). Moreover, crop breeding and the production of new, more tolerant genotypes are essential (Kaiser 1987, Chaves et al. 2008, Tuberosa 2012). However, to increase the efficiency of crop improvement processes, it is essential to apply appropriate physiological criteria and use efficient and reliable techniques to support the selection process toward the creation of new crop varieties that demonstrate a higher tolerance to environmental stress (Monneveux et al. 2012).

Thus, this study aims to characterise the specific responses of a diverse collection of wheat genotypes to progressive drought based on analyses of fast chlorophyll fluorescence kinetics, including testing the key fluorescence parameter sensitivity and genotypic variations in response to dehydration. The presented data may provide useful information toward developing procedures for more efficient drought tolerance screening of wheat genotypes.

MATERIAL AND METHODS

Biological material. In the experiment, eight morphologically and physiologically different genotypes of winter wheat of different origins belonging to several taxa of *Triticum* sp. were used: Rajve (Slovenia), San Pastore (Italy), Nanjing P2 (China) (all Triticum aestivum L.); Dusan (Serbia), ANDW-7A (USA) (Triticum durum L.); Spelled Line 73 (Belgium) (Triticum spelta L.), AZESVK2009-90 (Georgia) (Triticum timopheevi Zhuk.); and GRCSVK2013-16 (Greece (*Triticum monococcum* L.)). The seeds were obtained from the Gene Bank of NAFC-RIPP in Piešťany (Slovak Republic). The pot experiment was established in autumn. The plants were sown during the regular agrotechnical period so that they could undergo vernalisation under natural conditions. Wheat was cultivated in 3 L plastic pots (one plant per pot) with the same quantity of Klassman 4 soil substrate in all pots and the addition of Osmocote fertiliser (5 g per pot). During ontogenesis, all plants were exposed to direct sunlight, and after the application of water stress (after anthesis; 21 May), the plants were placed under a transparent foil tunnel. The control group consisted of well-watered plants, while the stressed group consisted of plants exposed to dehydration. Drought was induced by reducing the dose of irrigation to 1/3 of the total dose intended for the control plants. The weighting of plants was performed, and the doses were calculated based on the water loss of the plants. The experiment was carried out in 2018-2019 in an outdoor experimental space of the Department of Plant Physiology at the Slovak University of Agriculture in Nitra (Slovak Republic). The measurements presented in this study were performed on fully developed flag leaves from 21 May to 21 June 2019; eight series of measurements were performed eight times during this period.

Fast kinetics of chlorophyll fluorescence. The chlorophyll fluorescence fast phase parameters were calculated based on measurements performed by a Handy-PEA analyser (Hansatech Instruments, King's Lynn, UK). The leaves were illuminated with continuous red light (wavelength peak at 650 nm). The light was provided by an array of 3 light-emitting diodes. The light pulse intensity was $3\,500\,\mu\text{mol/m}^2/\text{s}$, and the light pulse duration was 1 s. Measurements were performed in the middle part of the leaf away from the central vein after 30 min of dark adaptation using leaf clips. The fluorescence intensity curve is characterised by polyphase growth, with the O

phase representing zero, i.e., basal fluorescence, J representing a reduction of the primary electron acceptor Q_A in PSII to Q_A , I representing a change in the size of plastoquinones and P representing a reduction of all plastoquinones PQ to PQH₂.

Based on the chlorophyll fluorescence data, multiple biophysical parameters, including the JIP-test ratios, were calculated (Strasser et al. 2000, Stirbet and Govindjee 2011). F_v/F_m is the maximum photochemical efficiency of PSII, and it is determined in a state of almost maximum openness of all RCs (after dark adaptation of plants) and defined as the quantum yield of electron transport to the primary acceptor, with an optimal value of 0.834. RC/ABS is the ratio of the total number of reaction centers to the total number of photons absorbed by the chlorophyll molecules of all reaction centers, and it expresses the average size of the active reaction center (RC) antennas. PI_{abs} is the performance index calculated based on absorption, and it is defined as the vitality index of photosynthesis and expresses the energy savings from photons absorbed by PSII light-collecting antennas up to Q_B reduction (Strasser et al. 2000). PI_{tot} represents the total performance index calculated based on the chlorophyll content, and it is defined as the total photosynthesis performance (Stirbet and Govindjee 2011). W_{κ} represents the relative variable chlorophyll fluorescence at time K, and it is derived from the fast fluorescence kinetics (Strasser et al. 2000).

Determination of the relative water content **in leaves.** The leaf relative water content (RWC) analyses were realised to assess the dehydration level. To eliminate destructive measurements to an unnecessary minimum, the RWC was assessed on 14 June, when the visual symptoms of drought stress appeared and a significant decrease of fluorescence parameters was observed. The samples of flag leaves were collected, and their fresh weight (FW) was measured. Subsequently, the leaf segments were immersed in distilled water for 4 h, and then the leaf weight with full turgor (TW) was determined. The leaves were dried in an oven at 80 °C for 3 h to obtain the dry weight (DW). The relative water content in the leaves was then calculated according to the following formula:

RWC (%) =
$$\frac{[FW - DW]}{[TW - DW]} \times 100$$

Statistical analysis. Statistical analysis was performed using a two-factor analysis of variance (ANOVA) followed by the post hoc Tukey's HSD (honestly significant difference) test (P < 0.05) using

Statistica version 9.0 software (Statsoft Inc., Tulsa, USA). The factors analysed included the water supply (control vs. drought stress variant) and genotype (8 genotypes). Where measurements were repeated several times during the measuring period, repeated measures analyses were used. The experiment was designed in blocks; each variant consisted of 32 pots. Four pots were analysed in each genotype in both variants (n = 4). The experiment was designed in blocks. All interpretations used in the text are supported by the results of the statistical analyses.

RESULTS AND DISCUSSION

To detect the genotype-specific patterns of the drought stress responses, nondestructive measurements of fast fluorescence kinetics were performed in the collection of diverse wheat genotypes to characterise the physiological state of the plants and the functional state of the photosynthetic apparatus. The monitoring of the fluorescence values during the whole period of the test (May–June 2019) indicated the divergence of the fluorescence parameter values. For example, the mean maximum photochemical efficiency $(F_{\rm v}/F_{\rm m})$ values before stress reached a value of 0.80 ± 0.02 for all genotypes (Figure 1A), which reflected the optimal state of the photosynthetic apparatus.

During dehydration, the values of F_v/F_m significantly decreased below the values of the control plants to an average value of 0.67, which was obtained on 14 June. However, although the drought stress was not terminated, we observed a slow recovery afterwards. A similar trend with a much lower F_v/F_m decrease was observed in the control plants. This finding indicates a strong interaction of drought with cooccurring environmental factors, especially with high temperatures because the period from 10-15 June was very hot in a given location, with daily maxima over 30 °C (peak at 33.1 °C on 14 June), whereas in the period from 16 to 21 June, it was cloudier, with moderate temperatures below 30 °C. Our previous results (Zivcak et al. 2008, 2019, Chovancek et al. 2019) indicate that an increase in temperature above the optimum values can lead to a significant interaction effect with drought. Thus, the fluorescence values obviously followed the trend of daily temperature, and the combination of drought and the high temperature was needed to observe a substantial decrease in F_v/F_m values in drought-stressed plants below the values of well-hydrated plants.

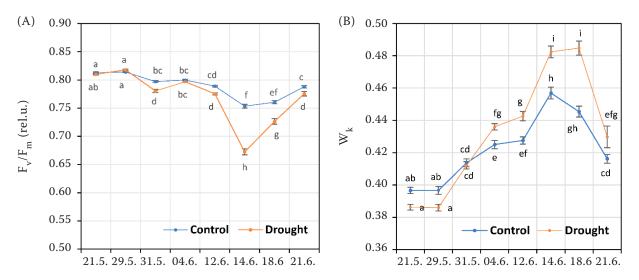


Figure 1. Analysis of the photosynthetic parameters in wheat varieties during the period of plant dehydration. Control includes well-hydrated plants, and drought includes plants with limited water supply. (A) Maximum photochemical efficiency of PSII (F_v/F_m) and (B) relative variable fluorescence at 0.3 ms (W_k). Mean values \pm standard error for all tested genotypes are presented. Different small letters indicate significant differences between treatments (P < 0.05) by Tukey's HSD (honestly significant difference) test

The low sensitivity of the F_v/F_m parameter to drought, when measured under moderate temperature observed in our study, corresponds well to the low sensitivity of PSII quantum efficiency to the drought that was previously clearly documented in wheat (Lu and Zhang 1999, Zivcak et al. 2008). Thus, we can preassume that F_v/F_m was probably more affected by the high temperatures and not by the drought itself.

To confirm the high-temperature interaction, we also analysed the relative variable fluorescence in 0.3 ms (K step of the fluorescence transient), indicated as W_k (Figure 1B). This parameter was previously found to be broadly insensitive to leaf dehydration but very sensitive to heat stress in wheat (Brestic and Zivcak 2013). The trends of W_k decrease followed the onset of the high-temperature period mentioned

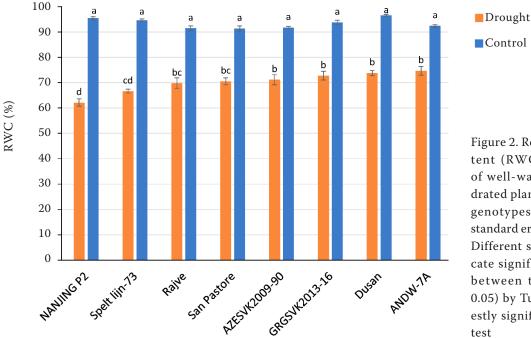


Figure 2. Relative water content (RWC) in the leaves of well-watered and dehydrated plants of eight wheat genotypes. Mean values \pm standard error are presented. Different small letters indicate significant differences between treatments (P < 0.05) by Tukey's HSD (honestly significant difference) test

above, both in the control and water-limited variants. However, the increase in W_k was significantly higher in drought-stressed plants, which may be associated with a higher leaf temperature due to the limited cooling effect of transpiration in plants exposed to water deficit (Ayeneh et al. 2002), and the difference in leaf temperature between well-watered and water-limited plants may exceed four degrees (Botyanszka 2019).

To analyse in detail the different responses to environmental stress between various genotypes, we analysed the data obtained in individual genotypes in addition to the stress (14 June). As the information on the dehydration level is crucial, we also analysed the leaf relative water content in both variants in all genotypes (Figure 2).

The RWC in the control plants fluctuated above 90%, which corresponds to the regular water content of well-hydrated plants. On the other hand,

wheat genotypes responded to the water deficit by decreasing the RWC, with a mean value of approximately 70%. A previous study found that 70% RWC represents the threshold value, in which the effect on metabolic functions in plants includes significant effects on the electron transport-related processes indicated by chlorophyll fluorescence (Masacci et al. 1996, Zivcak 2013). Among the genotypes assessed in the study, the differences in RWC in the drought-stress group were mostly insignificant, and although a lower value was found in Nanjing-P2, it was still above 60%.

During the period of the most intensive dehydration of plants, the maximum photochemical efficiency (F_v/F_m) decreased from 6% to 10% in the genotypes showing a more resistant response, from 10% to 15% for the mildly sensitive genotypes, and by over 15% for the most genotypes appearing the sensitive response (Figure 3A).

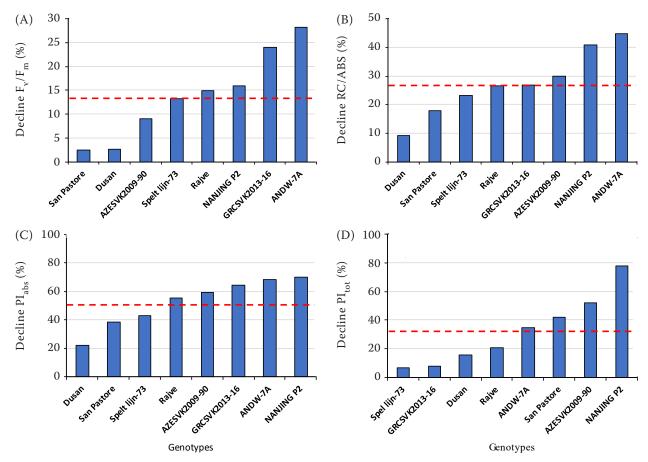


Figure 3. The average decline in drought compared to control in chlorophyll fluorescence fast phase parameters at terminal drought. (A) Maximum photochemical PSII efficiency (F_v/F_m) ; (B) ratio of active reaction centers to the absorption of chlorophyll molecules (RC/ABS); (C) performance index (PI_{abs}), and (D) total performance index (PI_{tot}). The columns represent the differences between the mean values observed in control and dehydrated plants. The red line indicates the mean value of the whole collection

We observed a significant decrease in F_v/F_m that reached 28% in the genotype of durum wheat ANDW-7A, which is typical of lower chlorophyll content and was previously found as more stress-sensitive. A similar change trend in the values induced by terminal drought was observed in the parameter that characterised the decrease in the number of active reaction centers for the absorption of chlorophyll molecules (RC/ABS). Compared to the other varieties, the genotype ANDW-7A showed a statistically significant decrease in RC/ABS that reached 45%, which was similar to that of F_v/F_m (Figure 3B). In this genotype, we previously identified a limited capacity to regulate electron transport (Zivcak et al. 2019), which may be a primary reason for its low-stress tolerance (Ferroni et al. 2020).

Interestingly, the integrative fluorescence parameters (performance indices, Figures 3C, D) indicated that the most substantial drought stress effect occurred in the cv. Nanjing-P2, which makes sense because the lowest RWC and hence the most severe stress were observed in this genotype. In particular, the parameter PI_{tot} indicated the highest level of drought stress-related damage in this genotype, which indicates that the drought stress in the genotype Nanjing-P2 strongly affected the electron transport chain at the PSII acceptor side, especially electron transport inhibition around PSI, as documented by the difference in $\mathrm{PI}_{\mathrm{tot}}$. A previous study found that fluorescence kinetics, including performance indices, can be relatively insensitive to a sudden decrease in the leaf hydration level (Goltsev et al. 2012). Unlike fast dehydration, changes in fluorescence kinetics were demonstrated in the case of prolonged drought stress. Specifically, $\mathrm{PI}_{\mathrm{tot}}$ is a reliable indicator of the nonstomatal limitations of photosynthesis associated with a reduction in the content of Rubisco content and activity and an increase in the number of non-reducing Q_{A} PSII centers (Jedmowski et al. 2014).

A comparison of the four parameters (Figure 3) indicates that F_v/F_m presents the lowest sensitivity, RC/ABS presents a slightly higher sensitivity, and the performance indices present high sensitivity, for which a decrease of over 70% was observed. A comparison of the two performance indices indicates that these two parameters, despite their similar interpretation, provide significantly different information. Chen et al. (2017) indicated that the response of PI_{abs} to temperature in a model plant was closely related to the normalised level of the K-peak. However, the I-P phase involved in PI_{tot} greatly modifies the values under combined drought and heat stress, as previously shown (Psidova et al. 2018). Therefore, it makes sense to use both of the performance indices together.

To analyse the effects specific to heat stress, we compared the K-step (W_k) values, which detect impairment of the oxygen-evolving complex by the direct effect of high temperature (Srivastava et al. 1997). The responses in genotypes analysed in the period with most apparent stress (14 June) indicated that overall changes in K-step were not clearly visible in the normalisation plot, which indicates that the effects of heat stress were not severe (Figure 4A). The only ex-

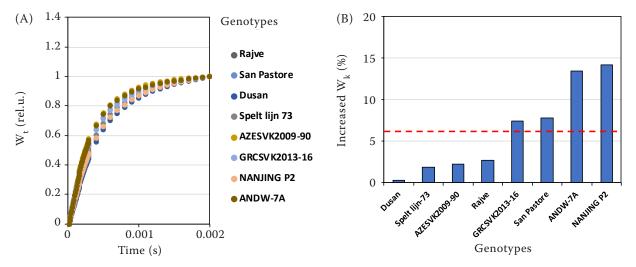


Figure 4. (A) Normalisation of chlorophyll fluorescence in phase J measured during drought for a group of 8 wheat genotypes, and (B) average increase in the variable fluorescence parameter of chlorophyll in phase K (W_k) in the drought-stress variant compared to the control. The red line indicates the mean value of the whole collection

ceptions were genotypes Nanjing-P2 and ANDW-7A (Figure 4B). In Nanjing-P2, the more severe OEC damage may be associated with its more profound dehydration associated with higher possible heating of the leaves, whereas in ANDW-7A, this damage may be associated with the overall sensitivity of its photosynthetic apparatus.

Thus, our results indicate that although the changes in PSII quantum efficiency ($F_{\rm v}/F_{\rm m}$) or a number of active reaction centers (RC/ABS) may reflect the differences in photoprotective capacities of the genotypes, the performance indices, especially ${\rm PI}_{\rm tot}$, can well indicate the direct effects of dehydration. This finding supports the idea that a multiparametric approach can provide more valuable and comprehensive information on the responses of crop plants to environmental stress compared with a single parameter (Kalaji et al. 2017, Stirbet et al. 2018).

Thus, our experiments confirmed the diversity in drought stress responses identified at the level of PSII photochemistry and beyond. The values of the fluorescence parameters, such as the maximum quantum efficiency of PSII photochemistry or the number of active reaction centers, were strongly influenced by the interaction of drought with high temperature, which was confirmed by the changes identified at the K-step of the fluorescence transient (W_k parameter). On the other hand, the integrative performance indices, especially the total performance index (PI_{tot}), were also sensitive to the changes associated with dehydration. Moreover, the overall sensitivity and ability to recognise the differences between the drought-sensitive and drought-tolerant genotypes were highest for PI_{tot}, thus confirming the exceptional suitability of this indicator in determining water stress effects and screening genotypes according to their drought tolerance. However, it is also evident that the multiparametric approach provides more complex information and may represent the most suitable way to practically include the method in the breeding process, thus leading to a more efficient selection of varieties that are tolerant to climatic extremes.

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