Influence of induced drought on photosynthetic performance in *Dactylis glomerata* varieties during the early growth stage

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**Abstract:** Drought significantly impacts the growth and yield of forage grasses, particularly its effect on *Dactylis glomerata* photosynthetic apparatus during the initial phase of development remains largely unknown. This study investigated the effects of drought on physiological parameters of various *D. glomerata* varieties. The seedlings obtained after seed germination under optimal and simulated drought conditions by PEG 6000 (three variants) were planted in small pots filled with garden substrate. Over a span of 42 days, the plants were initially kept well-watered (70% capillary water capacity, CWC), after which half of the seedlings from each variant were subjected to drought. This drought stress was applied during the tillering phase for 12 days. Subsequently, the plants were rehydrated (at 70% CWC) and allowed to recover for 14 days. Throughout both drought and recovery periods, measurements were taken. Leaf chlorophyll fluorescence parameters were assessed, and the JIP-test analysis was utilised to provide detailed insights into the functionality of *D. glomerata* photosynthetic apparatus under drought stress and post-recovery conditions. Several parameters were identified as indicative of the plants’ sensitivity to drought, such as performance indices PI ABS and PItot, along with quantum yield parameters Ψ E0, φE0, and φ P0. The results highlighted that var. Minora and Tukan exhibited greater tolerance to water deficit when compared to the other varieties studied. They showed a large increase in PI ABS and PItot values after drought stress as well as after the re-watering (recovery period) compared to control plants. This suggests their potential for better adaptation to drought conditions.

**Keywords:** chlorophyll a fluorescence transient, *Dactylis glomerata*, JIP-test, tillering phase, water deficit

**INTRODUCTION**

Amidst the context of climate change, water scarcity stands as a pivotal environmental factor that profoundly impacts plant development and yield conditions (Elsherey and Cao, 2008; Staniak and Kocoń, 2015; Omar et al., 2018). The repercussions of drought on grass growth exhibit significant variation, even within the same species (Mastalerczuk, Borawska-Jarmułowicz and Kalaji, 2017). *Dactylis glomerata* varieties play a crucial role in fodder grass mixtures across both permanent and temporary grasslands within temperate climate settings (Borawska-Jarmułowicz, Mastalerczuk and Janicka, 2016; Borawska-Jarmułowicz et al., 2022). This species demonstrates robust growth after seeding and strong competitiveness in relation to other mixture components throughout subsequent years of utilisation, along with notable drought tolerance during later stages of development.

The increasing prevalence of prolonged droughts has had a discernible impact on grassland productivity in Poland (Gabryszuk, Barszczewski and Wrobel, 2021). This can be attributed to disruptions in plant metabolism, particularly the photosynthetic activity (Kalaji et al., 2016; Fariaszewska et al., 2017). The measurement of chlorophyll a fluorescence (ChlF) parameters, closely linked to PSII functionality (Baker and
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Rosenqvist, 2004, has revealed responses to various stressors, including drought (Li et al., 2006; Elsheery and Cao, 2008; Kalaji et al., 2012; Kosmala et al., 2012; Borawska-Jarmulowicz et al., 2014; Guidi et al., 2016; Sousa de et al., 2017; Elsheery et al., 2020). The non-destructive analysis of the polyphasic fast chlorophyll transient, developed for assessing photosynthetic function, involves recording chlorophyll fluorescence emitted by a dark-adapted leaf during a brief (typically one-second) pulse of intense actinic light using a fluorimeter (Strasser, Tsimilli-Michael and Srivastava, 2004). High-resolution measurements of chlorophyll a fluorescence transient offer a swift means of extracting comprehensive information about PSII photochemical activity. Fast ChlF induction, a non-invasive spectroscopic technique, facilitates the detection and measurement of the effects of photosynthesis inhibitors in vivo (Baker, 2008).

The augmentation of ChlF intensity upon continuous bright light application to a dark-adapted sample is time-dependent and serves as a basis for calculating fast ChlF kinetics data. These data yield the ChlF transient curve (Schansker, Toth and Strasser, 2005; Stirbet and Govindjee, 2011). The ChlF method holds potential for genotypic screening for drought tolerance (Gaipa, Sengupta and Reddy, 2013). Evaluating plant vitality under stress conditions is feasible through analysis of the ChlF transient curve. Fluorescence parameters obtained from this curve, known as JIP-test (Force, Critchley and Rensen van, 2003; Strasser, Tsimilli-Michael and Srivastava, 2004), rapidly identify the influence of diverse stress factors on photosynthesis and demonstrate PSII functionality (Schansker, Toth and Strasser, 2005; Oukarroum et al., 2007). Chlorophyll fluorescence measurements, encompassing induction kinetics studies in dark-adapted samples followed by OJIP analysis, are commonly employed to study plant responses to abiotic stresses (Kalaji et al., 2016).

A typical fluorescence transient shows phases including the initial (F₀), represented on the fluorescence transient curve as O, followed by I (~2 ms), J (~30 ms), culminating in maximal (F₅₀) fluorescence (termed step P) (Strasser, Srivastava and Tsimilli-Michael, 2000; Strasser, Tsimilli-Michael and Srivastava, 2004). In photosynthetic samples kept in darkness, the electron acceptor side of PSII predominantly resides in the oxidised state (PSII reaction centres open, fluorescence intensity minimal). The swift O to J rise, referred to as the photochemical phase, is highly dependent on exciting light intensity, while the J–I and I–P segments of the fluorescence curve, known as thermal phases (temperature-sensitive), are much slower. Within less than 1 s, Chl a fluorescence reaches peak P.

The principal values are utilised to compute JIP-test parameters elucidating energy fluxes within and around the photosystem II (PSII) reaction centre (RC) (Strasser, Srivastava and Tsimilli-Michael, 2000). These parameters, expressed per active RC, denote specific energy fluxes, while per excited CS, they signify phenomenological energy fluxes. This framework enables evaluation of consecutive energy fluxes: average photon absorption (ABS), energy dissipation (DI), exciton trapping (TR), electron transport (ET), and reduction in electron acceptors at PSI’s acceptor side (RE) for assessing PSI functions. Other widely used parameters for providing insights into plant state under drought include the performance index (PI) and Fv/Fm (Force, Critchley and Rensen van, 2003; Živcák et al., 2008).

While *D. glomerata* is recognised as drought-tolerant during later development stages, scant attention has been paid to its early growth phase photosynthetic behaviour under drought stress. Our prior research demonstrated that induced drought conditions during seed germination positively impacted the photosynthetic efficiency and morphological characteristics of *D. glomerata* seedlings amid soil water deficit (Borawska-Jarmulowicz et al., 2020). This study employs the OJIP transient coupled with the JIP-test to uncover alterations in photosynthetic behaviours of *D. glomerata* plants during the tillering phase under drought conditions.

This study’s objective is to unveil insights into the photosynthetic apparatus functions of *D. glomerata* plants in the early growth stage under drought stress. Furthermore, it seeks to determine if seedlings obtained from seeds germinated under simulated drought conditions influences chlorophyll fluorescence parameters in *D. glomerata* subsequent developmental phase (tillering phase), and to identify which fluorescence parameters could serve as clear indicators of water stress prompted by drought.

**MATERIALS AND METHODS**

**PLANTS, GROWTH CONDITIONS AND EXPERIMENT DESIGN**

The impact of drought stress on physiological parameters of seedlings from various Polish *Dactylis glomerata* varieties — Amera (early), Krysta, Minor (medium early), and Tukan (medium late) — sourced from Poznańska Hodowla Roslin Ltd., was systematically investigated. The seedlings were obtained after seed germination under optimal and simulated drought conditions. Seed germination was conducted on Petri dishes within a controlled phytotron environment, maintaining specific conditions: air humidity at 60–65%, temperature at 25/16°C (day/night), and a photoperiod of 8 h light and 16 h darkness, supplemented with a light intensity of 1000 µmol·m⁻²·s⁻¹. Drought conditions were replicated through polyethylene glycol (PEG) 6000 on four variations: 0.0 MPa (control with laboratory paper at 60% humidity using deionised water), –0.4, –0.8, and –1.2 MPa. Each variety underwent three replications per variant, adhering to the approach outlined by Hardegree and Emmerich (1994). The osmotic potential of each solution was measured employing a cryoscopic osmometer Osmomat 030 (Gonotec, Germany).

After 21 days of germination, three replications of five plants each (seedlings possessing 2–3 leaves) from each variety of *D. glomerata* and induced variant of drought (during seed germination) were transplanted into pots (volume 1331 cm³), filled with garden substrate. It was characterised by pH_HCl 5.0–6.0, nitrogen content of 80–120 mg·cm⁻³, available phosphorus ranging from 22–44 mg·cm⁻³, and potassium within the range of 83–124 mg·cm⁻³. Following this, a nitrogen fertilisation of 0.85 mg per pot (equivalent to 50 kg·ha⁻¹, applied as urea) was administered after 21 days of vegetation.

To maintain optimal conditions, the plants were watered every two days until the soil and pots reached the desired weight, thereby sustaining 70% capillary water capacity (CWC) over the course of the next 42 days. Subsequently, during the tillering phase (characterised by 1–2 shoots per plant), half of the seedlings from each variant were maintained at 70% CWC (serving as the control), while the other half were subjected to a drought with no...
merata varieties, modifications in the phases within the relative variable fluorescence kinetics were scrutinised by doubly normalising the OJIP transients. In order to visually depict the impact of drought stress on the trajectory of the chlorophyll fluorescence induction curve, the relative variable fluorescence (Vt) was calculated using the formula: \( V_t = (F_t - F_0)/(F_M - F_0) \). The distinction in relative variable fluorescence curves stemming from drought stress was evaluated by contrasting the normalised fluorescence values (between O and P steps) observed in control plants under drought conditions (Strasser et al., 2010).

The induction curve generated was further used for the computation of various parameters signifying PSII performance. Specific energy fluxes for individual PSII reaction centres (RCs) and phenomenological energy fluxes assessed for the photosynthetic sample’s area (CS) at \( t = 0 \) were determined, alongside performance indices calculated on an absorption basis (PI ABS, PI tot). Biophysical parameters were deduced from the OJIP transients, including quantum yields (\( \Phi_P, \Phi_E, \Phi_R, \Psi_E, \delta_R \)), specific activities per reaction centre (RC), performance indices (PI), and structure-function indices (VJ, Sm, N) (Strasser et al., 2010). The descriptions and abbreviations of the employed fluorescence parameters are provided in Table 1.

Table 1. Definition of the selected fluorescence parameters (all fluorescence parameters are in relative units)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
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<tr>
<td>F M</td>
<td>time (in ms) to reach the maximal fluorescence intensity ( F_M )</td>
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<tr>
<td>V J = (F J – F 0)/(F M – F 0)</td>
<td>relative variable fluorescence at the J-step at moment ( t = 2 ) ms; ( F_J ) = fluorescence level at that moment; ( F_0 ) = minimal fluorescence level; ( F_M ) = maximal fluorescence level</td>
</tr>
<tr>
<td>S m = (Area)/(F M – F 0)</td>
<td>normalised total area above the OJIP curve (reflecting multiple turnover of QA reductions)</td>
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<tr>
<td>N = S m ∙ (M 0/V J)</td>
<td>number indicating how many times QA is reduced until fluorescence reaches its maximal value ( F_M ) (number of QA redox turnovers until ( F_M ) is reached)</td>
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</table>

Specific energy fluxes (per primary electron acceptor in PSII) – Quinone A (QA) – reducing PSII reaction centre – RC

ABS/RC = (1 − γRC)/γRC | absorbed energy flux per RC; absorption flux per one active RC, a ratio of active to inactive RCs |
DL/RC = (ABS/RC – R 0/RC) | dissipated energy flux per RC (at \( t = 0 \)); energy flux not intercepted by an RC, dissipated in the form of heat, fluorescence, or transfer to other systems, at time \( t = 0 \) |
TR 0/RC = M 0/(1/V J) | trapped energy flux per RC; energy flux trapped by one active RC at \( t = 0 \) |
ET 0/RC = M 0/(1/V J)Ψ E | electron transport flux per RC; rate of electron transport by one active RC at \( t = 0 \) |

The quantum yield for primary photochemistry

\( \Phi_P = 1 – F_0/F_M \) | maximum quantum yield of primary photochemistry (at \( t = 0 \)) |
\( \Phi_E = (1 – F_0/F_M)/(1 – V J) \) | quantum yield of electron transport (at \( t = 0 \)) |
\( \Phi_R = (1 – F_0/F_M)/(1 – V J) \) | quantum yield of reduction of end electron acceptors at the PSI acceptor side (RE) |
\( \Psi_E = 1 – V J \) | probability (at \( t = 0 \)) that a trapped exciton moves an electron into the electron transport chain beyond QA |
\( \delta_R = (1 – V J)/(1 – V J) \) | efficiency/probability with which an electron from the intersystem electron carriers moves to reduce an electron acceptors at the PSI acceptor side (RE) |

Phenomenological fluxes

ABS/CS 0 | absorbed energy flux per excited cross section (CS 0) |
DL/CS 0 | dissipated energy flux per CS 0 |
TR 0/CS 0 | trapped energy flux per CS 0 |
ET 0/CS 0 | electron transport flux per CS 0 |

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Influence of induced drought on photosynthetic performance in Dactylis glomerata varieties during the early growth stage

Statistical analysis of the data obtained during drought period and recovery of plants in the tillering phase was conducted through a multivariate analysis of variance (ANOVA) using Statistica 13.3 software (Statsoft Inc., Tulsa, OK, USA). The experiment was replicated three times. The key experimental factors encompassed: (A) soil moisture during the plants growth (optimum, drought), (B) simulated drought during seed germination (0.0 MPa, -0.4 MPa, -0.8 MPa, and -1.2 MPa), (C) D. glomerata variety (Amera, Krysta, Minora, and Tukan), and (D) measurement term (1, 2, 3 – corresponding to drought period, and 4 – post-recovery period). Additionally, interactions between soil moisture (optimum and drought) and the PEG variant (germination conditions) were examined. To assess significant differences, the means of each evaluated parameter were compared using Tukey’s HSD test at a significance level of $p \leq 0.05$. A principal component analysis (PCA) was performed to examine relationships between the tested parameters and characterise the multivariate distinctions among them in relation to the plant growth conditions.

RESULTS AND DISCUSSION

**OJIP FLUORESCENCE TRANSIENTS AND CALCULATED CURVES**

Drought stands as a significant factor capable of impeding plant functionality (Heeren van Swanepoel and Kruger, 2009). Despite numerous investigations aimed at unravelling the intricate mechanism through which drought stress influences plant photosynthesis, this subject remains an ongoing area of exploration (Lawson et al., 2003). Furthermore, thus far, no research has been conducted to ascertain the potential impact of drought-induced changes in seeds (resulting from increased surface tension with PEG 6000) on the subsequent performance of the photosynthetic apparatus in sprouted seedlings, which inevitably operate within water deficit conditions. The mechanism constraining photosynthesis during water scarcity is intricate, particularly as drought escalates to severe levels. Our research indeed validated the hypothesis that seed hardening does influence the ensuing resilience of Dactylis glomerata in the face of this stress. Nevertheless, the stress response was notably contingent on the variety in question and the duration of the drought (Tab. 2).

Throughout the examination, the tested D. glomerata varieties consistently exhibited typical OJIP chlorophyll fluorescence transients both during drought application and after plant recovery (Figs. 1–4). The trajectory of Chl $a$ fluorescence transitions across the distinct phases O–J, J–I, and I–P directly correlated with the physiological state of the photosynthetic subject. Our conclusions indicated that when maintained at 70% CWC soil moisture (as a control), no discernible alterations in the OJIP curve’s trajectory were observed. However, following drought stress, the values of OJIP-test parameters exhibited clear discrepancies among the studied varieties, contingent upon the specific accession. As per Baker (2008), drought stress has the potential to alter the characteristic points of the OJIP curve and lead to a reduction in fluorescence intensity at the J, I, and P steps. However, the extent of this change is variably influenced by the specific plant variety. Our observations align with this notion, revealing that the application of drought induced a reduction in the progression of induction curves across all tested plants, in comparison to the control. Particularly, the I–P phase bore the brunt of this impact, which can be attributed to a decline in electron acceptors. Similar phenomena were documented by Oukarroum et al. (2007) in barley plants. Stirbet and Govindjee (2012) elucidate that during the J–I–P phase, PSI reaction centres gradually close while the plastoquinone pool concurrently experiences reduction.

In our investigation, plants of var. Amera, originating from seeds germinated under induced drought conditions of -1.2 MPa, then subjected to 12 days of drought, exhibited a curve with a steeper trajectory than those of the other conditions. Notable disparities were evident at points I and P (Fig. 1B). Conversely, the curves obtained for the remaining variants closely resembled the trajectory of plants not subjected to stress. After recovery under 70% CWC conditions, it remained apparent that the curve generated for plants originating from seeds germinated under -1.2 MPa drought maintained the steepest course (Fig. 1D). All these shifts predominantly occurred within the last section of
Table 2. Significance of the effect of factors and their interactions on the analysed variables based on the analysis of variance

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soil moisture (A)</th>
<th>Induced drought – during seed germination, MPa (B)</th>
<th>Variety (C)</th>
<th>Term of measurement (D)</th>
<th>AxB</th>
<th>AxC</th>
<th>AxD</th>
<th>BxC</th>
<th>BxD</th>
<th>CxD</th>
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Explanations: soil moisture: O = optimum, D = drought; variety: A = Amera, K = Krysta, M = Minora, T = Tukan; term of measurement: 1 = beginning of drought, 2 = seven days after treatment of drought (7 DAT), 3 = 12 DAT and 4 = 14 days after re-watering (14 DAR). Abbreviations of fluorescence parameters see in Table 1; * significant effect (p ≤ 0.05), a = the lowest value, d = the highest value, ns = not significant.

Source: own study.
Fig. 1. Effect of drought on chlorophyll a fluorescence transients of dark adapted leaves of var. Amera; induction curves in 12 DAT (A – control, B – drought stress) and after re-watering (14 DAR) (C – control, D – recovery after drought); the fluorescence kinetics normalised – differential curves of delta Vt in 12 DAT (E – control, F – drought stress), and after re-watering (14 DAR) (G – control, H – recovery); 12 DAT = 12 days after treatment of drought, 14 DAR = 14 days after re-watering; values are means (n = 9); double normalised curves presenting the relative variable fluorescence Vt in O–P transient of chlorophyll fluorescence; source: own study.
Fig. 2. Effect of drought on chlorophyll a fluorescence transients of dark adapted leaves of var. Krysta; induction curves in 12 DAT (A – control, B – drought stress) and after re-watering (14 DAR) (C – control, D – recovery after drought); the fluorescence kinetics normalised – differential curves of delta V_i in 12 DAT (E – control, F – drought stress), and after re-watering (14 DAR) (G – control, H – recovery); 12 DAT = 12 days after treatment of drought, 14 DAR = 14 days after re-watering; values are means (n = 9); double normalised curves presenting the relative variable fluorescence V_i in O–P transient of chlorophyll fluorescence; source: own study
Fig. 3. Effect of drought on chlorophyll a fluorescence transients of dark adapted leaves of var. Minora; induction curves in 12 DAT (A – control, B – drought stress) and after re-watering (14 DAR) (C – control, D – recovery after drought); the fluorescence kinetics normalised – differential curves of delta V_t in 12 DAT (E – control, F – drought stress), and after re-watering (14 DAR) (G – control, H – recovery); 12 DAT = 12 days after treatment of drought, 14 DAR = 14 days after re-watering; values are means (n = 9); double normalised curves presenting the relative variable fluorescence V_t in O–P transient of chlorophyll fluorescence; source: own study.
Fig. 4. Effect of drought on chlorophyll a fluorescence transients of dark adapted leaves of var. Tukan; induction curves in 12 DAT (A – control, B – drought stress) and after re-watering (14 DAR) (C – control, D – recovery after drought); the fluorescence kinetics normalised – differential curves of delta $\Delta V_t$ in 12 DAT (E – control, F – drought stress), and after re-watering (14 DAR) (G – control, H – recovery); 12 DAT = 12 days after treatment of drought, 14 DAR = 14 days after re-watering; values are means ($n = 9$); double normalised curves presenting the relative variable fluorescence $V_t$ in O–P transient of chlorophyll fluorescence; source: own study.
the curve, namely the I–P phase. Normalised curves affirm that the most significant variations in seedlings under drought stress emerged from plants whose seeds germinated under –1.2 MPa drought (Fig. 1E–H).

In the case of var. Krysta, alterations in the curve’s trajectory were observed after the conclusion of the drought phase, particularly in the J–I phase, termed as the thermal phase (spanning 2–30 ms), which is additionally influenced by PSI activity as outlined by Stribet and Govindjee (2012). Notably, changes were also apparent in the I–P section (Fig. 2B).

They were arranged higher in plants for variants 0.0 MPa and –1.2 MPa during seed germination. While plants maintained under the conditions of 70% CWC showed a lower course of curves for these variants of seed germination compared to –0.4 MPa and –0.8 MPa (Fig. 2A). After the recovery period, the curve for –1.2 MPa was much lower compared to other curves (Fig. 2D). At the same time similarly arranged curves were found for plants that were kept constantly under the conditions of 70% CWC (regardless of the variant of seed germination, Fig. 2C).

Under the influence of drought stress, there was a visible differentiation in the course of the curves also in Minora variety (Fig. 3).

In the I–P section, the trajectory of curves exhibited a higher course in plants stemming from the –1.2 MPa and 0.0 MPa germination variants, as opposed to plants originating from seeds germinated at –0.4 MPa and –0.8 MPa. Subsequent to the recovery period, the curve progression was consistent irrespective of the seed germination variant (Fig. 3D). Upon analysing the normalised curves, it can be deduced that the most pronounced alterations in seedlings subjected to drought stress were observed in cases where seeds had germinated under –0.8 MPa drought conditions. These changes were discernible across the O–J and J–I sections. Additionally, it was verified that plants deriving from seeds treated with –1.2 MPa showcased the most robust recovery (Fig. 3E–H).

Concerning var. Tukan, upon the culmination of the 12-day drought period, it was evident that the curve progression across all variants was notably lower compared to the control (0.0 MPa) (Fig. 4). The most significant shifts (evident even within the O–J section) were observed in plants resulting from seeds treated with –1.2 MPa. For –0.4 MPa and –0.8 MPa variants, the curve progression was notably similar and exhibited higher values compared to the –1.2 MPa condition. The decrease in the maximal amplitude during the I–P phase implies a reduction in the PSI end electron acceptor pool size, potentially leading to an excessive reduction of the PSI acceptor side, as discussed by Kalachanis and Manetas (2010). Conversely, following the recovery period, the curve for the –0.4 MPa variant remained elevated compared to the others, with the overall course maintaining similarity. Analysing the normalised curves confirms that the most pronounced variations were observable in plants originating from seeds treated with –1.2 MPa (Fig. 4 E–H).

**JIP-TEST PARAMETERS CALCULATED FROM CHLOROPHYLL FLUORESCENCE TRANSIENTS**

Although distinct alterations in the trajectory of OJIP curves were not evident across the varieties prior to the stress test, individual parameters of the JIP-test exhibited changes (Figs. 5–8).

Amid optimal soil moisture conditions (control, 70% CWC), the values of PIABS and PITOT in var. Amera plants showcased an increase for the –0.4 MPa and –1.2 MPa germination variants, relative to the 0.0 MPa variant (Fig. 5A). However, upon the conclusion of the drought stress phase, the PIABS and PITOT parameters exhibited a decrease, particularly pronounced in plants originating from seeds treated with –1.2 MPa.
–1.2 MPa. Nevertheless, these values remained elevated when compared to the 0.0 MPa condition.

Furthermore, it was discernible that heightened drought conditions (indicated by higher MPa values) during seed germination were associated with a reduction in ABS/RC in seedlings (Fig. 5B). Subsequent to the recovery period, plants originating from seeds germinated under –1.2 MPa conditions displayed PI parameter values below that of the 0.0 MPa condition. Nevertheless, higher values were registered for several parameters, particularly phenomenological fluxes DI0/CS0 and DI0/CSm, as well as the t(FM) parameter (which was also observed across other seed germination variants) (Fig. 5D).

Fig. 6. A radar plot presentation of some important JIP-test parameters quantifying the behaviour of PSII of var. Krysta in 12 DAT (A – control, B – drought stress) and after re-watering (14 DAR) (C – control, D – recovery after drought). All values are expressed in comparison to the values of control. Values are means (n = 9); source: own study

Fig. 7. A radar plot presentation of some important JIP-test parameters quantifying the behaviour of PSII of var. Minora in 12 DAT (A – control, B – drought stress) and after re-watering (14 DAR) (C – control, D – recovery after drought). All values are expressed in comparison to the values of control. Values are means (n = 9); source: own study
In the case of Krysta variety, under the conditions of optimum moisture (control, 70% CWC), elevated values were found for specific energy fluxes per primary electron acceptor in PSII – ET_{0}/CS_{0}, RE_{0}/CS_{0}, along with performance indices PI_{ABS} and PI_{tot} solely in plants originating from seeds treated with −0.4 MPa (Fig. 6A). However, following 12 days of drought, a decline in the values of parameters converted to CS_{0}, as well as PI_{ABS} and PI_{tot} was evident across all seed germination variants (Fig. 6B). Simultaneously, augmented values of S_{m} and N parameters were recorded in the −1.2 MPa variant. After the plants underwent recovery, the majority of individual parameter values were generally lower in comparison to the control condition (0.0 MPa at 70% CWC), with a notable decline observed for PI_{ABS} and PI_{tot} indices (Fig. 6D).

Both PI_{ABS}, used to quantify PSII performance, and PI_{tot}, utilised to measure performance up to the reduction of PSI end electron acceptors, displayed remarkable sensitivity under drought conditions. Our study affirms that the PI_{ABS} parameter is among the most responsive within the JIP-tests for this particular type of stress. This aligns with conclusions drawn from the research conducted by Dąbrowski et al. (2019) on lawn varieties of *Lolium perenne*. According to Zivčak et al. (2013), ChlF measurements suggest an enhanced safeguarding of PSII and PSI photochemistry under drought conditions through the adjustment of energy distribution between photosystems and the activation of alternative electron sinks.

In var. Minora, under control conditions (70% CWC), an elevation in the DI_{0}/RC, DI_{0}/CS_{0}, and DI_{0}/CS_{m} parameters was discerned for the −0.4 MPa variant (Fig. 7A). Following the drought period, augmented values were recorded for the PI_{ABS} and PI_{tot} parameters (vitality indices) in plants arising from seeds treated with −0.4 MPa and −0.8 MPa (Fig. 7B). Subsequent to the recovery period, higher values of PI_{ABS} and PI_{tot} were similarly evident, particularly pronounced for the −1.2 MPa variant (Fig. 7D). Conversely, among plants maintained under 70% CWC conditions, the highest values were observed for the −0.8 MPa variant (Fig. 7C). It is noteworthy that both PI_{ABS} employed to quantify PSII performance, and PI_{tot}, used to measure performance up to the reduction of PSI end electron acceptors, underwent modification due to drought stress. In var. Minora, substantial increments in the values of these indices were observed directly after the stress period, as well as following re-watering, when compared to control plants. This implies a more effective adaptation to drought conditions. Dąbrowski et al. (2019) demonstrated that performance index values significantly decreased following the application of drought stress in both varieties of *Lolium perenne*. Regardless of the variety and degree of drought, re-watering induced partial recovery of these parameters to the same values as in control plants. In our study, an increase in the values of S_{m} and N parameters was also evident (particularly for −0.8 MPa and −1.2 MPa).

In the case of Tukan variety, the application of induced drought during seed germination (from −0.4 MPa to −1.2 MPa) resulted in an increase in the values of the PI_{ABS} and PI_{tot} parameters under control conditions (Fig. 8A). Following the conclusion of the drought treatment, values for most parameters decreased across all variants, falling below the value observed for the 0.0 MPa condition. However, subsequent to the recovery period, values for the assessed parameters increased across all seed germination conditions and approached the levels of the 0.0 MPa condition (with PI_{ABS} and PI_{tot} values even surpassing the initial levels) (Fig. 8D). This pattern suggests, similar to the Minora variety, an enhanced adaptation to drought conditions.

After 7 days, the initial signs of stress emerged in the form of a reduction in the DI_{0}/RC parameter value. Subsequently, following 12 days of drought, a notable decline was observed in the values of...
several parameters: specific energy fluxes (ET₀/RC and RE₀/RC), quantum yields (Ψₐ₀, φₐ₀), and vitality indices (PIₜot), with PIABS exhibiting a non-significant decrease (Tab. 2). Concurrently, following a 14-day recovery period with optimal soil moisture (70% CWC), these parameter values significantly rebounded compared to the post-drought period. Additionally, within these conditions, a marked increase in the maximum quantum yield of primary photochemistry (φₚ₀) was recorded, implying a heightened efficiency in photosynthetic electron transport.

Furthermore, it was noted that PIABS and PIₜot values experienced the most substantial increase during the recovery period, irrespective of the variety and the PEG variant used during seed germination. Due to PIABS incorporation of various parameters evaluated from the OJIP transient, remains closely tied to photosynthetic apparatus activity (as indicated by Slabbert and Krüger, 2011). It serves as the most sensitive indicator reflecting photosynthetic performance.

Perlikowski et al. (2016) previously reported that their JIP-test analysis of turf varieties of *Lolium perenne* indicated a decrease in the PIABS parameter under drought conditions when compared to non-stressed plants. This led the authors to conclude that PIABS is an accurate and sensitive indicator of the physiological state of plants. The literature, including works by Dąbrowski et al. (2016) and Mehta et al. (2010), has also indicated that both PIABS, used to quantify PSII behaviour, and PIₜot, utilised to measure performance up to the reduction of PSI end-electron acceptors (RE), are significantly influenced by salt stress, resulting in decreased values of these parameters.

In our study, we similarly observed an increase in the values of certain parameters after the application of stress, followed by lower values during the recovery period. For instance, V₁ and dissipated energy flux per reaction centre (DI₀/RC) and per cross-section (DI₀/CSₜ) exhibited this pattern. Conversely, phenomenological fluxes including RE₀/CSₜ, ABS/CSₜ, TR₀/CSₜ, and ET₀/CSₜ significantly increased following the recovery period. Interestingly, regardless of the variety, the specific energy flux parameters such as ABS/RC, TR₀/RC, and DI₀/RC remained relatively stable during the drought phase but exhibited decreased values after re-watering.

Towards the conclusion of the drought stress application, significant interactions were observed between soil moisture during plant growth (optimum 70% CWC and drought), the conditions during seed germination (variants of drought induced by PEG), and the plant varieties for all the assessed parameters. A similar relationship was found between varieties and the measurement terms. In our studies, no correlation was found between the chlorophyll and fluorescence transient shapes and the radar plots of some important JIP-test parameters. The JIP-test parameters effectively describe plant reactions to stress conditions, especially when integrated with other parameters examined in the study. The principal component analysis (PCA) aids in comprehending the collective effect of stressors on the photosynthetic machinery. Our PCA analysis indicated that, following the drought period, the first PC1 component explained around 56.5% of the variability, with the second PC2 accounting for 27.2% (Fig. 9A–B). Growth conditions during the drought...
period exerted the most pronounced influence on the TR/RC parameter. Furthermore, strong positive correlations were identified among $\Psi_{ER}$, PI$_{ABS}$, PL$_{tot}$, and $\varphi_E$. Conversely, $\varphi_P$ exhibited a negative correlation with ABS/RC and DL$_E$/RC after the drought period. These parameters attained peak values in plants that originated from seeds germinated under the most severe drought conditions and subsequently underwent drought stress during the tillering phase (D$_{MPa}$ = –1.2). In contrast, optimal moisture conditions during the growth phase of plants derived from seeds germinated under drought conditions (O$_{MPa}$ = –1.2) favoured high $\varphi_P$ values.

Following the recovery period, the first principal component (PC1) now accounts for approximately 70.3% of the variability observed, while the second principal component (PC2) accounts for 14.7% (Fig. 9 C–D). Among the parameters, growth conditions continued to exert the most significant influence on TR/RC and DL$_E$/CS$_{so}$, which were positively correlated. Additionally, a strong positive correlation was identified between PI$_{ABS}$ and PI$_{tot}$ (performance indices), $\Psi_{ER}$, $\varphi_E$, and $\varphi_P$ (parameters associated with quantum yield for primary photochemistry), as well as between $V_t$ (normalised data), DL$_E$/RC, and ABS/RC (specific energy flux parameters) (Fig. 9C).

Performance indices and parameters linked to quantum yield for primary photochemistry reached their highest values under the D$_{MPa}$ = –0.4 conditions. Conversely, the highest values for specific energy flux parameters and $V_t$ were registered under optimal soil moisture conditions (O) (Fig. 9D).

**CONCLUSIONS**

In our study, we observed distinct responses of *Dactylis glomerata* varieties to drought stress. We identified several parameters that serve as indicators of reduced photosynthetic efficiency in these varieties when subjected to drought conditions. Notably, we reaffirmed that the PI$_{ABS}$ parameter within the JIP-test framework is particularly sensitive to drought stress. Our investigation further demonstrated significant alterations in quantum yield parameters ($\Psi_{ER}$, $\varphi_E$, and $\varphi_P$) across all tested *D. glomerata* varieties. These parameters are recognised for their accuracy in reflecting the impact of abiotic stresses, as they signify a decline in the efficiency of regulating the dissipation of excitation energy by the photosynthetic membrane. Additionally, specific energy flux parameters (DL$_E$/RC and ABS/RC) also exhibited sensitivity to drought, although the degree of response varied among varieties. Notably, the Minora and Tukan varieties displayed greater tolerance to water deficit compared to other varieties as they showed a large increase in PI$_{ABS}$ and PI$_{tot}$ values after drought stress as well as after the re-watering (recovery period) compared to control plants. In summary, our data analysis underscores the complexity of the photosynthetic machinery’s reaction to drought stress in *Dactylis glomerata*. Our findings highlight that drought-resistant varieties can maintain higher photosynthetic activity during drought stress. We have also established a correlation indicating that seed hardening plays a pivotal role in enhancing the subsequent drought resistance of plants in the tested varieties. Importantly, our study suggests that seed germination under drought conditions might enhance the efficiency of this process.

**CONFLICT OF INTERESTS**

The authors declare no conflict of interests.

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