Physiological and Biochemical Responses of Forage Grass Varieties to Mild Drought Stress Under Field Conditions

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Abstract

In view of the expected increase in drought periods, researchers and breeders are searching for forage grasses that are more tolerant to drought stress. This study wanted to examine the physiological and biochemical reactions of nine forage grass varieties belonging to *Festuca*, *Lolium* and *Festulolium* under mild drought stress conditions in a semi-controlled field experiment. A mild drought stress treatment was applied in the period between cut II and cut III using three large mobile rain-out shelters equipped with sprinkler irrigation systems. The experiment consisted of two soil moisture treatments: (1) control where a soil moisture level of around 20% v/v was maintained and (2) drought stress where the soil moisture level decreased to 7.5% v/v. The experiment was cut 5 times in 2014 and 4 times in 2015. A total of nine varieties from five species of forage grasses were evaluated: *L. perenne*, *L. multiflorum*, *F. pratensis*, *F. arundinacea* and *F. braunii*. Dry matter yield, gas exchange parameters and chlorophyll fluorescence were significantly lower in drought stress than under control conditions and the physiological parameters reacted within 2 weeks after the start of the drought treatment in all species. In contrast, drought stress significantly increased water use efficiency, the content of proline, phenolic acids, flavonoids, water soluble carbohydrates and decreased neutral and acid detergent fibre on both years. Based on total dry matter yield and tolerance indices the most drought tolerant species were *L. multiflorum* in the first and *F. arundinacea* in the second investigated year.

Keywords Drought stress · Forage grasses · Rain-out shelter · Photosynthesis · Proline · Phenolic compounds

Introduction

Increased frequency and intensity of drought events are among the main effects of climate change and global warming. Water deficit mainly causes reduced crop yields and inhibits their development, which is related to series of morphological, physiological and biochemical changes in plant tissue. The loss of yield and survival of crop plants depends on duration and intensity of water stress (Bahrani et al. 2010; Abbasi et al. 2014). Physiological responses may depend on

plant genotype, but in general, reactions related to drought stress include reduction of CO₂ assimilation rate, stomatal conductance and photosynthetic efficiency of photosystem II (PSII). PSII shows considerable sensitivity to water stress: the photosynthetic electron transport through PSII is inhibited and oxygen involving complex of PSII and PSII reaction centres are damaged (He et al. 1995; Lu and Zhang 1999). One of the best known reaction of drought stress is a closure of stomata to avoid loss of water through the transpiration process (Cornic 1994; Lawlor 2002; Hura et al. 2007). Many studies also confirm an increase of osmoprotectants, such as proline, glycine betaine, alcohols and sugars (Bates et al. 1973; Sairam et al. 2002). These components prevent the loss of water from the cells by decreasing the osmotic potential (Bray 1993, 1997). The increase of some secondary metabolites like phenolic compounds under drought stress conditions is often observed in plants and could be an important strategy to cope with the adverse effects of water deficit (Jaafar et al. 2012). They have a strong radical scavenging activity contributed to the minimization of oxidative and drought stress in relation to the photosynthesis apparatus

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(Kikuzaki et al. 2002; Nakabayashi et al. 2014). *Lolium perenne*, one of the most important forage grass species in Northwestern Europe, is susceptible to drought stress and the increase in drought periods triggered researchers to find out the responses of species and varieties of forage grasses to water deficit. The knowledge of tolerance strategies to changing water availability makes a targeted selection of drought tolerant genotypes possible. Studies under highly controlled conditions in pots allow a detailed evaluation of the physiological reaction of plants to drought, but there is a need for an extrapolation of these results to the field (Fariaszewska et al. 2016). In order to identify drought tolerant genotypes, many indices recommended on the basis of yield under non-stress and drought stress conditions exist. The Stress Tolerance Index (STI) is one of the most useful indices to recognize tolerant genotypes (Fernandez 1992).

The main aims of this study were (1) to evaluate the response of varieties of different grass species to drought by measuring dynamics and changes in yield, forage quality, gas exchange parameters, chlorophyll fluorescence, accumulation of proline and phenolic compounds during two years in a rain-out shelter experiment and (2) to compare the obtained results from this semi-controlled field experiment with the results of an earlier published pot experiment where root growth was restricted (Fariaszewska et al. 2016).

Materials and Methods

Plant Material

Nine varieties of forage grasses were evaluated in the field experiment: *Lolium perenne* ‘Melluck’ (2n=2x), ‘Meltador’ (2n=4x), *Lolium multiflorum* ‘Meldiva’ (2n=2x), ‘Melmia’ (2n=4x), *Festuca pratensis* ‘Merifest’ (2n=2x), ‘Merifest Tp’ (2n=4x), *Festuca arundinacea* ‘Barolex’ (2n=6x), ‘Callina’ (2n=6x) and *Festulolium braunii* ‘Felopa’ (2n=4x). These varieties were the same varieties that were used in a Mitscherlich pot experiment under controlled conditions (Fariaszewska et al. 2016).

Experimental Design

A split-plot experimental design was installed in a field with three mobile rain-out shelters at the Institute for Agricultural and Fisheries Research (ILVO) in Melle (Belgium, 50°59'29" N, 3°47'04" E) with irrigation as whole-plot factor and variety as split-plot factor. The soil had a sandy loam texture with 76% sand, 16% silt and 8% clay and contained 1.6% C with a pH-KCl of 5.73. One shelter covered an area of 300 m² (30 m × 10 m) and

served as one replicate. Each shelter was split into two parts (drought–control) of 150 m² (15 m × 10 m) and each part had its own sprinkler irrigation system. One part contained twelve plots in an arrangement of three rows × four columns and was surrounded by border plots. The nine varieties used in the experiment were randomised over this twelve plots and the three remaining plots were used for breeding populations and were not included in this study. The plots were sown with an Oyord sowing machine on 2 October 2013. One plot had a surface of 3.1 m² (2.3 m × 1.35 m) after trimming and contained 10 rows with a distance of 13.5 cm between the rows. The sowing density was 1000 viable seeds per m².

All grasses were cut five times in 2014 (5 May, 6 June, 17 July, 5 August and 25 September) and four times in 2015 (21 May, 25 June, 3 August and 1 October). We only harvested four cuts in 2015 because the first cut in 2015 started later due to a colder winter and spring (Fig. 1). The plots were fertilised in 2014 with 81 + 100 kg N + K ha⁻¹ before cut I, 68 + 10 + 83 kg N + P + K ha⁻¹ after cut I, 68 + 33 kg N + K ha⁻¹ after cut II, 39 + 8 + 57 kg N + P + K ha⁻¹ after cut III and 41 kg N ha⁻¹ after cut IV and in 2015 with 81 + 100 kg N + K ha⁻¹ before the cut I, 59 + 12 + 86 kg N + P + K ha⁻¹ after cut I, 68 + 33 kg N + K ha⁻¹ after cut II and 52 + 11 + 76 kg N + P + K ha⁻¹ after cut III using a Rauch pneumatic fertiliser spreader. There were no pesticides used during the experiment.

A mild drought stress treatment was realised by covering the trial with the rain-out shelters in the period between cut II and cut III. Coverage period was between 11 June–14 July in 2014 and 1 July–3 August in 2015. The drought treatment was between 13 June and 14 July in 2014 and between 3 July and 3 August in 2015. The field experiment consisted of two soil moisture treatments: in the control treatment a soil moisture level of around 20% v/v (just above field capacity, Fig. 2) was obtained using the irrigation system, while in the drought stress treatment plants were only irrigated when the soil moisture level was less than 7.5% v/v. No irrigation was used beside the coverage period. The irrigation water was ground water with a pH of 6.9 and an EC of 0.482 mS cm⁻¹. Volumetric water content was measured hourly using 48 time domain reflectometry (TDR) sensors (type CS616, Campbell Scientific Inc., UK) installed in the plots. The two-pronged sensors made of stainless steel were 30 cm long and 3.2 mm in diameter, and were installed vertically at a depth of 10 cm in the soil. Data were collected using a datalogger and multiplexer (type CR1000 + AM16/32, Campbell Scientific Inc., UK) and LoggerNet software.

Fig. 1 Average monthly temperatures and precipitation in 2014–2015 and over the last 20 years in Merelbeke (Belgium)

Fig. 2 Changes in volumetric water content at the 0–30 cm soil depth between cut II and cut III in 2014 (a) and 2015 (b). The arrow in the figures indicate the date when the drought treatment started

Climatology data

Monthly rainfall totals and mean temperatures for each month in 2014–2015 and over the last 20 years are based on the data at the meteorological station in Merelbeke (50°59'40" N, 3°44'46" E) (Fig. 1). In 2015, lower temperatures were recorded in late winter and spring in comparison with 2014. In general, summer (June, July and August) was much drier in 2015 than in 2014.

Measurements

Dry Matter Yield and Dry Matter Content

Dry matter yield (10^3 kg ha^{-1}) and dry matter content (%) were measured at each cut. Plots were mown and fresh weight was determined using a plot harvester (Haldrup, Løgstør, Denmark) and a sample of 200 g of fresh plant material was taken to estimate the dry matter content.

The Stress Tolerance Index (STI) was calculated using the following relationship (Fernandez 1992):

$$STI = \frac{Y_p \cdot Y_s}{Mp^2} \quad (1)$$

In the above equation, Y_p , Y_s and Mp represent the yield of each variety in non-stress conditions, the yield in stress conditions and the average yield of varieties in non-stress conditions, respectively. We used the total annual dry matter yield to calculate the STI for 2014 and 2015.

Gas Exchange Parameters

During the drought treatment period gas exchange parameters (maximum photosynthesis rate (A_{max}), transpiration rate (E), stomatal conductance (g_s)) were measured using a LI6400 (Li-Cor Biosciences, Lincoln, Nebraska) at 25 °C, 1200 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ and 400 ppm CO_2 . Water use efficiency was calculated as A_{max}/E ratio. Measurements started on day 11 after the start of the drought treatment and were continued during the following 3 weeks. All measurements were determined on the second fully developed leaf in six replicates (two replicates per plot), twice a week (Monday and Thursday), between 8.30 and 12.00 am during the third cut in 2014 and 2015.

Chlorophyll *a* Fluorescence

Maximum quantum efficiency of PSII (F_v/F_m ratio) measurements started on day 8 after the start of the drought period and continued during the following 3 weeks. All measurements were done in fifteen replicates (five replicates per plot), twice a week (Thursday and Friday) during the third cut in 2014 and 2015, using a Pocket PEA ultra-portable chlorophyll fluorimeter (Hansatech Instruments, Norfolk, USA).

Quality Parameters

In both years, a sample of 1 g dried and ground leaf material collected at cut III was analysed in triplicate by Near Infra-Red Spectroscopy (NIRS) to quantify the water soluble carbohydrate content (WSC) according to the method of Wiseman et al. (1960), the organic matter digestibility (OMD) according to the method of De Boever et al. (1988), the crude protein content (CP) and the neutral detergent fibre digestibility (NDFD). The NIRS calibration of the NDFD was based on in vitro digestibility (Goering and Van Soest 1970) and NDF (neutral detergent fibre), ADF (acid detergent fibre) analysis (Van Soest et al. 1991).

Phytochemical Analysis

Plant material was collected from the field experiment at cut III in 2014, lyophilised for 3 days, grounded in the grinder and kept in a dried and dark place for analysis of proline and phenolic compounds content. Proline was extracted from 100 mg of lyophilised plant material with 50% (v/v) acetonitrile in three replicates. Extraction conditions, UPLC-PDA-ESI-MS/MS system setup and chromatographic conditions were according to Fariaszewska et al. (2016). The concentration of proline was expressed in $\mu\text{g g}^{-1}$ of dry plant material.

The concentration of flavonoids and phenolic acids was determined by the extraction of 100 mg lyophilised plant material in three replicates using Thermo Scientific Dionex ASE 150 Accelerated Solvent Extractor. Extraction process and UPLC-PDA-ESI-MS/MS was based on methods described in detail by Fariaszewska et al. (2016). The content of flavonoids and phenolic acids was expressed in $\mu\text{g mg}^{-1}$ of dry plant material. The standards solution were obtained from Sigma-Aldrich (St Louis, MO, USA).

Normalised Difference Vegetation Index (NDVI) Measurements

The Normalised Differential Vegetation Index (NDVI) was calculated based on the crop reflection in the visual (VIS) and Near Infra-Red (NIR) range: $\text{NDVI} = (\text{NIR} - \text{VIS}) / (\text{NIR} + \text{VIS})$, and has played an important role for satellite-based vegetation drought monitoring (Gu et al. 2007; Brown and de Beurs 2008). NDVI was measured in four technical replicates on each plot at the end of drought period (July 7 in 2014 and July 17 in 2015) using a handheld NDVI sensor (GreenSeeker, Trimble, Sunnyvale, CA, USA).

Statistical Analysis

The experiment was composed of nine varieties of grasses, two irrigation levels and three shelters (=replicates). Gas exchange and chlorophyll fluorescence parameters measurements were made per shelter during third regrowth, twice and five times, respectively. One biological and two technical samples were used for proline and phenolic compounds analysis for one shelter in the third regrowth. Quality parameters (NDF, AND, CP, WSC, OMD, NDFD) were analysed in one replicate per shelter. Dry matter yield and dry matter content were analysed in three replicates in five cuts in the first year and four cuts in the second year of field experiment. One-way and two-way ANOVA was used to analysis significant differences between varieties and irrigation levels. Tukey's test at a significance level of $\alpha = 0.05$ was applied to determine the interaction between varieties and irrigation levels for all parameters and cuts using StatGraphic Centurion XVI. Simple Pearson's correlation among indices and

yield in two water conditions and bi-plot graphs were performed by PQSTAT SOFTWARE 1.6.0 (PQStatSoftware, Poznań, Poland).

Results

Soil Moisture Level

Volumetric water content at 0–30 cm soil depth was maintained on average at 21.0–23.3% in 2014 and at 19.0–22.5% in 2015 for well-watered plants in cut III of the rain-out shelter experiment (Fig. 2). By the eighth day of the drought treatment, soil moisture level declined on average from 21.0 to 17.4% in 2014 and from 14.6 to 10.0% in 2015. By the eleventh day it had declined on average to 15.0% in 2014 and 8.0% in 2015 of the experiment. At the 27th day soil moisture level decreased on average to 9.0% in 2014 and 7.7% in 2015.

Dry Matter Yield

Drought stress significantly decreased dry matter yield (DMY) in cut III in all tested varieties in 2014 and 2015. The reduction was lower in 2014 compared with 2015 (Table 1). Total dry matter yield slightly decreased in 2014 (on average by 4.3%) and much more in 2015 (on average by 18.6%). In the first year of experiment, the two varieties of *L. multiflorum* ('Melmia', 'Meldiva') had the highest total DMY in the control and drought conditions, respectively: 20.2 and 22.8 t ha⁻¹, while the *F. pratensis* varieties had the lowest total dry matter yield for irrigated and non-irrigated plots. Under drought stress conditions the highest reduction of total DMY in 2014 was found for 'Barolex' (–10.1%), while 'Meltador' even showed a slight increase in total DMY (+6.7%). 'Barolex' had the highest total DMY in the control conditions, while the lowest was found for 'Felopa' in 2015. After the drought period in cut III, 'Barolex' had the lowest reduction in total dry matter yield of 2015 (–7.8%), while 'Merifest Tp.' had the highest reduction (–35.2%).

Based on the STI both *L. multiflorum* varieties showed the highest tolerance to drought in 2014, the lowest tolerance was found for *F. pratensis* (Table 1). In 2015 however, analysis of total DMY and STI resulted in *F. arundinacea* as the most tolerant to drought, while *F. pratensis* and *Festulolium* were less tolerant.

Dry Matter Content

Under drought stress dry matter content significantly increased in cut III in the 2 years of the rain-out shelter experiment: with 44.0% on average in 2014 and 81.8% on average in 2015 (Table 2). The highest increase was

observed in 'Felopa' (61.8%) and the lowest in 'Meldiva' (33.5%) in 2014. In contrast, the highest increase of dry matter content was found for 'Meltador' (167.2%) and the lowest for both *F. arundinacea* varieties in 2015. In both regrowth periods, a significantly higher dry matter content remained for all varieties under drought conditions.

Gas Exchange Parameters

Maximum Rate of Photosynthesis ($A_{\max}$)

Maximum rate of photosynthesis ($A_{\max}$) values during the entire period of water deficit treatment are presented in Fig. 3. Drought stress reduced CO₂ assimilation in 2014 with on average 29.2% and in 2015 with on average 28.4%. The highest decrease was recorded in 'Merifest' (on average 37.5%), 'Meldiva' (on average 35.4%) in 2014 and in 'Meldiva' (on average 34.4%) in 2015. The lowest decrease of photosynthesis intensity was found for 'Barolex' (on average 15.3%) in 2014 and for 'Merifest' and 'Meltador' in 2015. The highest decrease of CO₂ assimilation was determined at the 5th and 6th measurement for almost all varieties in both year of experiment, except 'Melmia' and 'Merifest', which characterised the highest decrease at the 4th and 2nd measurement, respectively.

Transpiration Rate (E)

All tested varieties reacted for drought stress by a decrease in transpiration: on average 58.2% in 2014 and 53.6% in 2015 compared to well-watered conditions (Fig. 4). The transpiration rate at the first measurement (11 days after the start of the drought) was clearly lower in 2015 compared with 2014. The highest reduction of this process was found for 'Melluck' (on average 67.4%) in 2014 and for 'Felopa' (on average 59.5%) in 2015. 'Callina' had the lowest average decline of transpiration rate in 2014 and 'Merifest' and 'Merifest Tp' in 2015.

WUE (Water Use Efficiency)

Water use efficiency was quite different within the tested grass varieties. There was a higher increase of WUE in 2014 (on average by 77.8%) compared with 2015 (on average by 65.7%) under drought stress versus control treatment. The highest increase of WUE was found in 2014 for 'Melluck' in 2014 and 2015. In contrast, the lowest increase was recorded in 'Merifest' in both investigated years (Fig. 5).

Stomatal Conductance (g_s)

Stomatal conductance reduction in drought conditions was higher in 2015 on average 65.7% compared with 39.9%

Table 1 Dry matter yield (DMY, 10^3 kg/ha⁻¹) and stress tolerance index (STI) of different grass species and varieties grown in the rain-out shelter experiment under optimal and drought treatment conditions in 2014 and 2015

Varieties	Total DMY ¹		Cut I + II Pre-treatment		Cut III Drought	Cut IV(+V) ² Regrowth		STI	
	C ³	D ⁴	Cut I + II Pre-treatment			C	D		
			C	D					
2014									
<i>L. perenne</i> 'Melluck'	16.7 abc	16.0 abc	7.1 abc	7.8 bc	4.9 abcdef	4.0 abcde	4.7 ab	4.2 ab	0.93 ab
<i>L. perenne</i> 'Meltador'	16.4 abc	17.5 abcd	7.1 abc	8.5 cd	5.0 bcdef	4.7 abcdef	4.3 ab	4.3 ab	1.01 ab
<i>L. multiflorum</i> 'Meldiva'	22.9 e	21.6 de	12.3 e	12.3 e	5.6 f	5.2 def	5.0 b	4.1 ab	1.71 c
<i>L. multiflorum</i> 'Melmia'	20.1 cde	19.3 bcde	10.8 de	11.5 e	5.0 bcdef	4.3abcde	4.3 ab	3.5 ab	1.34 bc
<i>F. pratensis</i> 'Merifest'	14.9 ab	14.9 ab	5.2 a	6.1 ab	5.1 cdef	4.1 abcd	4.6 ab	4.7 ab	0.77 a
<i>F. pratensis</i> 'Merifest Tp'	14.0 a	13.0 a	5.1 a	5.3 a	4.9 abcdef	3.8 ab	4.0 ab	3.9 ab	0.63 a
<i>F. arundinacea</i> 'Barolex'	15.9 abc	14.3 a	5.2 a	5.0 a	5.2 def	3.9 abc	5.5 b	5.4 b	0.78 a
<i>F. arundinacea</i> 'Callina'	16.2 abc	15.3 ab	5.6 ab	5.9 ab	5.4 ef	4.2 abcde	5.2 b	5.2 b	0.86 a
<i>F. braunii</i> 'Felopa'	16.1 abc	15.1 ab	7.9 bc	8.8 cd	4.8 abcdef	3.7 a	3.4 ab	2.6 a	0.86 a
Average	17.0 A	16.3 A	7.4 A	7.9 A	5.1 B	4.2 A	4.6 A	4.2 A	
Treatment	ns		*		***		ns		***
Variety	***		***		**		**		***
T×V	ns		ns		ns		ns		
2015									
<i>L. perenne</i> 'Melluck'	16.9 bcdef		8.1 ab	8.9 abcd	4.4 ef	1.3 ab	4.4 def	4.8 def	0.75 ab
<i>L. perenne</i> 'Meltador'	15.9 bcde		7.6 a	8.8 abcd	4.0 def	1.1 ab	4.3 de	4.4 def	0.68 ab
<i>L. multiflorum</i> 'Meldiva'	20.7 ef		12.0 bcd	12.8 d	4.9 f	1.7 ab	3.8 bcd	2.3 abc	1.04 cd
<i>L. multiflorum</i> 'Melmia'	19.8 def		10.9 abcd	12.00 bcd	4.9 f	1.6 ab	4.0 cd	2.3 ab	0.94 bc
<i>F. pratensis</i> 'Merifest'	17.6 cdef		9.2 abcd	9.4 abcd	3.8 def	0.9 a	4.6 def	2.3 abc	0.65 ab
<i>F. pratensis</i> 'Merifest Tp'	16.2 bcde		8.4 ab	8.6 abc	3.6 de	0.6 a	4.2 de	1.3 a	0.50 a
<i>F. arundinacea</i> 'Barolex'	21.8 f		11.2 abcd	12.7 cd	4.5 ef	2.1 bc	6.1 f	5.3 def	1.30 d
<i>F. arundinacea</i> 'Callina'	20.6 ef		10.4 abcd	11.9 bcd	4.4 ef	1.7 ab	5.8 ef	4.5 def	1.10 cd
<i>F. braunii</i> 'Felopa'	15.6 abcde		9.0 abcd	9.4 abcd	3.0 cd	0.7 a	3.6 bcd	1.8 a	0.56 a
Average	18.4 B		9.6 A	10.5 B	4.2 B	1.3 A	4.6	3.2	
Treatment	***		*		***		***		***
Variety	***		**		***		***		***
T×V	ns		ns		ns		***		***

Data are representing total DMY per year and the DMY before, during and after the drought treatment

Different upper-case letters indicate significant differences in each row and different lower-case indicate letters significant differences in each column according to Tukey's test ($\alpha = 0.05$)

Significant differences: ***($p < 0.001$); **($p < 0.01$); *($p < 0.05$)

¹Sum of DMY over the five cuts in 2014 or four cuts in 2015

²2014: sum of cut IV and V, 2015: only cut IV

³C: control treatment, plots were irrigated between cut II and cut III to keep volumetric water content above field capacity (20% v/v)

⁴D: drought treatment, plots irrigated were only when volumetric water content declined below 7.5% v/v between cut II and cut III

Table 2 Dry matter content (%) of grass species and varieties grown in the rain-out shelter experiment under optimal and drought treatment conditions in 2014 and 2015

Varieties	2014										2015																			
	Cut I + II Pre-treatment					Cut III Drought					Cut IV + V Regrowth					Cut I + II Pre-treatment					Cut III Drought					Cut IV Regrowth				
	C ¹	D ²				C	D				C	D				C	D				C	d				C	D			
<i>L. perenne</i> ‘Melluck’	22.6 ef	21.3 def	16.9 abc	22.8 cde	21.5 ab	23.5 b	27.3 abc	27.1 abc	19.4 ab	40.0 ef	28.6 fg	24.8 bcd																		
<i>L. perenne</i> ‘Meltador’	22.2 ef	20.4 bcdef	15.9 a	22.6 cde	20.5 ab	21.5 ab	27.1 abc	25.4 abc	17.7 a	47.3 g	29.6 g	21.3 a																		
<i>L. multiflorum</i> ‘Meldiva’	19.9 abcde	18.1 abcd	18.2 abcd	24.3 e	19.3 ab	20.6 ab	26.0 abc	25.2 abc	24.6 c	42.7 f	25.8 cde	21.6 a																		
<i>L. multiflorum</i> ‘Melmia’	17.9 abc	16.6 a	16.4 ab	23.5 de	16.9 a	20.3 ab	23.5 a	24.2 abc	22.6 bc	41.4 f	24.8 bcd	20.5 a																		
<i>F. pratensis</i> ‘Merifest’	22.8 ef	23.6 f	17.7 abcd	27.2 e	22.3 ab	23.5 b	26.6 abc	26.8 abc	21.4 abc	44.2 fg	31.4 h	25.7 cde																		
<i>F. pratensis</i> ‘Merifest Tp’	21.0 cdef	20.4 bcdef	16.0 a	24.3 e	20.1 ab	21.7 ab	25.3 abc	24.4 abc	18.5 ab	43.6 fg	25.5 cde	24.4 bcd																		
<i>F. arundinacea</i> ‘Barolex’	22.2 ef	21.8 ef	16.2 a	22.5 cde	19.2 ab	21.3 ab	26.8 abc	26.1 abc	19.7 ab	31.4 d	27.0 ef	25.9 de																		
<i>F. arundinacea</i> ‘Callina’	22.6 ef	22.6 ef	16.0 a	22.2 bcde	20.1 ab	21.5 ab	28.5 c	28.0 bc	22.8 bc	36.3 e	27.9 fg	25.5 cde																		
<i>F. F. braunii</i> ‘Felopa’	18.3 abcd	17.3 ab	15.7 a	25.4 e	19.7 ab	20.9 ab	24.0 ab	23.6 a	20.2 ab	44.2 fg	24.2 bcd	21.2 a																		
Average	21.2 B	20.2 A	16.6 A	23.9 B	20.0 A	21.6 B	26.1 A	25.7 A	22.0	40.0	27.2	23.4																		
Treatment	*		***		**		ns		***		***																			
Variety	***		**		*		**		*		***																			
TxV	ns		ns		ns		ns		ns		*																			

Data are means (n = 3)

Different upper-case letters indicate significant differences in each row and different lower letters significant differences in each column according to Tukey's test ($\alpha=0.05$)Significant differences: ***($p<0.001$); **($p<0.01$); *($p<0.05$)¹C: control treatment, plots were irrigated between cut II and cut III to keep volumetric water content above field capacity (20% v/v)²D: drought treatment, plots irrigated were only when volumetric water content declined below 7.5% v/v between cut II and cut III

Fig. 3 Maximum rate of photosynthesis ($A_{\max}$) of grass species and varieties grown in the field under optimal and drought treatment conditions in 2014 and 2015. Data are mean $\pm$ SE ($n=6$)

in 2014. The highest reduction of the stomatal conductance under drought stress was found for ‘Felopa’ (on average 48.2%) in 2014 and for ‘Melmia’ (on average 48.1%) in 2015. The lowest decrease in stomatal closure was recorded in ‘Meldiva’ (on average 28.0%) in 2014 and ‘Barolex’ (on average 56.1%) in 2015 (Fig. 6).

Chlorophyll *a* Fluorescence

Quantum Efficiency Photosystem II (F_v/F_m Ratio)

Quantum efficiency of photosystem II decreased with progressive soil drying in both investigated years (Fig. 7). The average decrease amounted to 1.2% in 2014 year and 2.4% in 2015 year in all tested varieties (Fig. 7). The quantum efficiency of PSII dropped clearly at the sixth measurement for the varieties ‘Meltador’, ‘Melmia’ and ‘Barolex’ in 2014. A more gradual decrease was found for ‘Melluck’ and ‘Merifest’. In 2015 the highest average decrease of F_v/F_m ratio was found in ‘Melluck’ (3.2%). Almost all tested varieties had already higher difference between control and drought from the first measurement in 2015 compared to 2014.

Forage Quality Parameters

The crude protein content decreased with 8.7% on average in 2014 in drought conditions, but increased with 20.3% on average in 2015 (Table 3). The highest reduction of crude protein was determined in *L. multiflorum* varieties in 2014. The highest increase of CP was found for both *F. pratensis* varieties and the lowest for ‘Barolex’. Under drought stress conditions WSC content significantly increased with 30.9% on average in all tested varieties in 2014 and for almost varieties with 5.8% on average in 2015. The highest increase of WSC content was found for both *F. arundinacea* varieties in 2014 and in 2015 for both *F. pratensis* varieties. Drought stress significantly decreased the acid detergent fibre (ADF) content with 9.4% on average in 2014 and with 15.1% on average in 2015. The NDF content decreased also by 2.6% on average in 2014 and 3.4% in 2015. In the first year of the experiment the ADF decreased most in ‘Merifest’ and ‘Felopa’. However, decrease of the ADF content was highest in ‘Meltador’, ‘Merifest’ in 2015. The highest significant decrease under water deficit in NDF was determined in ‘Meltador’ in 2014 and in ‘Merifest’ in 2015. Water stress didn’t significantly affect organic matter digestibility (OMD) in 2014, while a significant increase (by 3.7%)

Fig. 4 Transpiration rate (E) of grass species and varieties grown in the field under optimal and drought treatment conditions in 2014 and 2015. Data are mean $\pm$ SE (n=6)

was determined in 2015. Under drought stress the highest increase of OMD was found for *F. pratensis* varieties, while ‘Felopa’ had a slight decrease (by 4.0%). Over all, *L. perenne* and *F. pratensis* had the highest digestibility, while *L. multiflorum* varieties had the lowest digestibility, which is explained by the presence of stems at cut III. Under drought stress treatment NDFD significantly decreased with 3.1% on average in 2014 and with 1.7% on average in 2015. The highest decrease in NDFD was determined in ‘Melmia’ in 2014 and in ‘Felopa’ in 2015.

Proline and Phenolic Compounds Concentration

The proline concentration significantly increased under drought treatment (on average 69.4%) in all varieties (Table 4). The highest increase of proline content was found for ‘Merifest Tp’ (229.1%) and the lowest for ‘Melmia’ (36.9%) under drought stress. ‘Felopa’ accumulated $960 \mu\text{g g}^{-1}$ of proline under water deficit. In contrast, in the drought stress treatment the lowest concentration of this osmolite was determined in ‘Callina’ and ‘Barolex’. The concentration of flavonoids significantly increased under drought stress (by average 37.9%) in all varieties (Table 4). The highest increase of flavonoids concentration

had both varieties of Italian ryegrass, 66.5% for ‘Meldiva’ and 66.0% for ‘Melmia’. In contrast, the lowest increase was found for ‘Felopa’ and ‘Meltador’: 18.0 and 18.7%, respectively. Water deficit caused a significant increase in the concentration of phenolic acids in all varieties (by average 19.1%). The highest increase of this compounds was found for ‘Felopa’ (38.0%) and ‘Meldiva’ (34.5%).

Normalised Difference Vegetation Index (NDVI) Measurements

Overall, NDVI of the drought treatment plots was significantly smaller than in the control conditions in 2014 and 2015, but a higher decrease was observed in 2015 (on average by 13.8%) than in 2014 (on average by 2.5%) (Table 5). The highest values of NDVI were found for ‘Melluck’ under control conditions in both investigated years, while the lowest were observed in ‘Melmia’ in 2014 and ‘Felopa’ in 2015 under drought stress conditions. Moreover, *F. arundinacea* varieties showed no decrease in NDVI in drought stress conditions in 2014 and only a low decrease in 2015.

Fig. 5 Water use efficiency of grass species and varieties grown in the field under optimal and drought treatment conditions in 2014 and 2015. Data are mean $\pm$ SE ($n=6$)

Principal Components and Correlation Analysis

Principal component analysis (PCA) revealed that two dimensions explained 66.8% and 84.1% of total variation in drought stress in 2014 and 2015, respectively (Fig. 8). Two main clusters were separated by dimension 1: on the left side the control treatment and on the right side the drought treatment. Dimension 1 had a high positive correlation with DMY, ADF, NDF, F_v/F_m ratio and all gas exchange parameters and a negative correlation with DMC, WUE and WSC in both years, while dimension 2 had a high positive correlation with OMD and NDFD in both years. Dimension 2 was negatively correlated with PA and FI in 2014. Bi-plot graphs showed that *F. braunii* ‘Felopa’ was similar in reaction as *F. pratensis* and *L. perenne* in both investigated years under control and drought stress conditions. *L. multiflorum* and *F. arundinacea* varieties clearly clustered as separate groups under drought and control conditions in 2014 and 2015. The main driver for *L. multiflorum* was its low OMD (and associated parameters) in both years and its high DMY in 2014. The main driver for *F. arundinacea* was its high DMY (and associated photosynthesis parameters), especially in 2015.

Table 6 shows that the accumulation of phenolic acids had a positive and significant correlation with DMY in

control and drought stress treatment in 2014. DMC was significantly negatively correlated with DMY and STI under drought treatment in 2015. The transpiration rate and stomatal conductance were clearly associated in the drought treatment of both years and even in the control treatment of 2015 (Table 6).

Discussion

Many authors confirmed that mild drought stress causes changes in productivity, physiological processes and chemical composition of crop plants (Mafakheri et al. 2010; Ball 2001; Abdelgawad et al. 2015). Our results showed a significant reduction of dry matter yield in *Festuca*, *Lolium* and *Festulolium* in the field experiment, confirming the results of other authors in many grass species like *F. arundinacea*, *Poa pratensis*, *Phleum pratense*, *Phalaris canariensis* (Kallida et al. 2008; Turner et al. 2012). In our studies the drought treatment reduced dry matter yield more in 2015 than in 2014. *L. multiflorum* had the highest dry matter production in drought and control conditions in 2014, while *F. arundinacea* had the highest dry matter production in both conditions in 2015. Moreover, *F. pratensis* and *F. arundinacea*

Fig. 6 Stomatal conductance (g_s) of grass species and varieties grown in the field under optimal and drought treatment conditions in 2014 and 2015. Data are mean $\pm$ SE ($n=6$)

showed a higher production in optimal water conditions in 2015 compared with 2014, which may be related to a deeper and better developed root system in the second year (Deru et al. 2012; Coughon et al. 2017). All tetraploids varieties had a tendency for a lower dry matter production under control and drought stress conditions, except for *L. perenne*, where a slightly better total dry matter yield was found in the tetraploid variety, but only in the first year after sowing under drought conditions. The order of drought tolerance based on the total DMY and STI under drought stress was $Lm > Lp > Fa > Felo > Fp$ in 2014. A first reason for the differences between 2014 and 2015 was the lower precipitation in May and June in 2015. This resulted in a low soil moisture level before the start of the drought period under the rain-out shelter in 2015. Reduction of dry matter yield could also be related to development of the sward and age of the plants, confirming the results of Staniak (2013). Younger grasses better withstand water deficit in the soil and they didn't have a destroyed sward through a previous stress factor (here the same drought plots of 2014 received a drought treatment in 2015). *F. arundinacea* as a tolerant species had the highest dry matter production and the lowest reduction in drought stress conditions from all tested varieties and species in 2015. In contrast, drought treatment during 3 weeks

in the pot experiment had the highest impact on DMY of *F. arundinacea* compared to other tested species of grasses (Fariaszewska et al. 2016), which could be due to a under-developed root system. Generally, the order in drought tolerance based of total DMY and STI of different species under drought stress was following: $Fa > Lm > Lp > Felo > Fp$ in 2015. Moreover, a high STI index is considered as higher yield in stress and non-stress conditions. Under irrigation and subtropical field conditions Neal et al. (2009) noted that *F. arundinacea* had a higher production of dry matter yield than *L. perenne*. Results of Ebrahimiyan et al. (2012) confirmed this trend and showed a lower reduction of DMY and better production of *F. arundinacea* in the second year of control and drought treatment. Jeronimo et al. (2014) showed a significant dry matter yield reduction in *L. perenne* (by 16%) and *F. pratensis* (by 12%) during drought conditions in the case of a grazing exploitation.

Many researchers proved that photosynthesis decreases under drought stress as an effect of stomata closure (leading to a lower CO_2 availability), damaged photosynthetic apparatus including PSI and PSII, intensified photo-inhibition, changes in photosynthetic pigments or damage to chloroplast biochemistry under stress factors (Fu and Huang 2001; Flexas and Medrano 2002; Kong et al. 2015). Reduction of

Fig. 7 F_v/F_m ratio of grass species and varieties grown in the field under optimal and drought treatment conditions in 2014 and 2015. Data are mean $\pm$ SE ($n = 15$)

photosynthesis rate was similar in 2014 and 2015 and the highest reduction of this process was found for *L. multiflorum* in both years and for *F. pratensis* in 2014. The previous pot experiment of Fariaszewska et al. (2016) showed that intensity of photosynthesis decreased the most in *F. pratensis*. Under drought stress conditions plants loss turgor pressure. The plant reacts through closing the stoma, in order to reduce the transpiration rate and to keep more water into the cells. *Festuca* species and *F. braunii* had a higher reduction of stomatal conductance in 2014. In 2015, both stomatal conductance and transpiration rate were similar for all species. Significant reduction of transpiration rate due to drought in forage grasses (*F. arundinacea*, *L. perenne*, *D. glomerata*, *L. chinesis*) and *T. aestivum* varieties were found in many studies: Huang and Gao (1999), Xu and Zhou (2005), Olszewska et al. (2010), Staniak (2013), and Guan et al. (2015). Changes in the intensity of physiological processes under drought stress conditions can also be the result of the repair mechanisms, e.g. induction of LEA protein, lipid transfer protein, protease inhibitors (Starck 2002) or they are associated with a decrease in the export of assimilates from the leaf blade to other plant organs (Stark 1995). Water use efficiency (WUE) is often regarded as an indicator of yield under drought stress and even an element

of plant drought resistance. Under drought treatment, WUE increased in all varieties more in 2014 (average by 78%) than 2015 (on average by 66%). It could be related to a better developed root system, a higher reduction of the transpiration process or a more efficient use of a finite water supply in 2015 compared to 2014. In general, *L. perenne* had high values of WUE in 2014 and *Lolium* species and *F. braunii* in 2015 in drought stress conditions. Transpiration rate and WUE had a significant negative correlation coefficient in optimal and drought stress conditions (Fig. 8 and Table 6) Rumasz-Rudnicka (2010) and Fariaszewska et al. (2016) confirmed this negative correlation between WUE and transpiration in different forage grasses and the increase of WUE during lack of watering in the pot experiments. In recent years, the measurements of chlorophyll fluorescence makes it possible to evaluate of the health and functionality of the photosynthetic apparatus during the process of CO_2 assimilation under environmental stresses (Strasser et al. 2000; Sayed 2003; Clark et al. (2000). In general, our results did not show clear differences between the tested species and varieties. Nevertheless, the F_v/F_m decreased more in 2015 than in 2014. A clear decrease of F_v/F_m ratio was observed after 2 weeks of drought treatment in most of tested varieties in 2014 and at the 8th day of the drought treatment in 2015.

Table 3 Forage quality parameters at cut III of grass species and varieties grown in the rain-out shelter experiment under control and drought treatment conditions. Values are expressed as % of absolute dry matter. Data are means (n = 3)

Varieties	CP ¹	WSC		ADF		NDF		OMD		NDFD		
		D ³	C	D	C	C	D	C	D	C	D	
2014												
<i>L. perenne</i> 'Melluck'	13.4 b	12.1 ab	13.2 abc	18.6 cde	30.1 bcde	27.2 ab	51.2 ab	51.2 ab	79.7 ef	81.0 f	77.2 bcd	75.6 defgh
<i>L. perenne</i> 'Meltador'	12.6 ab	11.7 ab	16.5 bcde	20.5 e	28.5 abc	26.3 a	53.9 abc	49.7 a	82.9 f	82.8 f	78.4 fgh	76.1 defgh
<i>L. multiflorum</i> 'Meldiva'	11.4 ab	9.4 a	15.1 abcde	19.5 de	30.9 cdef	29.8 bcd	56.0 cde	55.5 bcd	67.2 abc	64.2 a	64.0 ab	60.4 a
<i>L. multiflorum</i> 'Melmia'	12.0 ab	9.9 a	14.0 abcd	18.3 cde	31.0 cdef	29.5 abcd	55.8 bcde	55.6 bcd	68.8 abc	65.5 ab	65.8 abc	60.1 a
<i>F. pratensis</i> 'Merifest'	13.3 b	11.7 ab	10.7 ab	13.6 abc	34.5 g	30.0 bcde	60.2 e	57.0 cde	80.9 f	80.5 f	79.1 gh	78.0 fgh
<i>F. pratensis</i> 'Merifest Tp'	11.7 ab	11.3 ab	11.2 ab	13.4 abcde	33.3 efg	30.8 cdef	57.8 cde	57.3cde	77.8 ef	80.2 f	80.5 h	78.0 fgh
<i>F. arundinacea</i> 'Barolex'	13.8 b	12.8 ab	9.3 a	13.4 abcd	32.1 defg	28.9 abcd	59.2 de	58.3 cde	70.4bcd	71.3 cd	73.5 defg	72.3 def
<i>F. arundinacea</i> 'Callina'	13.9 b	12.5 ab	9.4 a	13.9 abcd	32.2 defg	29.3 abcd	59.1 de	58.0 cde	70.4 bcd	69.7abcd	72.8 defg	70.2 bcd
<i>F. braunii</i> 'Felopa'	11.7 ab	12.4 ab	10.0 a	13.7 abcd	34.0 fg	29.6 abcd	59.0 de	56.0 cde	72.6 cd	74.4 de	72.7 def	70.9 cde
Average	12.6 B	11.5 A	12.3 A	16.1 B	31.9 B	28.9 A	56.9 B	55.4 A	74.5 A	74.4 A	73.6 B	71.3 A
Treatment	**		***		***		**		ns		***	
Variety	**		**		***		***		***		***	
T×V	ns		ns		ns		ns		ns		ns	
2015												
<i>L. perenne</i> 'Melluck'	14.6 abcd	17.5 de	12.8 abc	12.8 abc	27.9 defgh	23.7 abc	52.1 abcd	50.7 ab	79.7 h	80.4 ij	76.5 f	75.4 f
<i>L. perenne</i> 'Meltador'	14.1 abcd	17.7 de	13.5 bc	13.7 bc	28.0 defgh	23.1 a	51.6 abc	50.1 a	81.4 j	82.6 jk	79.2 g	77.3 fg
<i>L. multiflorum</i> 'Meldiva'	10.8 a	12.4 abc	12.8 abc	16.1 c	30.1 fgh	26.8 bcdefg	57.2 def	56.2 cdef	60.0 a	64.3 b	57.2 a	59.8 b
<i>L. multiflorum</i> 'Melmia'	11.1 ab	13.1 abc	16.4 c	15.8 c	29.6 efgh	26.1 abcde	56.0 bcdef	55.1 abcdef	63.0 b	65.5 b	60.0 b	61.4 bc
<i>F. pratensis</i> 'Merifest'	15.2 cd	19.4 e	6.8 a	10.0 abc	30.8 h	23.2 ab	59.8 f	53.7 abcde	75.6 fg	83.9 k	77.3 fg	74.8 ef
<i>F. pratensis</i> 'Merifest Tp'	15.1 cd	19.3 e	7.3 ab	10.8 abc	30.1 fgh	24.2 abc	58.0 ef	54.6 abcdef	76.8 g	84.8 k	80.8 h	73.7 e
<i>F. arundinacea</i> 'Barolex'	13.9 abcd	14.8 bcd	10.0 abc	12.5 abc	30.0 fgh	26.4 abcdef	58.4 ef	56.3 cdef	67.8 cd	69.0 d	72.5 de	72.8 e
<i>F. arundinacea</i> 'Callina'	13.5 abc	16.0 cde	13.4 bc	13.1 abc	27.3 cdefgh	24.5 abcd	55.6 bcdef	55.1 abcdef	68.7 d	70.1 d	69.9 c	71.6 d
<i>F. braunii</i> 'Felopa'	15.5 cde	19.1 e	11.7 abc	10.0 abc	27.9 defgh	24.4 abcd	54.1 abcde	54.0 abcde	76.7 g	73.6 e	74.8 ef	70.2 d
Average	13.8 A	16.6 B	12.0 A	12.7 A	29.1 A	24.7 B	55.9 B	54.0 A	72.2 A	74.9 B	72.0 B	70.8 A
Treatment	***		ns		**		***		***		*	
Variety	**		**		***		**		***		***	
T×V	ns		ns		*		ns		***		***	

¹ CP-crude protein, WSC-water-soluble sugars, ADF-acid detergent fiber, NDF-neutral detergent fiber, OMD-organic matter digestibility, NDFD-cell wall digestibility² C: control treatment, plots were irrigated between cut II and cut III to keep volumetric water content above field capacity (20% v/v)³ D: drought treatment, plots irrigated were only when volumetric water content declined below 7.5% v/v between cut II and cut IIIDifferent upper-case letters indicate significant differences in each row and different lower letters significant differences in each column according to Tukey's test ($\alpha=0.05$)Significant differences: ***($p<0.001$); **($p<0.01$); *($p<0.05$)

Table 4 Concentration of proline, flavonoids and phenolic acids at cut III of grass species and varieties under optimal and drought treatment conditions in 2014

Varieties	Proline ($\mu\text{g g}^{-1}$)		Flavonoids ($\mu\text{g mg}^{-1}$)			Phenolic acid ($\mu\text{g mg}^{-1}$)
	C ¹	D ²	C	D	C	D
<i>L. perenne</i> ‘Melluck’	369.4 de	666.9 h	24.6 hi	35.3 l	34.5 e	40.4 h
<i>L. perenne</i> ‘Meltador’	195.6 ab	563.9 gh	15.5 cd	18.4 ef	37.6 fg	41.8 ij
<i>L. multiflorum</i> ‘Meldiva’	343.6 cd	470.4 efg	23.3 ghi	38.8 m	41.5 hi	55.8 m
<i>L. multiflorum</i> ‘Melmia’	378.6 de	391.9 def	14.4 bc	23.9 ghi	38.0 g	49.1 k
<i>F. pratensis</i> ‘Merifest’	302.8 bcd	466.9 efg	20.3 f	26.8 j	38.5 g	43.0 j
<i>F. pratensis</i> ‘Merifest Tp’	151.4 a	498.2 fg	18.4 ef	22.5 g	26.6 c	30.5 d
<i>F. arundinacea</i> ‘Barolex’	167.4 a	232.6 abc	17.3 de	22.6 gh	29.5 d	34.0 e
<i>F. arundinacea</i> ‘Callina’	141.7 a	206.2 ab	24.7 i	32.9 k	52.0 l	58.3 m
<i>F. braunii</i> ‘Felopa’	580.0 gh	960.6 i	12.2 a	14.4 bc	12.9 a	17.8 b
Average	292.3 A	495.3 B	19.0 A	26.2 B	34.6 A	41.2 B
Treatment	***		***		***	
Variety	***		***		***	
TxV	***		***		***	

¹C: control treatment, plots were irrigated between cut II and cut III to keep volumetric water content above field capacity (20% v/v)

²D: drought treatment, plots irrigated were only when volumetric water content declined below 7.5% v/v between cut II and cut III

Different upper-case letters indicate significant differences in each row and different lower letters significant differences in each column according to Tukey’s test ($\alpha=0.05$)

Significant differences: *** ($p<0.001$); ** ($p<0.01$); * ($p<0.05$)

Table 5 Normalized Differential Vegetation Index (NDVI) of the different grass species and varieties grown in the rain-out shelter experiment under optimal and drought treatment conditions in 2014 and 2015

Varieties	2014		2015	
	C	D	C	D
<i>L. perenne</i> ‘Melluck’	0.894 a	0.856 ab	0.896 a	0.829 ab
<i>L. perenne</i> ‘Meltador’	0.886 a	0.852 ab	0.872 bc	0.795 ab
<i>L. multiflorum</i> ‘Meldiva’	0.848 ab	0.835 ab	0.849 cd	0.724 ab
<i>L. multiflorum</i> ‘Melmia’	0.866 ab	0.822 b	0.854 cd	0.751 ab
<i>F. pratensis</i> ‘Merifest’	0.875 ab	0.858 ab	0.888 ab	0.697 ab
<i>F. pratensis</i> ‘Merifest Tp’	0.881 ab	0.862 ab	0.878 ab	0.674 ab
<i>F. arundinacea</i> ‘Barolex’	0.856 ab	0.875 a	0.879 ab	0.847 a
<i>F. arundinacea</i> ‘Callina’	0.839 b	0.839 ab	0.848 d	0.810 ab
<i>F. braunii</i> ‘Felopa’	0.884 ab	0.833 ab	0.852 cd	0.601 b
Average	0.870 B	0.848 A	0.868 B	0.748 A
Treatment	***		***	
Variety	***		**	
TxV	*		*	

Different letters indicate significant differences within the columns ($p<0.05$)

Significant differences: *** ($p<0.001$); ** ($p<0.01$); * ($p<0.05$)

Huang and Gao (1999) showed a significant reduction of F_v/F_m ratio after 3 weeks of stop irrigation (RWC 40%) in *F. arundinacea* varieties (on average by 3.7%) with variation between varieties.

The length of the growing season may also affect water availability and grasses ability to maintain forage quality under drought stress. As is known, forage quality decrease with plant age, maturity stage at harvest and environmental conditions, because leaf/steam ratio decreases (Nelson 1994; Peterson et al. 1992; Buxton 1995). In drought stress conditions ADF and NDF decreased in all tested grasses in both years of our field experiment. Our study showed the highest decrease of ADF in *F. pratensis* and NDF in *L. perenne* in both years. Reduction of NDF and ADF content was found also by Staniak (2013) and Küchenmeister et al. (2013) in *F. braunii*, *L. perenne*, *L. multiflorum*, *F. pratensis* and *T. repens* under water deficit. High temperature may increase lignin accumulation and decrease quality, but drought stress may actually increase forage quality by delaying maturity which explains much of the improved digestibility (Buxton 1995; Cherney and Hall 2008). Our study showed a significant increase of organic matter digestibility in 2015.

The crude protein content (CP) increased during the drought period of 2015 and this was in accordance with other studies with *F. pratensis*, *P. pratense*, *F. pratensis*, *L. perenne*, *L. multiflorum* and *F. braunii* (Madziar and Latanowicz 1996; Olszewska 2009; Trzaskoś et al. 2001; Staniak

Fig. 8 The bi-plot display of dry matter yield, physiological attributes and forage quality parameters of grass varieties under control and drought treatment conditions in 2014 (**a**) and 2015 (**b**). *DMY* dry matter yield, *DMC* dry matter content, *A_{max}* maximum rate of photosynthesis, *E* transpiration rate, *g_s* stomatal conductance, *F_v/F_m*

ratio-maximum quantum efficiency of PSII, *Pro* proline content, *FL* flavonoids content, *PA* phenolic acid content, *CP* crude protein, *WSC* water-soluble sugars, *ADF* acid detergent fiber, *NDF* neutral detergent fiber, *OMD* organic matter digestibility, *NDFD* cell wall digestibility

2013). Increase of CP, might be explained by delayed maturity and changes in the leaf/stem ratio (Peterson et al. 1992). Moreover, if both nitrogen and water are present in the same soil horizon, they may be taken up together and forage CP concentration may be unaffected or may be increased if soil nitrogen is more available than water (Buxton 1995).

Under drought stress conditions a significant increase of WSC was found only in 2014. In addition, *Festuca* species had a higher increase of WSC than *Lolium* species, mainly *F. arundinacea* in 2014 and *F. pratensis* in 2015. Kozłowski and Kukułka (1996), Thomas and James (1999), and Küchenmeister et al. (2013) confirmed the increase of WSC concentration in *L. perenne* under drought stress. Moreover Kozłowski and Goliński (1996) signalised an increase of WSC in *F. pratensis* and Turner et al. (2012) in *F. arundinacea* under drought conditions. Similar relationships were demonstrated by Staniak (2013), in the second year of vegetation: under water deficit in the soil: *F. pratensis* accumulated more WSC compared to well-watered conditions, while *L. multiflorum* and *F. braunii* decreased the accumulation of sugars. The accumulation of WSC has been often associated with an increase in drought tolerance and survival of grasses (Volaire and Thomas 1995; Volaire and Lelièvre 1998). According to many studies, increase of sugars content may change the osmotic potential, which is a physiological

mechanism in responses to drought (Morgan 1984; DaCosta and Huang 2006). Additionally, the reserve of sugars could be utilised as the primary source for continued biomass production following drought stress (Chaves 1991).

Our results showed that proline concentration increased in all grass species and varieties in response to drought. According to Bandurska and Jóźwiak (2010) accumulation of proline depends on many factors such as plant's age, which may explain a higher concentration of proline in *L. perenne* and *F. braunii* in 2014. *F. pratensis* and *L. perenne* were characterised by the highest increase of this osmolyte. Proline seems to be associated with a better drought tolerance in *Festuca* compared with *Lolium* species, which was also suggested by Perlikowski et al. (2014) and Fariaszewska et al. (2016).

Phenolic compounds such as phenolic acids and flavonoids have regulative and anti-oxidative properties and it is believed that they can be an important element to responses to environmental stresses (Weidner et al. 2007; Rosicka-Kaczmarek 2004). The synthesis and behaviour of phenolic compounds such as flavonoids and phenolic acids during abiotic stress is still not well-documented in forage grasses and other crops. The phenolic compounds have a benzene ring and are therefore very reactive structures. The benzene ring as a π -electron system can neutralize UV

Table 6 Correlation coefficient of different traits under optimal (below diagonal) and drought treatment (above diagonal) conditions during cut III in 2014 and 2015

Trait	DMY	$A_{\max}$	E	WUE	g_s	F_v/F_m	DMC	STI	Pro	Fl	PA
2014											
DMY		0.31	0.07	−0.24	0.53*	−0.27	−0.17	0.97***	−0.21	0.49*	0.68*
$A_{\max}$	0.11		0.58*	−0.22	0.10	−0.05	−0.36	−0.24	−0.42	−0.48*	−0.25
E	0.41	0.09		−0.87**	0.65*	−0.29	−0.41	0.22	−0.77*	0.08	0.50
WUE	−0.32	0.24	−0.94***		−0.60*	0.16	−0.09	−0.39	0.66*	−0.16	−0.62*
g_s	0.22	−0.06	−0.15	0.08		−0.25	−0.46	0.67**	−0.68*	0.71*	0.74**
F_v/F_m	−0.81***	−0.29	−0.53	0.38	−0.04		0.13	−0.26	0.34	−0.37	−0.54
DMC	0.55*	0.09	−0.42	0.46	0.34	−0.19		−0.17	0.41	−0.17	−0.30
STI	0.84***	−0.01	0.08	−0.09	0.32	−0.65*	0.58*		−0.35	0.56	0.76**
Pro	−0.32	−0.23	−0.70**	0.65*	−0.13	−0.19	0.13	−0.17		−0.39	−0.66*
Fl	0.54*	−0.31	0.17	−0.23	0.14	−0.45	0.54*	0.37	0.54		0.75**
PA	0.71**	0.01	0.46	−0.43	0.24	−0.75*	0.38	0.65*	0.38	0.67	
2015											
DMY		0.09	0.32	−0.52*	0.49	−0.20	−0.78***	0.99***			
$A_{\max}$	0.12		0.51*	−0.60**	0.56*	0.39	−0.02	0.02			
E	−0.01	0.83***		−0.91*	0.86***	0.38	−0.60**	0.26			
WUE	0.30	−0.17	−0.68**		−0.92***	−0.52*	0.44	−0.21			
g_s	−0.10	0.84***	0.92***	−0.55		0.49	−0.52*	0.17			
F_v/F_m	−0.19	0.61**	0.39	−0.12	0.62*		0.04	−0.27			
DMC	0.56*	0.29	0.15	0.23	0.24	0.11		−0.71**			
STI	0.89***	0.42	0.33	0.11	0.17	0.03	0.53*				

DMY dry matter yield, $A_{\max}$ maximum rate of photosynthesis, E transpiration rate, g_s stomatal conductance, F_v/F_m ratio maximum quantum efficiency of PSII, DMC-dry matter content, STI stress tolerance index, Pro proline content, Fl flavonoids content, PA phenolic acid content

Significant correlation: *** ($p < 0.001$); ** ($p < 0.01$); * ($p < 0.05$)

and visible radiation (Bilger et al. 2001). *Lolium* species had a higher increase of these compounds than *Festuca* species, but the highest increase of phenolic acids was presented in *F. braunii*. Hura et al. (2007) and Sullivan and Teramura (1990) suggested that the protection of the photosynthetic apparatus during drought stress may additionally depend on the high concentration of phenolic compounds in the leaf tissue. Moreover, many authors confirmed the accumulation of phenolic compounds as an effect of water deficit: e.g. in spring *Triticosecale*, *M. sativa*, *T. aestivum* and *Festuca*, *Lolium*, *Festulolium* species (Hura et al. 2008; Kang et al. 2011; Gregorová et al. 2015; Fariaszewska et al. 2016) and it may be closely related to drought tolerance. Correlation analysis demonstrated that intensity of photosynthesis had a significant negative correlation with flavonoids, suggesting that the up-regulation of the shikimic acid pathway involved in production of secondary metabolites was under down-regulated photosynthesis (Jaafar et al. 2008). The synthesis of the basic skeleton of secondary metabolites is dependent on the carbon assimilated during photosynthesis (Asada 1994). The increase in production of secondary metabolites under low photosynthesis might be due to increased

shikimic acid pathway activity under stressed conditions, especially under water deficit and low photosynthesis (Jaafar et al. 2012).

NDVI, which is the normalised difference between a visible red reflectance and near infrared (NIR), has played an important role for satellite-based vegetation drought monitoring (Gu et al. 2007; Brown and de Beurs 2008). In our studies, the retention of the NDVI, exhibited the fluctuation of soil moisture and stress in tested forage grasses under drought stress (Table 5). Our results showed a relationship among NDVI and drought conditions, which confirms the results of Gu et al. (2007) and Gu et al. (2008) who estimated drought in grasslands in central United States. Generally, high NDVI values reflect greater vigour and photosynthesis process of vegetation canopy (Gao 1996). This was confirmed by our results especially in *F. arundinacea* varieties, which had a high photosynthesis and NDVI values under drought stress conditions in both years of the experiment.

Conclusions

Mild drought stress has a significant impact on the production and quality of forage grasses, hence breeding of more drought tolerant species and varieties of forage grasses is key in adaptation to future drought periods. Physiological parameters show that plants react within 2 weeks after the start of the drought stress. In general, the physiological parameters show that photosynthesis is reduced and this results clearly in a decreased dry matter yield due to drought. This is also true for the accumulation of biochemical parameters. However, the correlations between dry matter yield and the examined parameters are not always clear and straightforward. Also, the differential growth reaction of the different grass species towards drought is not clearly linked to a specific parameter or process. The rain-out shelter experiment confirms the results of the previously reported pot experiment (Fariaszewska et al. 2016) that *L. multiflorum* is less impacted by a drought period especially in the first mowing year after establishment. In contrast, the drought tolerance reaction of *F. arundinacea* in the second mowing year was not picked up by the pot experiment. This can be mainly attributed to root growth (in deeper soil layers), that could not be assessed in the pot experiment.

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