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Improving tolerance in seedlings of some Polish varieties of *Dactylis glomerata* to water deficit by application of simulated drought during seed germination

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Abstract

Effects of drought stress on the physiological and morphological parameters of *Dactylis glomerata* varieties were studied. Seeds were germinated under simulated drought during 21 d and then subjected either to drought or optimal soil moisture conditions for 12 d. After this period all plants were optimally watered. Plants derived from seeds germinated under drought showed higher or similar photosynthetic efficiency as compared to those germinated under optimum moisture not only under stress but also after regeneration period. Application of drought during the seed germination improved nitrogen balance index (NBI) and chlorophyll content (Chl) in plants after regeneration, whereas flavonoids content (Flv) was similar to plants derived from the seeds germinated under optimum moisture. Water deficit during seed germination can positively affect the maintenance of the quantum efficiency of PSII of plants under drought stress. NBI, Chl, and Flv indices can be used to assess the response of *Dactylis glomerata* to drought during the initial growth of plants.

Additional key words: chlorophyll *a* fluorescence; cocksfoot; drought stress; tillering phase.

Introduction

Under natural conditions, plants are exposed to many environmental stress factors. Among them, drought has become an important problem in agriculture. Shortage of water in the soil can disrupt the photosynthetic apparatus, causing the inhibition of the growth, development, and decrease of plant productivity (Hura *et al.* 2007). Understanding of the mechanisms behind drought resistance, which can lead to the restitution of physiological functions and to hardening of plants under drought stresses, is essential to achieve these aims (Xu *et al.* 2011).

Photosynthesis is the most significant physiological process and is affected by stress factors in all its phases. The mechanism of photosynthesis involves various components, including photosynthetic pigments and photosystems and CO₂ reduction pathways (Ashraf and Harris 2013).

First, stress factors inhibit the intensity of photosynthesis by closing the stomata and limiting the availability and flow of carbon dioxide in the intercellular spaces (Hura *et al.* 2007). Stress factors can damage plants and, as a result, reduce their overall photosynthesis efficiency. Chlorophyll (Chl) *a* fluorescence measurements have been used to study plants reaction to various kinds of environmental stresses (Strasser *et al.* 2004, Elsheery and Cao 2008, Borawska-Jarmułowicz *et al.* 2014, Dąbrowski *et al.* 2016, Mastalerczuk *et al.* 2019) and represents a noninvasive, simple, and inexpensive tool for analyzing light-dependent photosynthetic reactions and for indirectly estimating Chl content in the same tissue sample (Kalaji *et al.* 2016). Measurements of Chl fluorescence parameters are a source of accurate information on the state of the photosynthetic apparatus of plants and can help to estimate the influence of stress conditions on the growth and yield of plants. The measured parameters are closely correlated

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Abbreviations: Area – area over the fluorescence induction curve between F₀ and F_m; AWC – absolute water content; DM – dry mass; Flv – index related to the flavonol content, epidermal UV-absorbance; F_v/F_m – maximal quantum yield of PSII photochemistry; NBI – nitrogen balance index measured as the ratio of Chl and Flv; PI_{abs} – performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors; PI_{tot} – performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors.

with the functioning of PSII (Baker and Rosenqvist 2004). PSII has high resistance to water deficit and negative impacts therefore occur only under conditions of extreme drought (Lauriano *et al.* 2006). The performance index (PI) and F_v/F_m are the most widely used parameters related to Chl fluorescence which provides information about the general state of plants and their vitality under drought stress (Force *et al.* 2003). According to Guha *et al.* (2013), the Chl fluorescence method is potentially useful for screening genotypes for drought tolerance. It has been reported that it is possible to estimate the content of Chl, anthocyanins, and flavonols based on the absolute fluorescence intensities, simple and complex fluorescence ratios and light transmission (Cerovic *et al.* 2012). Potential use of specific indices, *i.e.*, nitrogen balance index (NBI), relative content of Chl and flavonoids (Flv) for sensing different stresses has not yet been fully exploited (Bürling *et al.* 2013). For this reason, it is necessary to identify in advance the appropriate indices and to validate them using diverse genotypes grown under different conditions.

Effects of drought on plant growth vary highly even within a grass species (Mastalerczuk *et al.* 2017). Moreover, the responses to water deficit may change at the different stages of plant growth, not only on mature plants and at an early stage of growth, but also during seed germination. The current state of knowledge on physiological and genetic response of seedlings of forage grasses to drought is not yet sufficiently defined (Xu *et al.* 2006). Moreover, it is also important to know the impact of moisture conditions during seed germination at early growth and plant development.

Dactylis glomerata L. is one of the most popular cool-season, long-lived perennial forage grass considered to be one of major economic importance due to its high forage production (Jafari and Naseri 2007). This species is considered to be relatively tolerant to drought stress in the stage of full plant maturity. However, there are little studies on the response of varieties of *Dactylis glomerata* to water deficit in the early stages of plant development and indicating the possibility of improving the sensitivity of plants to drought. Finding out the responses of individual species and varieties of grasses to this environmental factor and their possible adaptation and acclimatization to changing water conditions may allow for the selection of more tolerant varieties to water shortages in the soil.

The present study was designed to examine the reaction of selected *Dactylis glomerata* varieties to a short-term stress of water deficit in the soil, by assessing the physiological and morphological parameters. We wanted: (1) to find out whether the use of simulated drought conditions during seed germination is a method to improve the tolerance of seedlings to drought; (2) to evaluate tolerance of *Dactylis glomerata* varieties in the tillering phase of plants to water deficit by comparing the efficiency of the photosynthetic apparatus of plants; (3) to reveal whether the physiological parameters are suitable for such a study.

Materials and methods

Plant material and experimental design: The experiment

focused on monitoring the effects of drought stress on the physiological and morphological parameters of seedlings of Polish varieties of *Dactylis glomerata* differing in earliness and drought tolerance: Amera (early, tolerant), Krysta (medium early), Minora (medium early, tolerant), and Tukan (medium late). The study was conducted in phytotron under a set of controlled conditions: air humidity of 60–65%, temperature of 25/16°C (day/night) at 8-h photoperiod under light of 95 W m⁻². In the experiment, the seedlings of each variety obtained after seed germination on Petri dishes under conditions of drought simulated with polyethylene glycol (PEG) 6000 were used. Seeds were germinated in constant temperature 25°C (day/night) under cool, white light of 20 W m⁻² and variable photoperiod of 12/12 h (day/night).

The amount of PEG applied to create each level of drought stress was mixed with water obtaining osmotic potential (Hardegree and Emmerich 1994), measured with a cryoscopic osmometer *Osmomat 030* (Gonotec, Germany). Four variants were used: 0.0 MPa (A) (laboratory paper moistened of 60% with deionised water), -0.4 MPa (B), -0.8 MPa (C), and -1.2 MPa (D) in three replications for each variety (Fig. 1). After 21 d of germination, five plants from each variant (in the 2–3-leaf phase) were planted in three replications in containers (11 × 11 cm) filled with garden substrate. The chemical properties of soil were as follows: pH 5.0–6.0 (in mol L⁻¹ KCl), total nitrogen content of 80–120 mg(N_{tot}) L⁻¹, 22–44 mg(available P) L⁻¹, and 83–124 mg(K) L⁻¹. Subsequently after 21 d of growth, nitrogen fertilization (at a dose of 50 kg ha⁻¹, in the form of urea), 0.85 mg per container (5 ml of solution) was applied. The plants were watered every two days to a given mass of the soil and containers maintaining optimum moisture at 70% capillary water capacity (CWC). At 42 d after planting the seedlings, half of the plants from each variant was subjected to drought conditions (no watering) and half of them was left at 70% CWC (Fig. 1). Stress conditions were sustained at the tillering phase (1–2 shoots per plant) for 12 d. Then, plants were rewatered and allowed to recover under 70% CWC of soil moisture during 14 d. Measurements were carried out in eight terms: 1–6 (I) in the period of applying drought conditions (1, 3, 5, 7, 10, 12 d under treatment) and 7–8 (II) after drought (19 and 26 d of experiment).

Physiological measurements: Leaf Chl fluorescence parameters were measured using *Pocket PEA (DataPEA Plus ver. 1.10, Hansatech Instruments Ltd., UK)*. Following parameters were assessed after dark adaptation of plants for 30 min: maximal quantum yield of PSII photochemistry (F_v/F_m), performance index for energy conservation from exciton to the reduction of intersystem electron acceptors (PI_{abs}), performance index for energy conservation from exciton to the reduction of PSI end acceptors (PI_{tot}), and area over the fluorescence induction curve between F_0 and F_m (Area). The Chl and flavonoids content of leaves (Chl and Flv indices), as well as nitrogen balance index (NBI), which is the ratio of Chl to epidermal flavonol (Flv) leaf content, were evaluated *in situ* on the sampling day using the noninvasive leaf clip sensor *Dualex Scientific+™* (Force-A, Orsay, France) (Cerovic *et al.* 2012).

Fig. 1. Scheme of investigations for each variety of *Dactylis glomerata*. Simulated drought during seed germination (A: 0.0 MPa; B: -0.4 MPa; C: -0.8 MPa; D: -1.2 MPa).

All measurements were performed on the youngest fully expanded leaf on nine plants from each combination. At the end of the experiment absolute water content (AWC) was assessed. Plants from the individual variants were cut and the fresh and dry mass (after drying in an oven at 105°C for 24 h) was determined. AWC was calculated using the formula: $AWC = [(FM - DM)/FM] \times 100$, where: FM – fresh mass [mg]; DM – dry mass [mg] (Hall and MacHardy 1981).

Morphological measurements: Number of shoots and leaves [number per plant], as well as the aboveground mass of plants [mg per plant] were assessed at the end of the experiment.

The statistical analysis of the data was performed on the basis of three-way analysis of variance (ANOVA) using the *Statistica 13.3* software (Statsoft Inc., Tulsa, OK, USA). The experimental factors were the soil moisture during plant growth, the conditions of seed germination, and the variety. In order to verify differences in morphological traits between *Dactylis glomerata* varieties depending on seed germination conditions and soil moisture during plant growth, one-way ANOVA was performed. The means of each trait were compared according to the Tukey's HSD test at $p \leq 0.05$. Relations between mass of plants and other traits were assessed by correlation analysis ($p \leq 0.05$).

Results

Physiological measurements: Changes in fluorescence parameters were observed in response to drought conditions induced during seed germination and soil moisture during

plant growth (Table 1). Seedlings of *Dactylis glomerata* subjected to drought stress, regardless of the variety and seed germination conditions, showed a significant decrease in PI_{abs} and PI_{tot} , while values of other parameters (F_v/F_m and Area) did not differ compared to optimum moisture. The results obtained showed also that plants derived from the seeds subjected to drought were characterized by higher PI_{abs} and F_v/F_m values (significantly at -0.8 MPa and -1.2 MPa for PI_{abs} and at -1.2 MPa for F_v/F_m) under water deficit in relation to plants with seeds germinated in optimum moisture conditions (A). However, the value of the Area parameter was the highest when seeds were not subjected to drought conditions. Differences in the responses of varieties to drought were also evident, Amara and Tukan exhibited significantly higher values of these parameters (except Area) in comparison to Krysta and Minora.

Subjecting the plants after drought stress to 70% CWC resulted in increasing values of fluorescence parameters, which were significantly higher in comparison to plants maintained in optimum moisture. This was particularly evident for PI_{abs} and PI_{tot} parameters (Table 1). Values of examined parameters were also higher compared to previously applied stress. Moreover, plants derived from seeds germinated under drought showed similar (Area, PI_{abs}) or even greater values of evaluated parameters (F_v/F_m) compared to plants germinated under optimum moisture. Simultaneously, we noted higher values of the assessed parameters for all varieties. Moreover, cv. Tukan was characterized by significantly higher values for most parameters (except Area), while Krysta showed the smallest values.

Table 1. Values of physiological parameters of *Dactylis glomerata* plants under soil moisture conditions (optimum, drought) depending on simulated drought during seed germination (A: 0.0 MPa, B: -0.4 MPa, C: -0.8 MPa, D: -1.2 MPa). Mean values marked by the same lowercase letters (between levels of individual factor in columns) do not differ significantly according to Tukey's test ($p \leq 0.05$). F_v/F_m – maximal quantum yield of PSII photochemistry; PI_{abs} – performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors; PI_{tot} – performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors; Area – area over the fluorescence induction curve between F_0 and F_m ; NBI – nitrogen balance index; Chl – chlorophyll content index; Flv – flavonol content index.

Factor	F_v/F_m	PI_{abs}	PI_{tot}	Area	NBI	Chl	Flv
Drought period							
Optimum	0.62 ^a	0.60 ^b	0.36 ^b	255,021 ^a	5.31 ^a	7.29 ^a	1.49 ^a
Drought	0.62 ^a	0.56 ^a	0.31 ^a	261,976 ^a	5.26 ^a	7.24 ^a	1.48 ^a
A	0.61 ^a	0.54 ^a	0.33 ^{ab}	272,031 ^c	5.52 ^b	7.22 ^a	1.43 ^a
B	0.62 ^{ab}	0.57 ^{ab}	0.32 ^a	256,123 ^{ab}	5.25 ^{ab}	7.21 ^a	1.50 ^b
C	0.62 ^{ab}	0.60 ^b	0.34 ^{ab}	244,343 ^a	5.07 ^a	7.13 ^a	1.48 ^b
D	0.63 ^b	0.61 ^b	0.35 ^b	261,496 ^{bc}	5.29 ^{ab}	7.49 ^a	1.51 ^b
Amera	0.63 ^b	0.68 ^b	0.37 ^c	247,416 ^a	4.48 ^a	5.61 ^a	1.33 ^a
Krysta	0.60 ^a	0.50 ^a	0.31 ^b	261,159 ^{ab}	5.60 ^b	7.71 ^b	1.53 ^b
Minora	0.60 ^a	0.47 ^a	0.27 ^a	266,808 ^b	5.46 ^b	7.88 ^b	1.54 ^b
Tukan	0.64 ^b	0.66 ^b	0.39 ^c	258,610 ^{ab}	5.60 ^b	7.86 ^b	1.53 ^b
Rewatering							
Optimum	0.64 ^a	0.83 ^a	0.44 ^a	327,715 ^a	8.16 ^a	12.79 ^a	1.61 ^a
Drought	0.67 ^b	1.08 ^b	0.58 ^b	353,051 ^b	8.96 ^b	12.74 ^a	1.58 ^a
A	0.65 ^a	0.91 ^a	0.51 ^{ab}	347,115 ^a	8.04 ^a	11.82 ^a	1.62 ^a
B	0.66 ^b	0.97 ^a	0.53 ^b	346,620 ^a	8.71 ^b	12.94 ^b	1.59 ^a
C	0.66 ^b	1.02 ^a	0.56 ^b	333,577 ^a	8.78 ^b	12.84 ^b	1.56 ^a
D	0.66 ^b	0.92 ^a	0.46 ^a	334,221 ^a	8.72 ^b	13.45 ^b	1.62 ^a
Amera	0.66 ^b	0.90 ^b	0.46 ^a	313,211 ^a	8.08 ^a	10.22 ^a	1.46 ^a
Krysta	0.64 ^a	0.77 ^a	0.44 ^a	319,623 ^a	8.39 ^a	13.35 ^b	1.63 ^b
Minora	0.64 ^a	0.89 ^{ab}	0.50 ^a	379,716 ^c	8.06 ^a	13.14 ^b	1.72 ^c
Tukan	0.69 ^c	1.25 ^c	0.66 ^b	348,984 ^b	9.72 ^b	14.35 ^c	1.57 ^b

Values of NBI, Chl, and Flv indices of *Dactylis glomerata* plants were similar both under conditions of optimum moisture and during drought, but only regardless of seed germination conditions and variety (Table 1). However, differences were found due to conditions during the seed germination and the variety. The use of simulated drought in the period of seed germination resulted in lower values of NBI, significantly for -0.8 MPa (C) in comparison to optimum (A). Plants derived from seeds germinated under drought exhibited similar values of Chl but significantly higher Flv compared to plants obtained from seeds under optimum moisture. We noticed that Amera was distinguished by lower values of all measured parameters among tested varieties. Furthermore, after drought period, plants obtained significantly higher values only in relation to NBI (Table 1). We also found that drought induced during seed germination increased values of Chl and NBI, but it did not affect the flavonoid content (Flv). Moreover, the differences between varieties were also marked and Amera still showed lower values of these parameters in relation to the others, while Tukan exhibited significantly higher for Chl and NBI. Values of NBI index varied in terms of measurements depending on drought

during seed germination and soil moisture (optimum, drought) during plant growth (Fig. 2). Measurements in periods of 1–4 (period of drought stress) showed that NBI values were lower for all variants of drought induced during seed germination in comparison to measurements after drought period. As a result, at the end of measurements, NBI values in optimum soil conditions were similar for all treatments, while plants subjected to drought showed even higher NBI, especially for -0.8 MPa. Similar trend was visible with respect to Chl index. At the end of the drought period (6th term), values of Flv were similar, except D variant (-1.2 MPa), for plants with seeds germinated under induced drought compared to ones germinated in optimum (A). Whereas after regeneration period, Flv index was similar for all variants of induced drought. At the same time, *Dactylis glomerata* plants subjected to drought conditions were characterized by higher AWC values with respect to plants that grew under optimum water conditions (Table 2). We found that application of simulated drought at the level of -0.4 and -0.8 MPa during seed germination and then exposure of plants to drought soil stress increased also AWC values in relation to plants that were not stressed.

Fig. 2. Changes of the values of nitrogen balance index (NBI), chlorophyll content index (Chl), and flavonol content index (Flv) (shown as relative units) of *Dactylis glomerata* plants under optimum and drought soil moisture conditions in terms during the period of drought stress (1–6) and after stress (7–8) depending on simulated drought during seed germination (A: 0.0 MPa; B: –0.4 MPa; C: –0.8 MPa; D: –1.2 MPa). Bars on each point refer to LSD.

Morphological measurements: Plants derived from seeds germinated under induced drought and then subjected to drought conditions showed higher mass compared to ones maintained under optimum moisture (Table 2). Moreover, after a period of drought stress, the number of leaves on the plant, regardless of the variety, was higher when simulated drought during seed germination was applied [especially, at –0.4 (B) and –0.8 MPa (C)]. It was also shown that plants derived from seeds germinated under drought were characterized by a higher dry mass, significantly at –0.8 MPa, in relation to plants whose seeds were germinated under optimum conditions (A). Besides, cv. Tukan formed the most shoots and leaves regardless of moisture conditions during seed germination and plant growth, while cv. Amara showed the least values. We also

showed that application of simulated drought during seed germination influenced a higher number of shoots and leaves (except D variant) as well as higher mass of plants (significantly at –0.8 MPa) of *Dactylis glomerata* varieties grown in drought stress conditions in relation to plants in optimum moisture. We noted stronger and statistically significant correlations between the mass of plants and F_v/F_m parameter as well as NBI and Chl indices in drought soil conditions in comparison to optimum moisture (Fig. 3).

Discussion

We evaluated the impact of simulated drought conditions during seed germination on physiological and morphological response of seedlings of *Dactylis glomerata* varieties

Table 2. Number of shoots and leaves, dry mass (DM), and absolute water content (AWC) of plants of *Dactylis glomerata* varieties after drought stress depending on soil moisture (optimum, drought) and simulated drought during seed germination (A: 0.0 MPa, B: -0.4 MPa, C: -0.8 MPa, D: -1.2 MPa). Mean values marked by the same lowercase letters (between levels of individual factor in columns) and the same uppercase letters (for interactions: soil moisture and simulated drought during seed germination for individual variety) do not differ significantly according to Tukey's test ($p \leq 0.05$). DM – dry mass.

Factor	Trait	Shoots [plant ⁻¹]	Leaves [plant ⁻¹]				DM [mg plant ⁻¹]	AWC [%]										
Optimum Drought		1.48 ^a	7.31 ^a				0.512 ^a	72.84 ^a										
		1.66 ^a	7.15 ^a				0.586 ^b	75.04 ^b										
	A	1.68 ^b	6.83 ^{ab}				0.502 ^a	75.56 ^c										
	B	1.68 ^b	7.54 ^{bc}				0.536 ^a	75.46 ^c										
	C	1.68 ^b	7.88 ^c				0.609 ^b	74.12 ^b										
D		1.24 ^a	6.67 ^a				0.549 ^a	70.62 ^a										
		1.10 ^a	6.28 ^a				0.428 ^a	75.61 ^d										
Amera		1.53 ^a	7.65 ^b				0.536 ^b	74.64 ^c										
Krysta		1.43 ^a	6.67 ^a				0.511 ^b	73.60 ^b										
Minora		2.22 ^b	8.32 ^b				0.721 ^c	71.91 ^a										
Tukan		Simulated drought during seed germination																
	Soil moisture		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	Optimum		1.0 ^A	1.0 ^A	1.0 ^A	1.0 ^A	6.8 ^A	6.3 ^A	6.7 ^A	6.6 ^A	0.50 ^{ABC}	0.34 ^A	0.31 ^A	0.68 ^{CD}	74.3 ^A	74.5 ^A	74.1 ^A	73.8 ^A
	Drought		1.1 ^A	1.3 ^A	1.1 ^A	1.1 ^A	5.7 ^A	6.6 ^A	5.6 ^A	6.1 ^A	0.48 ^{AB}	0.57 ^{BC}	0.40 ^{AB}	0.82 ^D	77.1 ^B	79.6 ^C	79.0 ^{BC}	72.6 ^A
	Optimum		1.9 ^{AB}	1.3 ^{AB}	1.2 ^{AB}	1.2 ^{AB}	6.3 ^A	8.0 ^{AB}	7.4 ^{AB}	7.0 ^{AB}	0.50 ^A	0.58 ^A	0.57 ^A	0.54 ^A	76.1 ^C	72.3 ^B	73.4 ^B	70.2 ^A
	Drought		1.2 ^{AB}	1.9 ^{AB}	2.4 ^B	1.0 ^A	7.0 ^{AB}	9.2 ^{AB}	10.2 ^B	6.0 ^A	0.35 ^A	0.57 ^A	0.85 ^B	0.43 ^A	75.2 ^C	78.1 ^D	76.5 ^{CD}	75.3 ^{BC}
	Optimum		1.9 ^{AB}	1.3 ^{AB}	1.0 ^A	1.4 ^{AB}	6.9 ^{AB}	6.8 ^{AB}	6.2 ^{AB}	7.0 ^{AB}	1.11 ^B	0.62 ^A	0.38 ^A	0.64 ^A	75.2 ^B	74.9 ^B	72.4 ^{AB}	68.8 ^A
	Drought		1.0 ^A	1.8 ^{AB}	2.0 ^B	1.0 ^A	5.9 ^{AB}	7.2 ^{AB}	7.9 ^B	5.4 ^A	0.48 ^A	0.62 ^A	0.71 ^A	0.53 ^A	77.5 ^B	74.4 ^B	72.9 ^{AB}	72.6 ^{AB}
	Optimum		2.3 ^{AB}	2.0 ^{AB}	1.9 ^{AB}	2.1 ^{AB}	8.6 ^{AB}	8.0 ^{AB}	8.7 ^{AB}	9.7 ^B	0.88 ^A	0.65 ^A	0.72 ^A	0.92 ^A	72.3 ^C	72.1 ^C	70.9 ^{BC}	70.2 ^B
	Drought		3.0 ^B	2.7 ^{AB}	2.8 ^{AB}	1.0 ^A	7.6 ^{AB}	8.2 ^{AB}	10.3 ^B	5.6 ^A	0.72 ^A	0.71 ^A	1.05 ^A	0.91 ^A	76.9 ^D	77.8 ^D	73.7 ^C	61.4 ^A

Fig. 3. Correlation coefficients (r) between dry mass of plants (DM) and selected parameters of *Dactylis glomerata* plants depending on soil moisture conditions during plant growth ($n = 144$). The coefficient values marked by an asterisk are significant at $p \leq 0.05$. Area – area over the fluorescence induction curve between F_0 and F_m ; F_v/F_m – maximal quantum yield of PSII photochemistry; PI_{abs} – performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors; PI_{tot} – performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors; NBI – nitrogen balance index; Chl – chlorophyll content index; Flv – flavonol content index; AWC – absolute water content.

during water deficit and after rewatering period. In recent years, measurements of Chl *a* fluorescence have been widely used in plant physiology related to photosynthesis and become a tool for selecting varieties of fodder plants that are resistant to environmental stresses (Sayed 2003, Strasser *et al.* 2004, Kalaji *et al.* 2016).

Numerous authors confirmed that the PI index is a sensitive parameter describing Chl fluorescence under different environmental conditions and is used widely to compare the whole primary photochemical reactions (Strasser *et al.* 2000). According to Kalaji *et al.* (2016), PI index provides quantitative information about the general state of plants and their vitality and, in the opinion of Oukarroum *et al.* (2007), gives detailed analysis of plant response to the stressor. Our own studies showed that stress caused by the shortage of water significantly affected the decrease of PI_{abs} and PI_{tot} parameters of Chl fluorescence. This shows that primary photochemical reactions were affected by drought treatment. The results also showed that after the rewatering period, the PI values for plants subjected to drought conditions were significantly higher in relation to plants that were kept under optimal moisture conditions. At the same time, PI_{abs} and PI_{tot} values were greater in comparison to drought stress period. Similar pattern was also found for all levels of moisture during seed germination and for all investigated varieties.

Commonly used F_v/F_m parameter represents the conversion and capture efficiency of primary light energy and is an excellent measure for the evaluation of quantum yield of primary photochemistry of PSII (Strasser *et al.* 2004). Our own studies showed that stress caused by the shortage of water in the soil generally did not affect the decrease of values of other fluorescence parameters: F_v/F_m and Area of *Dactylis glomerata* plants. In turn, Baker and Rosenqvist (2004) noticed strong decrease of F_v/F_m parameter in dark-adapted plants caused by photodamage of PSII reaction centers induced by drought. Xu *et al.* (2011) also reported that drought decreased the F_v/F_m ratio in varieties of *Poa pratensis*. Our own studies have shown that in response to rewatering, values of F_v/F_m were even greater after plant regeneration. According to Xu *et al.*

(2011), the increase in the value of F_v/F_m parameter in grass species plants suggests that the functioning of PSII has been fully restored after reducing drought stress. Therefore, the results indicate that application of simulated 21-d drought during seeds germination (independently of amount of PEG solution) positively affected the maintenance of the quantum efficiency of PSII photochemistry. Our findings correspond also with Abid *et al.* (2018) who stated that wheat plants originated from the osmo-primed (30 h) seeds retained a long-lasting stress memory, which stabilized plant growth and productivity by altering physiological and biochemical responses to postgermination drought stress. In our studies in the period of water stress, the value of Area declined as the level of induced drought during seed germination (MPa) increased. This indicates that the electron transfer from reaction center to quinone pool was blocked. The maximum decrease obtained in Area was only 10% (for -0.8 MPa) and after regeneration of plants under optimum moisture, these values were similarly independent of seed germination conditions. This indicates small impairment of PSII photochemistry under drought stress (Kalaji *et al.* 2012).

According to literature, Flv and Chl indices can be considered as candidates for universal and genotype-independent parameters for drought in plants (Cerovic *et al.* 2012, Bürling *et al.* 2013). Our studies showed that the use of simulated drought in the period of *Dactylis glomerata* seed germination resulted after recovery of plants in lower values of NBI index, similar Chl and significantly higher of Flv indices in comparison to plants obtained from seeds germinated under optimum moisture. Bürling *et al.* (2013), in the study on wheat varieties in the vegetative phase under drought-induced stress, noticed that Chl content shown by NBI and Chl indices was reduced, while the content of flavones in the epidermis strongly increased. Taking this data into consideration, our results in relation to Chl index may indicate that the use of drought conditions during seed germination positively influenced the value of this parameter in seedlings of *Dactylis glomerata* during the period of drought stress. This statement may also refer

to the state of plants in the period after rewatering, as it was found that drought induced during seed germination increased values of NBI and Chl, but it did not affect the flavonoid content in plants. As a result, after regeneration period, Chl and NBI indices were significantly higher in comparison to optimum, whereas Flv index was similar for all variants of drought during seed germination. Furthermore, our results also showed significant effect of water stress on investigated varieties in respect of Chl fluorescence parameters and NBI, Chl, and Flv indices, which is confirmed by the results of many researchers (Bürling *et al.* 2013, Kalaji *et al.* 2016).

In our study, early Amara and medium late Tukan varieties during drought period were characterized by significantly higher values of Chl fluorescence parameters in comparison to Krysta and Minora (medium early). This was evident in Tukan plants also after the recovery period. In contrast, cv. Amara was characterized by the smallest values of NBI, Chl, and Flv indices in the period of drought as well as after the rewatering period.

Our results also revealed that *Dactylis glomerata* plants subjected to drought conditions were characterized by a higher mass after regeneration period with respect to plants that grew under optimum water conditions. Similar trend was observed in AWC values. We found that the application of simulated drought at the level of -0.4 and -0.8 MPa during seed germination and then exposure of plants to drought soil stress influenced the achievement of higher AWC values in relation to plants that were not stressed. We also recorded that plants derived from seeds germinated under drought formed higher dry mass in comparison to plants whose seeds were germinated under optimum water conditions. Our results confirmed the studies of Abid *et al.* (2018), who showed that seedlings of *Triticum aestivum* obtained higher dry mass under water deficit conditions when seeds were subjected to drought stress during the period of germination. We noted significant positive correlation between the mass of plants and F_v/F_m under drought conditions (Fig. 3). Our results are consistent with the results of Tobiasz-Salach *et al.* (2019) who found that the grain yield of oat was positively correlated with these parameters of Chl fluorescence in different soil moisture conditions, independently of tested cultivars. This study showed also the significant relationship between the mass of plants and NBI index under drought conditions, while for Flv it was noted under optimum moisture conditions. The Chl index was significantly correlated with the mass of plants under optimum moisture and drought conditions as well.

Conclusions: The use of simulated drought conditions during seed germination had a positive effect on photosynthetic efficiency and morphological characteristics of *Dactylis glomerata* seedlings under conditions of soil water deficit. Application of simulated drought at the level of -0.4 and -0.8 MPa during seed germination and then exposure of plants to drought soil stress increased AWC values in relation to plants that were not stressed. It indicates that this procedure can be applied to improve the tolerance of *Dactylis glomerata* to drought in the

initial period of plants growth. Differences in the reaction of varieties to drought were evident. The comparison of photosynthetic apparatus efficiency of tested varieties after rewatering period showed that the medium late cv. Tukan was characterized by better tolerance of seedlings to drought showing significantly higher values for most physiological parameters (except Area). Noninvasively measured parameters, such as NBI, Chl, and Flv, can be successfully used to assess the response of *Dactylis glomerata* to drought stress during the initial growth of plants.

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