

RESPONSE OF KENTUCKY BLUEGRASS LAWN PLANTS TO DROUGHT STRESS AT EARLY GROWTH STAGES

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We investigated the effect of water deficiency in soil on the morphological features and photosynthetic efficiency of Kentucky bluegrass during the early phase period of plants growth. Plants grown under stress-free conditions until tillering. Three levels of soil capillary water capacity (% CWC) were applied: (70%, 50% and drought stress at 35%). Plants were grown under these conditions for 21 days. Subsequently, all of them were subjected to well-watered conditions for 14 days (recovery). The study showed that values of relative water content, chlorophyll fluorescence parameters and morphological traits were lower under conditions of soil water deficiency and drought stress relative to the optimal soil moisture. Restoration of optimal soil water conditions increased all parameters characterizing the above ground part of biomass, while values of root traits did not vary. Plants subjected to well-watered post drought soil conditions, showed a rapid recovery of photosynthetic efficiency of Kentucky bluegrass. This work suggests that, this plant can survive short periods of drought stress and thus can be recommended for cultivation in the regions where drought conditions are expected to occur. Moreover, plant photosynthetic efficiency can substitute classical evaluation method of plant tolerance to drought stress based on plant water status.

Keywords: Chlorophyll fluorescence, drought, *Poa pratensis* L., relative water content, photosynthesis, specific root length.

INTRODUCTION

Drought is one of the most serious environmental stresses, occurring as a result of climate change (Ahuja *et al.*, 2010; Staniak and Kocon, 2015). In light of global warming and reduced fresh water resources it has become important to search for any species with good drought resistance. This is particularly important in the case of plants not intended for consumption, such as lawn species. The most widely grown lawn grass in Poland is Kentucky bluegrass (*Poa pratensis* L.). The water requirement of this species is rather low, depending on the variety (Richardson *et al.*, 2008; Mastalerczuk and Borawska-Jarmulowicz, 2009). Lawn varieties of Kentucky bluegrass are characterized by a steady sodding and high aesthetic qualities, especially on intensive lawns, if properly used. Any malfunctioning in the photosynthetic process under low water conditions can lead to the limitation of growth and development of plants (Staniak and Kocon, 2015).

Most of the previous studies, designed to understand and explain the effects of the environmental stresses and functioning of the photosynthetic process, were based on plant-gas exchange measurements (Hura *et al.*, 2007). Recently, the importance of assessing the physiological state of the photosynthetic process by measuring chlorophyll fluorescence ($\lambda_{max} = 685\text{nm}$), appearing in 95% of photosystem II (PSII), has notably increased. It is a simple and non-destructive measurement method, which permits

studying the reaction of the light dependent phase of photosynthesis, as well as estimates indirectly the chlorophyll content in the sample tissue. As a result of abiotic stress, there is a reduction in the photochemical efficiency of PSII and the changes in the values of chlorophyll *a* fluorescence. Improved measurements of chlorophyll *a* fluorescence technique, such as prompt, delayed fluorescence and quenching analysis, support studying the function of photosynthesis at different levels, e.g., pigments, reactions of primary light, electron transport and enzymatic dark in the stroma, as well as slow regulatory processes (Kalaji *et al.*, 2014). The effect of water deficiency on the light-dependent phase of photosynthesis is not well understood in agricultural plant species and previous studies have not provided conclusive results (Hura *et al.*, 2007). Therefore, it seems reasonable to continue the research, using measurement of chlorophyll *a* fluorescence parameters, allowing for the identification of water deficiency in plants.

An essential element of the assessment of tolerance to drought grass varieties can also be the size and structure of the root system, which plays an important physiological role in plant development (Stypczynska *et al.*, 2012). A well-developed root system of grasses is conducive to the stability and sustainability of plants. A water deficit leads to a loss of part of the roots of the upper soil layers so the possibility of a deeper penetration of the roots and regeneration of the root system after rehydration is important (Huang and Gao, 2000). Changes in the structure of the root system under periods of

water deficiency are recognized apart from the colour change of leaves or ceiling temperatures in order to establish a good criterion for assessing the tolerance of plants to drought (Grzesiak *et al.*, 2003). Knowledge of the grass root system reaction to water shortages is important for understanding further mechanisms for drought tolerance throughout the plant. Therefore, the objective of the present study is to evaluate the impact of water deficiency in the soil on the morphological features of shoots and roots of lawn Kentucky bluegrass plants during the initial period of growth. Moreover, to learn if photosynthetic machinery efficiency can be used as an indicator for their tolerance towards drought stress.

MATERIALS AND METHODS

The study was conducted under controlled conditions in a growth chamber of the Warsaw University of Life Sciences–SGGW in Poland. Plastic pots of 150 mm in diameter and 180 mm in height were filled with podzolic soil formed from loamy sand, which was collected from the upper 20 cm of sand at the SGGW Experimental Station farm field. The soil chemical characteristics were as follows: 9.5 g kg⁻¹ total organic C, 1.01 g kg⁻¹ N, 94.5 mg kg⁻¹ available P, 99.6 mg kg⁻¹ K and 40.0 mg kg⁻¹ Mg. The pH_{KCl} of the soil was 5.2. Before the experiment, the soil in each pot was mixed with 0.125 g N, 0.153 g P and 0.153 g K. Three seeds of lawn variety Sójka of Kentucky bluegrass were sown in four locations per pot in three replications. Directly after the germination phase, the poorly developed seedlings were removed and investigations were performed on four plants per pot.

The plants were grown under stress-free conditions until the tillering phase (40 days after sowing), and then three levels of soil moisture (capillary water capacity, % CWC) were applied: W - well-watered soil, control (70%); D1 - water deficiency (50%); D2 - drought stress, irrigation was withheld and soil was allowed to dry for 21 days (CWC of soil was decreased from 70% to 35%). Plants were grown in these differentiated soil water conditions for 21 days. Subsequently, all of them were subjected to well-watered conditions (70% CWC) for 14 days (recovery). The soil water regime was controlled every day by weighing each pot and adding water, as needed, to maintain the required level.

Conditions in a growth chamber were set and automatically controlled during the study period. A relative humidity of air day/night was 60–65%, the photo period for the day/night cycle was 16/8 h and radiation during the day was about 95 (W m⁻²) (350 PAR). The temperature was gradually increased (2°C per hour) from 12°C at night to 26°C during the day. The maximum temperature was maintained for four hours and then gradually reduced to 12°C.

The morphological traits and physiological parameters of the plants were set, starting from the date the plants were

subjected to stress conditions, in four terms: 7, 14, 21 days of stress, and 35 days (the end of the recovery) – after well-watered conditions.

Morphological measurements: The leaf and shoot numbers per plant were evaluated. Then the plants were cut at tillering nodes, and soil samples with roots were strained with a sieve having a mesh size of 3.0–0.3 mm, and then washed in a gentle stream of water to separate the plant roots from the soil. The collected root material was scanned by an optical scanner Epson Perfection V700 Photo (resolution 400 pdi) and converted to morphometric analysis using software WinRhizo 2012 (Regent Instruments Inc., Canada). The total root length (cm per plant), average diameter (mm) of roots and specific root length – root length per unit root dry mass (SRL, m g⁻¹) were calculated. Plant samples were dried at 105°C (consistent with weight) and the shoot and root dry matter (g per plant) were determined.

Plant photosynthetic efficiency (Chlorophyll fluorescence parameters): The quantum yield of photosystem II (Φ_{PSII}), maximal (F_m'), and steady-state (F_s) chlorophyll fluorescence yields of light-adapted samples were measured using a fluorometer FMS-2 (Hansatech Ltd. Kings Lynn, UK). Measurements were performed on each plant in the middle of the fully developed leaf blades. Chlorophyll fluorescence parameters are shown as relative units. The photosynthetic electron transport rate (ETR) was calculated as:

$$ETR = Y \times PAR \times 0.5 \times 0.84$$

where: Y represents the quantum yield of photosystem II (PSII) electron transport (Φ_{PSII}); 0.5 represents the fact that an electronic transmission needs to absorb two photons, assuming that the light energy absorbed by a photosynthetic system can be distributed to the photosynthetic system PSI and PSII by the same proportion, i.e., 50% each; 0.84 is the absorption coefficient indicating that a light energy incidence of only 84% can be absorbed by the leaves (Ralph *et al.*, 2005). PAR was measured automatically by a fluorometer.

Relative water content: To estimate relative water content (RWC, %), the leaves of Kentucky bluegrass plants were cut and their fresh weight was determined. Then, they were submerged for 8h in distilled water at room temperature (20°C) to determine their turgid weight. The RWC was calculated from the equation (Turner, 1981):

$$RWC (\%) = \frac{\text{leaf fresh weight} - \text{leaf dry weight}}{\text{turgid weight} - \text{fresh weight}} \times 100\%$$

The experimental data were analysed statistically using analysis of variance with Statistica 10.0 software (Statsoft, Inc. Tulsa, USA). The significance of differences between means were determined using the Tukey's test at the significance level of $P \leq 0.01$. The relations between some selected fluorescence parameters and the morphological traits were assessed by correlation analysis.

RESULTS AND DISCUSSION

The results indicate that water deficiency had a significant effect on the morphological traits of Kentucky bluegrass plants (Table 1). Under the conditions of soil moisture deficiency (D1), in addition to soil drying (D2), the

morphological traits of both the above- and below-ground parts of plants decreased, compared to the control (W). Plant response to drought stress was particularly evident with respect to the number of leaves and shoots, dry matter of shoots and roots, and the total length of roots (Fig. 1). Drought stress already had a significant inhibitory impact on the

Table 1. The effect of water conditions on the morphological features of Kentucky bluegrass plants (regardless of the measurement term), see text for abbreviations

| Water condition | Leaves/plant | Shoots/plant | Shoots dry matter (g/plant) | Total roots length (cm/plant) | Average root diameter (mm/plant) | Roots dry matter (g/plant) | SRL (m g ⁻¹) |
|-----------------|--------------|--------------|-----------------------------|-------------------------------|----------------------------------|----------------------------|--------------------------|
| W | 49.3a | 17.2a | 1.41a | 10478.2a | 0.68a | 0.50a | 214.1c |
| D1 | 37.4b | 13.2b | 0.86b | 6267.8b | 0.63a | 0.27b | 231.6b |
| D2 | 22.0c | 7.4c | 0.37c | 2315.0c | 0.55b | 0.09c | 268.8a |

Means followed by the same letter within a column were not significantly different ($P \leq 0.01$)

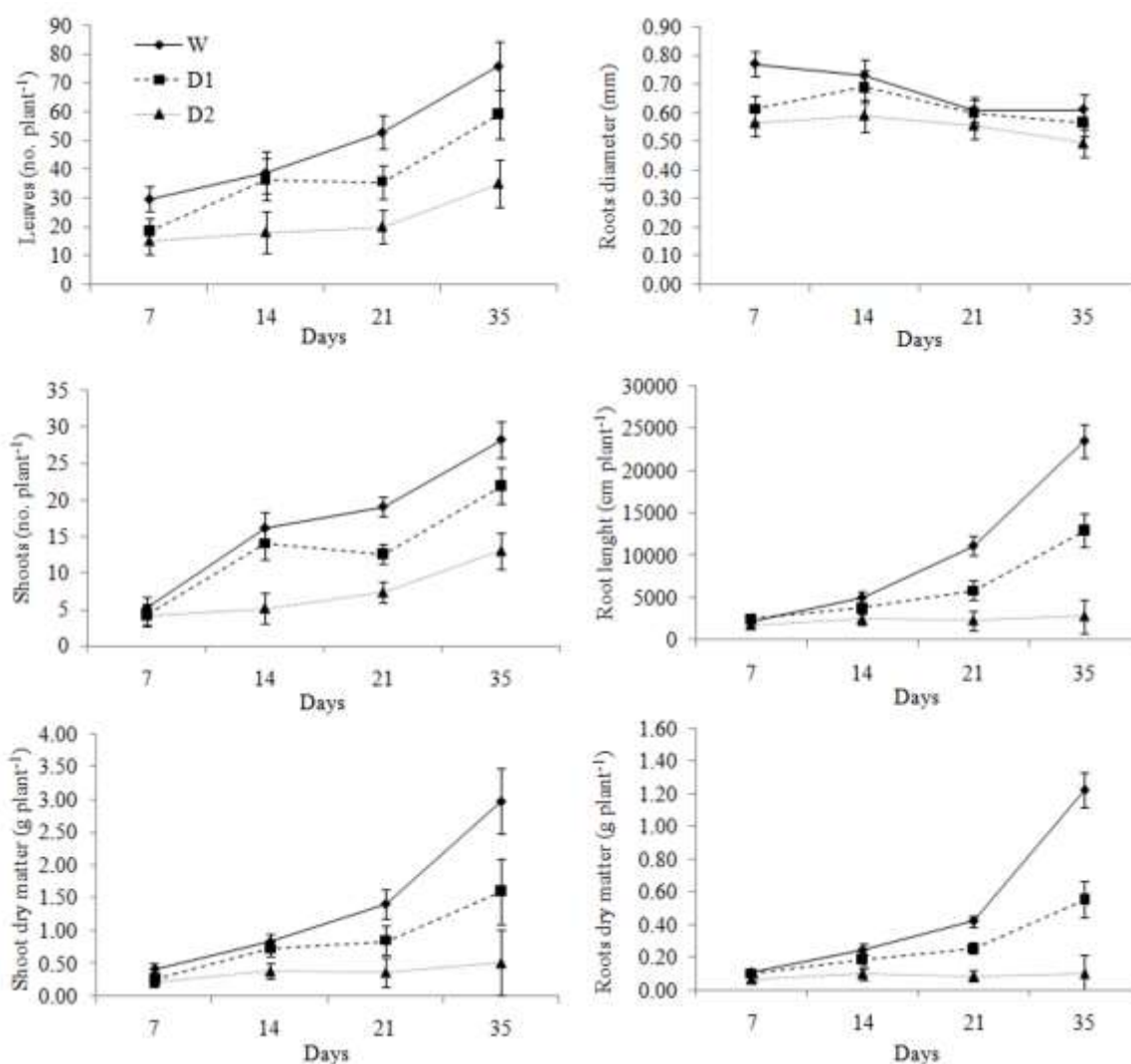


Figure 1. The effect of water conditions in the soil on the morphological features of Kentucky bluegrass plants in terms of measurement (7, 14, 21 days of stress, and on 35th days – at the end of the recovery).

formation of leaves after 7 days of soil water deficiency (D1, D2), with the formation of shoots being similar. It was noted that after 14 days, both the number of leaves and shoots was significantly lower in the soil-drying condition (D2), while in the third week of stress these features were differentiated under all levels of soil moisture. Consequently, less dry matter of shoots appeared, especially after 21 days of stress conditions. Optimal soil moisture conditions (70% CWC) within two weeks after the end of stress (recovery from 21st to 35th day) caused an increase in all parameters characterizing the above-ground biomass. It is well documented that drought stress reduces the weight and morphological traits of shoots and leaves (Riaz *et al.*, 2010; Stypczynska *et al.*, 2012). Carmo-Silva *et al.* (2009) found that the decreased shoot growth of grasses and photosynthetic CO₂ assimilation rates (Carmo-Silva *et al.*, 2008) may be an adaptive response to drought stress. Volaire (2003), Staniak and Kocon (2015) have shown that the dieback of plant leaves and shoots during a period of soil water deficiency facilitated the movement of specific proteins (dehydrins protecting cells from the harmful effects of dehydration) to the leaf buds, which initiates regrowth after stress.

Drought stress caused by a lack of watering (D2), inhibited the development of the below-ground parts of plants in the second week of stressful conditions, whereas water deficiency (D1) did so in the third week, compared to the control (Fig. 1). After three weeks of stress the length of roots was limited by about 47% for D1 and by 79% for D2, and root dry matter by about 38% and 81%, respectively. The recovery of roots after drought stress conditions was less than the regeneration of shoots. It was found that, after 14 days of growth in well-watered conditions, the length and dry matter of roots after stress partially increased in plants, growing by 50% CWC (D1), while the values of these parameters for soil drying conditions (D2) were not varied. Results obtained are similar to other studies that showed the length and dry matter of plant roots reduced by water stress (Riaz *et al.*, 2010; Stypczynska *et al.*, 2012). There are also some reports that show no significant effect of drought on grass root biomass (Su *et al.*, 2007; Skinner and Comas, 2010).

The studies have shown, that the specific root length (SRL) was significantly higher in plants grown under conditions of drying soil (D2) (Fig. 2). A lack of watering of Kentucky bluegrass plants resulted in an increase of SRL values in all periods under study, compared to other treatments. Moreover,

the average diameter of plant roots was significantly lower (about 19.1%) under conditions where CWC decreased from 70% to 35%, compared to the control (Fig. 1). The return of plants to optimal soil moisture conditions did not cause the alignment of SRL values between the used soil moisture levels. Greater values of specific root length, smaller root diameter and increased root length should improve plant acquisition of water and productivity under drought conditions (Wasson *et al.*, 2012; Comas *et al.*, 2013).

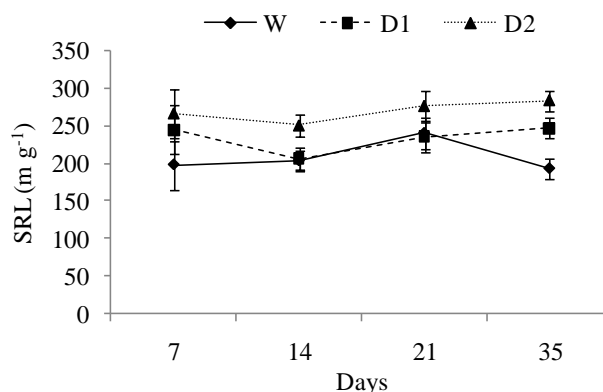


Figure 2. Specific root length (SRL) of Kentucky bluegrass plants in differentiated water condition in terms of measurement (7, 14, 21 days of stress, and on 35th days – at the end of the recovery)

Our own studies have shown that there was high positive correlation between the above- and below-ground morphological traits of plants, e.g. shoot and root dry matter ($r=0.97$) (Table 3). A comprehensive study of the water deficit resistance of the *Festuca* species presented by Stypczynska *et al.* (2012), reported that soil drought decreased root size and inhibited shoot growth, which resulted in a changing relationship between the parts of plants.

It was noted that drought stress has a significant effect on chlorophyll *a* fluorescence parameters evaluated in Kentucky bluegrass plants (Table 2). The values of potential quantum efficiency Φ_{PSII} in plants grown under conditions of soil drying (D2) were significantly reduced (by about 35.4%), as compared to the control. Plants under drought stress caused by soil drying from 70% to 35% CWC indicated low values of Φ_{PSII} after 14 days of stress by about 47%, compared to the control (Fig. 3). The extent of reduction in Φ_{PSII} after 21 days

Table 2. Effect of water deficiency conditions on Kentucky bluegrass plants (regardless of the measurement term), see text for abbreviations

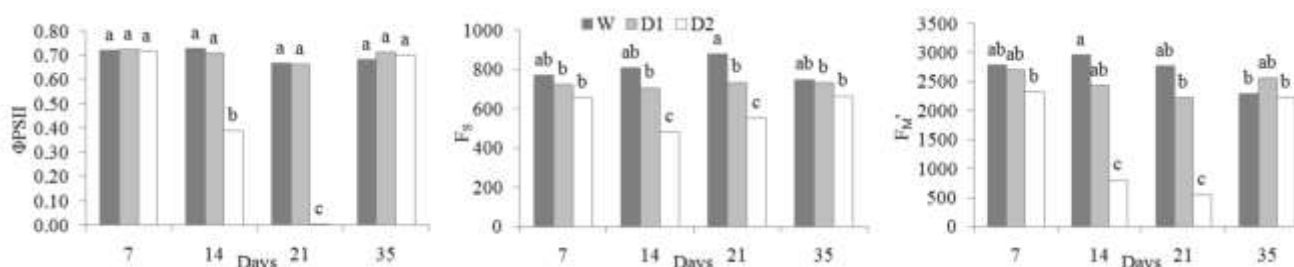
| Water condition | Φ_{PSII} | F_s | F_m' | ETR | RWC (%) |
|-----------------|---------------|--------|---------|--------|---------|
| W | 0.700a | 800.0a | 2701.3a | 94.82a | 87.3a |
| D1 | 0.702a | 724.2b | 2480.9b | 94.15a | 77.1a |
| D2 | 0.452b | 589.0c | 1477.0c | 60.18b | 60.2b |

Means followed by the same letter within a column were not significantly different ($P \leq 0.01$); Chlorophyll fluorescence parameters are shown as relative units

Table 3. Correlation coefficients between the morphological and physiological traits of Kentucky bluegrass plants, using data from all levels of soil moisture and terms of measurement (n = 36)

| | Shoots No. | Leaves No. | Shoots dry matter | Roots dry matter | Roots length | Roots diameter | SRL | F _s | F _m ' | ΦPSII | ETR |
|-------------------|------------|------------|-------------------|------------------|--------------|----------------|--------|----------------|------------------|-------|-------|
| Leaves No. | 0.96* | | | | | | | | | | |
| Shoots dry matter | 0.87* | 0.90* | | | | | | | | | |
| Roots dry matter | 0.89* | 0.88* | 0.97* | | | | | | | | |
| Roots length | 0.88* | 0.90* | 0.97* | 0.99* | | | | | | | |
| Roots diameter | -0.12 | -0.03 | -0.06 | -0.07 | -0.13 | | | | | | |
| SRL | -0.30 | -0.38* | -0.37* | -0.40* | -0.32 | -0.78* | | | | | |
| F _s | 0.47* | 0.52* | 0.38* | 0.35* | 0.39* | 0.31 | -0.35* | | | | |
| F _m ' | 0.31 | 0.36* | 0.23 | 0.21 | 0.22 | 0.35* | -0.39* | 0.85* | | | |
| ΦPSII | 0.28 | 0.35* | 0.22 | 0.22 | 0.22 | 0.25 | -0.37* | 0.63* | 0.90* | | |
| ETR | 0.44* | 0.45* | 0.28 | 0.26 | 0.29 | -0.13 | -0.03 | 0.45* | 0.66* | 0.80* | |
| RWC | 0.32 | 0.39* | 0.35* | 0.36* | 0.35* | 0.46* | -0.49* | 0.73* | 0.91* | 0.90* | 0.61* |

*Correlation is significant at the 0.01 level.

**Figure 3. The effect of water deficiency in the soil on chlorophyll *a* fluorescence of light-adapted samples (expressed in relative units) of Kentucky bluegrass plants in terms of measurement (7, 14, 21 days – drought stress, and on 35th days – at the end of the recovery). Means with the same letter were not significantly different ($P \leq 0.01$)**

of stress was higher and values were close to zero (0.004). Response of potential quantum efficiency Φ_{PSII} to water deficiency (D1) was similar to the control in all periods of study under observation. However, after 14 days of recovery, the potential quantum efficiency reached values similar to the control (0.700). This suggests that PSII of Kentucky bluegrass plants recovered from drought stress, completely. Two weeks of an optimal rehydration period revealed the almost fully recovered photosynthetic process of Kentucky lawn bluegrass. This is in agreement with the results of Hura *et al.* (2009). They found that Φ_{PSII} recovery in the triticale variety indicated drought resistance of the photosynthetic process and elicited an increase of the light absorption by chlorophyll *a* associated with the PSII used in photochemistry. The next sensitive parameters, highly correlated with Φ_{PSII} , were at a maximum (F_m') with a steady-state (F_s) chlorophyll fluorescence (correlation coefficient was 0.90 and 0.63, respectively) (Table 2). The studies have shown that the applied levels of soil moisture caused a significant differentiation in the values of these parameters. Lack of watering and reduced soil moisture up to 50% CWC resulted in decreased values of F_m' and F_s in Kentucky bluegrass

plants. Significant reduction of the F_m' and F_s values was observed after 14 and 21 days of applying stress (Fig. 3). Subjecting the plants after stress to well-watered conditions (70% CWC) resulted in increasing values of parameters tested.

The results indicate that applied levels of soil water, especially conditions with decreasing CWC from 70 to 35%, had a significant effect on the electron transport rate (ETR) (Table 2). Drought stress (D2) reduced ETR values on the second and third terms of measurement (Fig. 4). Applying optimal soil moisture conditions after a period of stress resulted in the alignment of the tested parameter of all plants. The studies have shown that correlations between ETR and Φ_{PSII} were significant ($r = 0.80$), as well (Table 3.). These observations are confirmed by the results of earlier studies on corn hybrids, wheat varieties and triticale genotypes (O'Neill *et al.*, 2006; Subrahmanyam *et al.*, 2006; Hura *et al.*, 2011) indicating that soil drought reduced the values of ETR and Φ_{PSII} .

The relative water content (RWC) in Kentucky bluegrass plants was varied significantly, depending on the applied levels of soil moisture. At a capillary water capacity of 70%

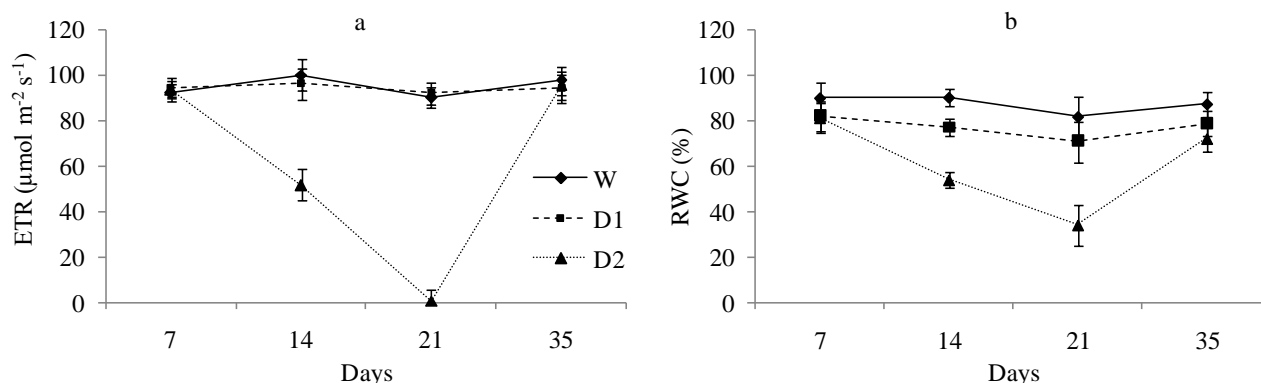


Figure 4. The effect of water deficiency in the soil on a) electron transport rate, ETR and b) relative water content, RWC of Kentucky bluegrass plants in terms of measurement (7, 14, 21 days –drought stress, and on 35th days – at the end of the recovery)

(W) and 50% (D1) values of RWC in plant were significantly higher compared to values achieved at water stress, caused by decreasing the CWC from 70 to 35% (D2). A differentiation of the RWC values was also found in measurements during subsequent periods under study (especially at 14 and 21 days of treatment) at all levels of soil moisture. A condition of drying soil caused a decrease in values of this parameter (40.2% and 58.3% at 14 and 21 days respectively). A higher value of RWC indicates that the plant is better adapted to water stress (Gracia *et al.*, 2002). Our study showed that restoring optimal water soil conditions increased the values of the RWC of plants. These results correspond to the Hu *et al.* (2010) and Xu *et al.* (2011) investigations that showed plants fully recovered after a decline in leaf RWC during drought treatment. The study also revealed a high positive correlation between RWC and chlorophyll *a* fluorescence parameters: Φ_{PSII} , F_s , F_m' and ETR (Table 3). Similar results were obtained by Goltsev *et al.* (2012). Our findings suggest that these parameters can be used as an important physiological indicator for the evaluation of plant adaptability to a state of water deficiency. According to Haffani *et al.*, (2013) there is a high correlation between the RWC and growth parameters of plants. In our studies, correlations between morphological traits and RWC were also significant and ranged from $r=0.35$ to $r=0.39$ for above-ground plant parts and from $r=0.35$ to $r=0.46$ for below-ground ones.

Conclusions: Kentucky bluegrass number of leaves and shoots, dry matter of shoots and roots, and total root length were decreased under conditions of soil water deficient. Restoring optimal water soil conditions, within 2 weeks after the end of stress (recovery period: 21st – 35th) enhanced the above-ground biomass, while the root traits did not show any changes. The specific root length (SRL) was significantly higher in plants grown under conditions of soil drying from 70% to 35% CWC, regardless of the duration of the stress. This points to a greater share of roots with a smaller diameter and better branching under drought stress conditions which

seems to be a survival strategy of Kentucky bluegrass under water stress. Photosynthetic efficiency of Kentucky bluegrass plants was reduced under drought conditions. All studied parameters were significantly correlated with plant water status, expressed as relative water content (RWC). This suggest that, parameters of photosynthetic efficiency can be used to evaluate plant tolerance toward drought stress, vastly and invasively (without need to measure plant water status estimation).

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