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CHANGES IN YIELD, LEAF AREA AND FLUORESCENCE CHLOROPHYLL PARAMETERS OF DIFFERENT FORAGE GRASSES CULTIVARS UNDER DROUGHT STRESS

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Abstract. The aim of the study was to assess the effects of water deficit on yield, leaf area and fluorescence chlorophyll parameters of forage grasses. The pot experiment was conducted in 2013 in IUNG-PIB's greenhouse in Puławy. Nine cultivars of five species (*Lolium perenne*, *Lolium multiflorum*, *Festuca pratensis*, *Festuca arundinacea* and *Festulolium braunii*) were investigated in well-watered conditions (70% field water capacity) as well as in a short-term drought stress conditions (40% field water capacity). The study showed, that dry matter of yield, chlorophyll fluorescence parameters: quantum of photosynthetic yield efficiency (*Fv/Fm* ratio) and performance index (*PI*) and leaf area were significantly lower in drought stress than under well-watered conditions in all cultivars in each regrowth. The data demonstrated the highest yield decrease for *F. arundunacea* cv. Barolex in the first, for *L. perenne* cv. Melluck and Meltador in the second and for *F. braunii* cv. Felopa in the third regrowth. The hybrid *F. braunii* was characterized by the lowest yield reduction and *PI* parameter in first and second regrowth. Based on the yield average, short drought resistance of different species was in the following order: *F. pratensis* > *F. braunii* – *L. multiflorum* > *F. arundinacea* > *L. perenne*. There was a significant relationship between dry matter yield and leaf area, and also between dry matter yield and fluorescence chlorophyll parameters.

Key words: *Festuca*, *Festulolium*, *Fv/Fm* ratio, *Lolium*, *PI* index, water deficit

INTRODUCTION

In recent years, drought has become an important threat to agriculture. Its most visible effect is the inhibition of the growth and development of plants, as well as

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lowering their yields. The shortage of water in the soil is caused by a lack of rainfalls, frequently accompanied by high air temperatures [Xu and Zhou 2006]. Plants growing in natural environments are often exposed to stress factors, which may cause adverse changes in their organisms. Water deficit in the soil can lead to serious disturbances in the course of physiological and metabolic processes in plant cells [Lipiec *et al.* 2013, Staniak 2013]. It is believed that stress factors, such as drought, first of all inhibit the intensity of photosynthesis by closing the stomata and reducing the availability and flow of CO_2 in the intercellular spaces [Kalaji and Łoboda 2010, Jones 1998, Lu and Zhang 1998, Hura *et al.* 2007]. The phenomenon of chlorophyll fluorescence is used to determine the efficiency of photosynthetic apparatus and the overall health of PSII [Baker and Rosenquist 2004]. Measurements of chlorophyll fluorescence parameters are a source of detailed information on the state of the photosynthetic apparatus and the damages within the chloroplasts. They also help to estimate the influence of environmental stresses on the growth and yield of plants [Maxwell and Johnson 2000]. Two parameters related to chlorophyll fluorescence: *PI* (Performace Index) and *Fv/Fm* are most commonly used as an indicators of susceptibility to drought stress. *PI* reflects the functioning of photosystem II and shows the efficiency of plants under stress conditions, while *Fv/Fm* parameter estimates a maximum quantum efficiency of PSII [Strasser *et al.* 2000, Force *et al.* 2003]. The evaluation of chlorophyll fluorescence, it may therefore be a useful tool to evaluate the energy and metabolic balance of photosynthesis and the level of yields of various plant species under conditions of water deficit [Araus *et al.* 1998].

The current state of knowledge on the physiological, metabolic and genetic response of forage grasses on environmental factors is insufficient. Finding out the responses of individual species and cultivars of forage grasses to adverse environmental factors and their possible adaptation and acclimatization to changing conditions may allow for the selection of more resistant cultivars and help in the cultivation of cultivars which have an increased adaptability and a higher tolerance to water shortages in the soil. The aim of the study was to compare the reactions of selected species and cultivars of forage grasses to a short-term stress associated with water shortage in the soil, by assessing the level of yield, leaf area and chlorophyll fluorescence parameters under optimal and limited moisture conditions.

MATERIAL AND METHODS

Two-factor pot experiment was performed in 2013 in a greenhouse of the Institute of Soil Science and Plant Cultivation – State Research Institute in Puławy (lubelskie voivodeship), in the completely randomized block method, with four replication. Nine cultivars of grasses, in different ploidy, were tested during experiment: *Lolium perenne* (L.) cv. Meltador (4n) and Melluck (2n), *Lolium multiflorum* (Lam.) cv. Meldiva (2n) and Melmia (4n), *Festuca pretenses* (L.) cv. Merifest (2n) and Merifest Tp (4n), *Festuca arundinacea* (Schreb.) cv. Barolex (6n) and Callina (6n), *Festulolium braunii* [(K. Richt.) A. Camus] cv. Felopa (4n). The seeds were sown in April in Mitcherlich pots filled 9 kg of a grey-brown podsolic soil from arable layer (0-30 cm). The contents of available nutrients (mg per 100 g soil) were: phosphorus 37.7, potassium 17.5 and magnesium 2.2. Soil pH_{KCl} was 7.6. The pots were fertilized at doses ($g \cdot pot^{-1}$): 3.6 N in three rates, 1.0 P, 1.5 K, 0.5 Mg in the form of solutions: NH_4NO_3, KH_2PO_4, K_2SO_4 and $MgSO_4$. Grasses were evaluated at two levels of soil moisture: 70% FWC (optimum soil moisture) and 40% FWC (drought stress) in four water regimes: 1) control: 70% FWC, 2) drought at first (spring) regrowth: plants were grown in optimal conditions 70% FWC and three weeks before defoliation the moisture level of soil was reduced to 40% FWC, 3) drought at second (summer) regrowth: plants were grown in optimal conditions 70% FWC in first regrowth and three weeks before defoliation in second regrowth the moisture level of soil was reduced to 40% FWC, 4) drought at third (autumn) regrowth: plants were grown in optimal conditions 70% FWC in first and second regrowth and three weeks before defoliation in third regrowth the moisture level of soil was reduced to 40% FWC. All tested grasses were cut after 5-6 weeks of regrowth, depending on their growth rate.

In order to maintain the appropriate soil moisture, water losses were made up on a daily basis, to achieve a specified weight of the pot with soil. All tested plants were defoliated after 3 weeks of drought stress. Dry matter yield of grasses, leaf area and parameters of chlorophyll fluorescence were evaluated. Chlorophyll fluorescence parameters (*Fv/Fm* ratio, *PI*) were measured using Pocket PEA ultra-portable chlorophyll fluorimeter (Hansatech Instruments, Norfolk, USA), after 2 weeks of water stress. Five fully developed leaves of grasses from each pot, under drought stress and optimal soil moisture, were selected to measure the parameters. All measurements were performed after 20 minutes of dark adaptation period. Leaf area (the total leaf area of all leaves on the plant) was measured using LI-3000C Portable Leaf Area Meter (LI-COR) just before each cut after three week of stress. Five plants from each pot under drought stress and well-watered conditions were selected. Leaf area of plant was integrated and calculated using Windows interface software according the formula:

LEAF AREA (cm^2) = LENGTH OF LEAF (cm) × WIDTH OF LEAF (cm)

Data collected during the pot experiment of this study were statistically analyzed using Multifactor AVOVA analysis of variance technique and Tukey’s test on 0.05 significant level using STATISTICA 10.0 and StatGraphic Centurion XVI.

RESULTS

A short-term water stress was an important factor which determines the height of dry matter yield of the species and cultivars of forage grasses. Under optimum soil moisture, the best yields were obtained from: in spring regrowth – *L. multiflorum* cultivars Melmia and Meldiva and *L. perenne* cv. Meltador (Fig. 1), in summer regrowth – *L. perenne* cv. Meltador (Fig. 2), while in autumn regrowth – *F. arundinacea* cv. Callina, *F. braunii* cv. Felopa and *F. pratensis* cv. Merifest Tp (Fig. 3). All the compared cultivars of grasses responded to the shortage of water in the soil with a generally significant decrease in dry matter yield, by an average of 17.4% in the first, 23.1% – in the second and 33.9% – in the third regrowth. The largest decrease in yield under stress conditions was found: in the spring regrowth in *F. arundinacea* cv. Barolex (by 33.5%), in the summer regrowth – *L. perenne* cv. Melluck (by 46.0%) i Meltador (by 42.1%) and in the autumn regrowth – *F. braunii* cv. Felopa (by 53.1%) and *L. perenne* Meltador (by 51.2%). The weakest reaction to a short-term drought stress was recorded for *F. braunii* cv. Felopa (2.9%) and *F. pratensis* cv. Merifest Tp (4.3%) in first regrowth, and for *F. braunii* cv. Felopa (5.6%) in the second regrowth (differences statistically insignificant).

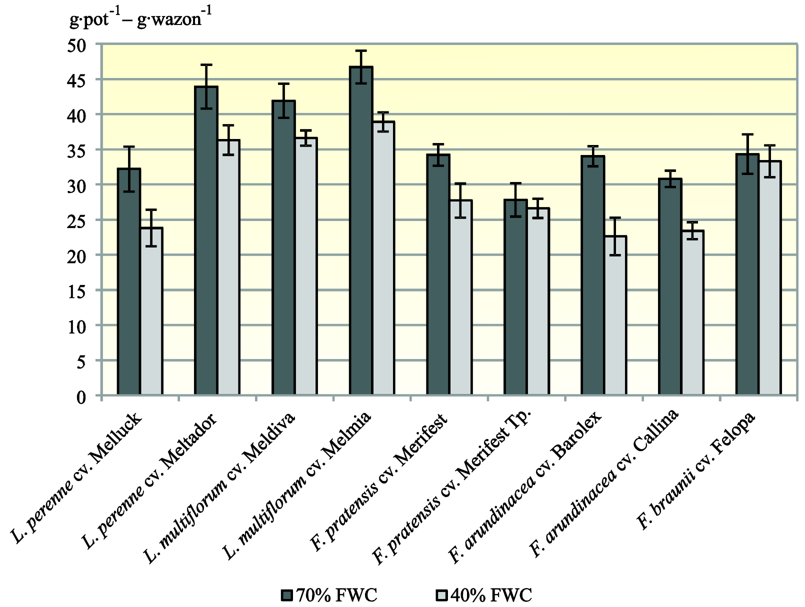

Fig. 1. Dry matter yield of grass cultivars (± standard deviation) under optimal (70% FWC) and limited (40% FWC) soil moisture in the first regrowth

Rys. 1. Plon suchej masy odmian traw (± odchylenie standardowe) w optymalnych (70% ppw) i ograniczonych (40% ppw) warunkach wilgotności gleby w pierwszym odroście

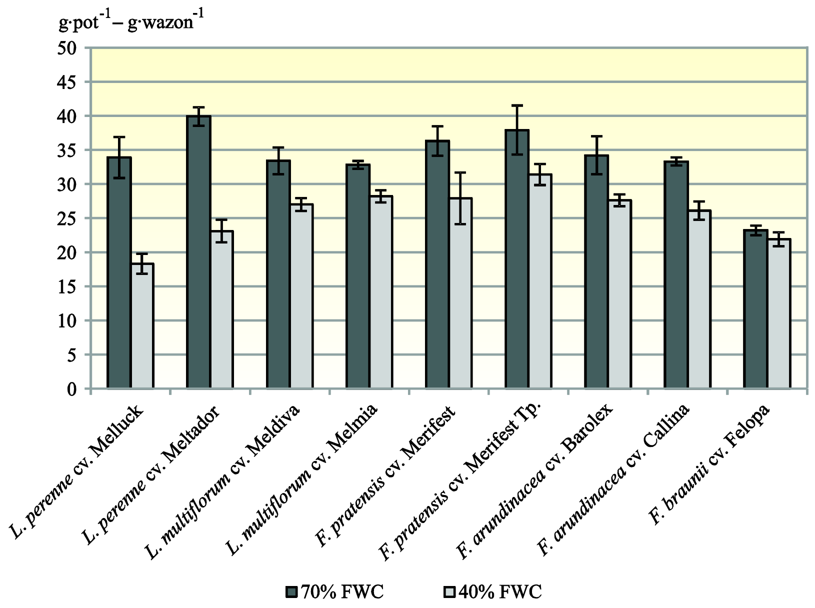

Fig. 2. Dry matter yield of grass cultivars (± standard deviation) under optimal (70% FWC) and limited (40% FWC) soil moisture in the second regrowth

Rys. 2. Plon suchej masy odmian traw (± odchylenie standardowe) w optymalnych (70% ppw) i ograniczonych (40% ppw) warunkach wilgotności gleby w drugim odroście

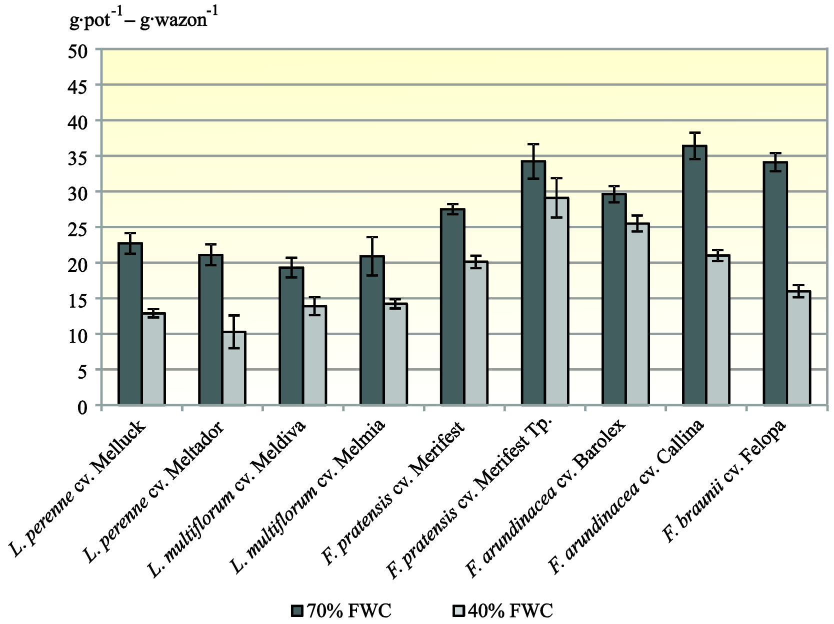

Fig. 3. Dry matter yield of grass cultivars (± standard deviation) under optimal (70% FWC) and limited (40% FWC) soil moisture in the third regrowth

Rys. 3. Plon suchej masy odmian traw (± odchylenie standardowe) w optymalnych (70% ppw) i ograniczonych (40% ppw) warunkach wilgotności gleby w trzecim odroście

The diversity of soil moisture significantly affected the total leaf area of all tested species and cultivars of grasses (Table 1, 3).

Table 1. Total leaf area (cm^2) of grass cultivars under optimal (70% FWC) and limited (40% FWC) soil moisture

Tabela 1. Całkowita powierzchnia liściowa (cm^2) odmian traw w optymalnych (70% ppw) i ograniczonych (40% ppw) warunkach wilgotności gleby

Cultivar of grass Odmiana trawy	Cut I – Pokos I		Cut II – Pokos II		Cut III – Pokos III	
	C[1]	S[1]	C	S	C	S
L. perenne cv. Melluck	36.57 b	28.12 a	18.51 bc	14.91a	19.20 b	14.75 a
L. perenne cv. Meltador	42.38 c	33.47 b	25.92 ef	18.50 bc	23.23 d	16.40 ab
L. multiflorum cv. Meldiva	71.34 i	36.37 b	31.18 g	17.38 b	39.25 h	25.44 d
L. multiflorum cv. Melmia	77.89 j	48.70 d	39.45 k	27.32 f	58.70 jk	34.40 fg
F. pratensis cv. Merifest	79.92 j	62.32 g	52.33 l	40.27 k	57.39 j	40.28 h
F. pratensis cv. Merifest Tp.	87.91 k	72.10 i	70.77 n	36.42 j	66.62 l	48.47 i
F. arundinacea cv. Barolex	84.02 k	59.20 fg	64.41 m	33.96 i	61.35 k	37.54 gh
F. arundinacea cv. Callina	86.50 k	54.67 e	65.13 m	35.47 ij	74.97 m	38.59 h
F. braunii cv. Felopa	66.67 h	58.56 f	33.82 hi	21.33 d	32.5 ef	22.66 cd
Average – Średnia	70.36 b	50.39 a	44.6 b	27.28 a	48.14 b	30.95 a

C[1] – 70% FWC – 70% ppw; S[1] – 40% FWC – 40% ppw
values mark the same letter did not differ significantly (P = 0.05) – wartości oznaczone takimi samymi literami nie różnią sie istotnie (P = 0,05)

Under the conditions of optimal hydration, the larger leaf area was recorded for both cultivars of *F. arundinacea* and *F. pratensis* cv. Merifest Tp, while under the conditions of reduced soil moisture – both cultivars of *F. pratensis*. The largest decrease in the total leaf area was observed with *F. arundinacea* cv. Callina (on average by 43.2%) and with *L. multiflorum* cv. Meldiva (by 44.1%). The leaf area decreased the least in the cultivars of *L. perenne* cv. Melluck and Meltador (respectively by 22.2 and 26.3%) and *F. braunii* cv. Felopa (by 22.9%). The biggest decrease of the total leaf area was recorded in the second regrowth (on average by 36.0%). The regression analysis showed a quite high, significant correlation between the yield of dry matter and the total leaf area, as indicated by a high determination coefficient $R^2 = 0.44$ (Fig. 4).

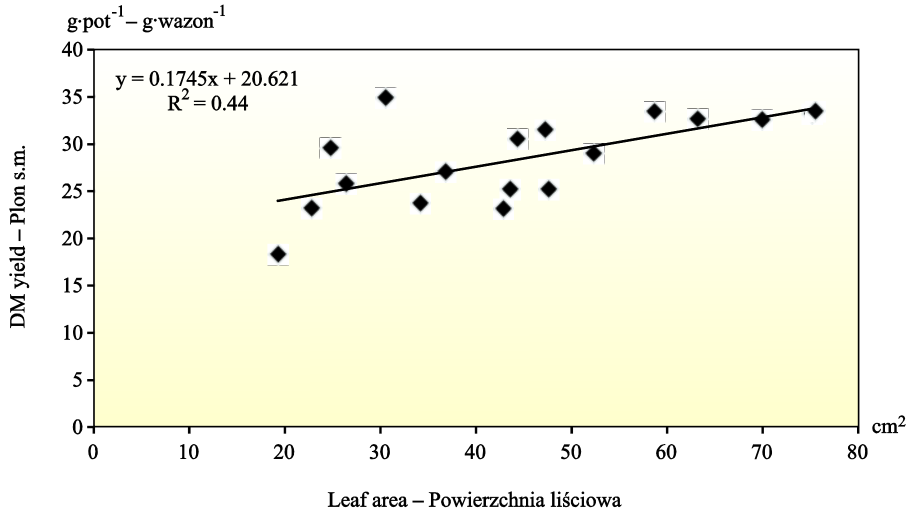

Fig. 4. Relationship between total leaf area and dry matter yield of grasses
Rys. 4. Zależność między całkowitą powierzchnią liściową a plonem suchej masy traw

The studies showed that stress caused by the shortage of water in the soil generally significantly affected the decrease in chlorophyll fluorescence parameters: *Fv/Fm* and *PI* (Table 2, 3). The *Fv/Fm* ratio decreased in the first regrowth by an average of 1.13%, in the second by 2.87%, and in the third by 0.74%. In all three regrowth, the most significant decrease of efficiency of photosystem II were observed with *F. pratensis* cv. Merifest, and they were respectively 1.38, 5.76 and 2.06%. The overall vitality of photosystem II in the tested grasses, which was determined by the parameter *PI* significantly decreased under drought stress compared to the conditions of optimal hydration. In the first regrowth, it decreased on average by 14.9%, in the second – by 28.0%, and in the third – by 20.4%. Similarly as in the case of *Fv/Fm* indicator, the highest decrease of PSII vitality was recorded for *F. pratensis* cv. Merifest (on average by 32.5%), and the lowest for *F. braunii* cv. Felopa (on average by 4.0%). Throughout the growing season, a hybrid *F. braunii* had the best photosynthetic performance index, which is confirmed by minimal decreases of *PI* parameter in the conditions of water deficit (by 3.4% in the first, 3.1% in the second, and 5.5% in the third regrowth). A high determination coefficient $R^2 = 0.68$ indicates a high dependency of the yields of grasses on *PI* parameter and $R^2 = 0.37$ on *Fv/Fm* parameter (Fig. 5, 6).

Table 2. Fluorescence chlorophyll parameters of grass cultivars under optimal (70% FWC) and limited (40% FWC) soil moisture
Tabela 2. Parametry fluorescencji chlorofilu u odmian traw w optymalnych (70% ppw) i ograniczonych (40% ppw) warunkach wilgotności gleby

Cultivar of grass Odmiana trawy	*Fv/Fm*						*PI*					
	cut I pokos I		cut II pokos II		cut III pokos III		cut I pokos I		cut II pokos II		cut III pokos III	
	C[1]	S[1]	C	S	C	S	C	S	C	S	C	S
L. perenne cv. Melluck	0.798	0.781	0.803	0.785	0.808	0.794	2.676	1.634	2.068	1.564	3.266	2.917
L. perenne cv. Meltador	0.798	0.794	0.803	0.795	0.808	0.805	2.580	2.090	2.091	1.914	3.387	2.579
L. multiflorum cv. Meldiva	0.800	0.791	0.805	0.764	0.810	0.805	2.604	2.474	2.411	1.687	2.433	2.243
L. multiflorum cv. Melmia	0.805	0.795	0.804	0.784	0.812	0.811	2.704	2.194	2663	2.042	3.390	2.581
F. pratensis cv. Merifest	0.796	0.785	0.799	0.753	0.825	0.808	2.501	2.203	2.808	1.084	4.626	3.513
F. pratensis cv. Merifest Tp.	0.798	0.797	0.806	0.758	0.811	0.804	2.564	2.355	2.309	1.258	4.741	3.637
F. arundinacea cv. Barolex	0.796	0.784	0.779	0.774	0.811	0.802	2.046	1.740	2.220	1.895	4.196	3.350
F. arundinacea cv. Callina	0.796	0.785	0.790	0.773	0.807	0.806	2.232	1.962	2.364	1.575	3.757	2.465
$LSD_{0.05}$ – $NIR_{0,05}$	0.005		0.007		0.004		0.222		0.494		0.332	

C[1] – 70% FWC – 70% ppw
S[1] – 40% FWC – 40% ppw

Table 3. Multifactor Anova testing for dry matter yield, leaf area and chlorophyll fluorescence parameters of grasses under optimal (70% FWC) and limited (40% FWC) soil moisture
Tabela 3. Wieloczynnikowa analiza wariancji dla plonu suchej masy, powierzchni liściowej i parametrów fluorescencji chlorofilu traw w optymalnych (70% ppw) i ograniczonych (40% ppw) warunkach wilgotności gleby

	Trait – Cecha	DMY	LA	*Fv/Fm*	*PI*
Cut I Pokos I	cultivar – odmiana (C)	***	***	***	***
	soil moisture – wilgotność gleby (M)	***	***	***	**
	C × M	*	***	**	***
Cut II Pokos II	cultivar – odmiana (C)	***	***	***	*
	soil moisture – wilgotność gleby (M)	***	***	***	***
	C × M	***	***	***	**
Cut III Pokos III	cultivar – odmiana (C)	***	***	***	***
	soil moisture – wilgotność gleby (M)	***	***	***	***
	C × M	***	***	**	***

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$
DMY: dry matter yield – plon suchej masy (N = 4), LA: leaf area – powierzchnia liściowa (N = 4), *Fv/Fm* (N = 20), *PI* (N=20)

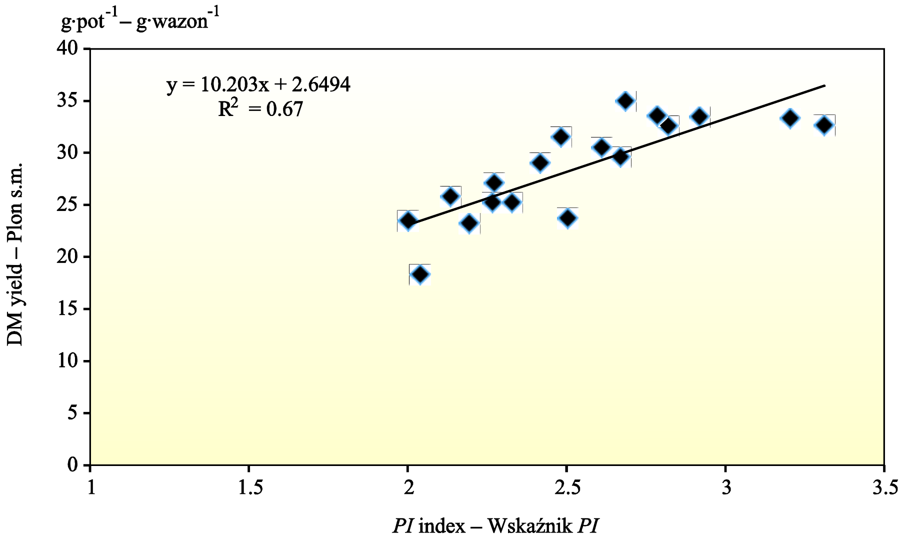

Fig. 5. Relationship between *PI* index and dry matter yield of grasses
Rys. 5. Zależność między wskaźnikiem *PI* a plonem suchej masy traw

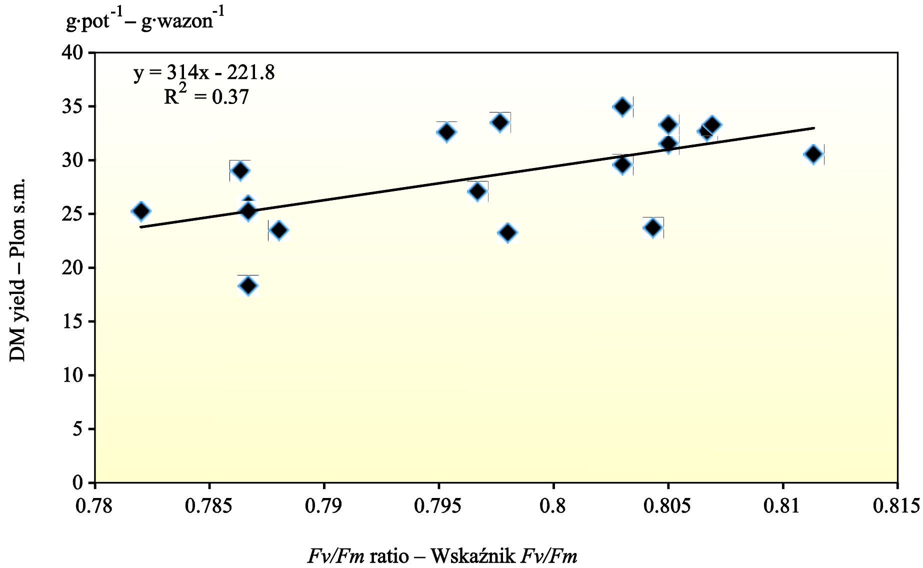

Fig. 6. Relationship between *Fv/Fm* ratio and dry matter yield of grasses
Rys. 6. Zależność miedzy wskaźnikiem *Fv/Fm* a plonem suchej masy traw

DISCUSSION

Forage grasses respond to water shortages in the soil with the reduction of yields, due to the inhibition of growth and development processes of plants. In such conditions, there is a decrease in the intensity of photosynthesis and there are a disruption in the transport of assimilates from the leaf blades to other organs [Lu and Zhang 1998, Starck 2010]. The results of own studies confirmed that *L. multiflorum*, *L. perenne*, *F. arundinacea*, *F. pratensis* and *F. braunii* react to a short-term stress connected with water deficits with a significant reduction of yields in each of the three regrowth. The lowest reduction of dry matter yield was recorded in the first, higher in the second, and the highest in the third regrowth. The highest reduction of yield in third regrowth could be related to the age of grasses. The older plants are more sensitive to drought, but it may be also the result of higher temperatures during summer and beginning of autumn period then in spring (first regrowth). Using the level of average yield decrease as the criterion of the resistance to stress allowed to draw the conclusion that tetraploid *F. pratensis* cv. Merifest Tp was the most resistant to water deficits in the soil, while *L. perenne* cv. Melluck and Meltador – the least. Significant reduction in the yields of certain fodder grasses (*F. pratensis, L. perenne*) under drought stress was recorded in the studies of Olszewska *et al.* [2010]. Also, Ebrahimiyan *et al.* [2013] demonstrated a reduction in the yield of the dry matter of *F. arundinacea* in conditions of limited moisture. A diverse reaction of species and cultivars of fodder grasses to the changes in soil moisture results from their genetic conditioning associated with the tolerance to stress [Dziadczyk 2002].

The reduction of soil moisture did not initially cause any visible morphological changes, but a greater severity of drought caused visible changes, such as curling of leaves, drying out of plants, and their weaker growth [Zagdańska 1992, Chaves *et al.* 2002, Chevea and Oliveira 2004]. These changes are intended to reduce the leaf area and limit the process of transpiration. The results of own studies showed a reduction in the leaf area in all tested species and cultivars of grasses in the conditions of drought. The largest reduction was recorded in *F. arundinacea* cv. Callina and *L. multiflorum* cv. Meldiva. According to Wilman *et al*. [1998], grasses which better manage water under the conditions of reduced moisture limit the development of their leaf blades and shoots.

Grasses reacted to drought stress with the reduction of the leaf area combined with the decline in the efficiency of the photosynthetic apparatus [Havaux 1992, Huang *et al.* 1998, Kościelniak *et al.* 2006]. In recent years, the measurements of chlorophyll fluorescence have become a tool for the selection of the cultivars of fodder plants which are resistant to environmental stresses [Sayed 2003]. According to Clark *et al.* [2000], chlorophyll fluorescence allows for the assessment of the health and integrity of the photosynthetic apparatus during the process of CO_2 assimilation. So far, there has been little literature on the impact of water deficit on chlorophyll fluorescence indicators in different species of fodder grasses. Own studies showed that all the tested grasses reacted to reduced soil moisture with the reduction of *Fv/Fm* and *PI* parameters. It was confirmed by the results of Huang *et al.* [1998], who also recorded the reduction in both parameters describing chlorophyll fluorescence under drought stress at *F. pratensis*. In turn, Yu and Jian [2013] recorded the decrease in the quantum efficiency of photosystem II in different hybrids of *Festulolium* and *L. perenne* under drought conditions. Numerous authors confirmed that the *PI* index is a sensitive parameter that differentiates plants during different environmental conditions [Strasser *et al.* 2000].

CONCLUSIONS

1. The study showed that *L. multiflorum*, *L. perenne*, *F. arundinacea*, *F. pratensis* and *F. braunii* react to a short-term stress connected with water deficits in the soil with a significant reduction of yields in each of the three regrowth. The most resistant to the drought stress was tetraploid *F. pratensis* cv. Merifest Tp, while the least – *L. perenne* cv. Melluck and Meltador.

2. The diversity of soil moisture significantly affected the leaf area of all tested species and cultivars of grasses. The largest decrease in the leaf area in condition of water shortage was observed with *F. arundinacea* cv. Callina and *L. multiflorum* cv. Meldiva.

3. Stress caused by the shortage of water in the soil generally significantly affected the decrease in chlorophyll fluorescence parameters: *Fv/Fm* and *PI.* In all three regrowth, the most significant decreases of both parameters were observed with *F. pratensis* cv. Merifest.

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ZMIANY W PLONACH, POWIERZCHNI LIŚCIOWEJ ORAZ WSKAŹNIKACH FLUORESCENCJI CHLOROFILU U RÓŻNYCH ODMIAN TRAW PASTEWNYCH W WARUNKACH STRESU SUSZY

Streszczenie. Doświadczenie wazonowe przeprowadzono w 2013 roku w hali wegetacyjnej IUNG-PIB w Puławach. Celem badań była ocena wpływu niedoboru wody w glebie na plonowanie, wielkość powierzchni liściowej oraz wskaźniki fluorescencji chlorofilu u wybranych traw pastewnych. Dziewięć odmian traw należących do pięciu gatunków (*Lolium perenne*, *Lolium multiflorum*, *Festuca pratensis*, *Festuca arundinacea* i *Festulolium braunii*) testowano w optymalnych warunkach wilgotnościowych (70% ppw) i podczas krótkotrwałego stresu suszy (40% ppw). Badania wykazały, że wskaźniki fluorescencji chlorofilu: maksymalna wydajność kwantowa fotosystemu II (*Fv/Fm*) i wskaźnik witalności (*PI*), a także plon suchej masy i całkowita powierzchnia liściowa wszystkich badanych traw były istotnie niższe w warunkach stresu suszy niż przy optymalnym uwilgotnieniu gleby. Największy spadek plonu suchej masy zanotowano: w pierwszym odroście u *F. arundunacea* odm. Barolex, w drugim – u *L. perenne* odm. Melluck i Meltador, zaś w trzecim – u *F. braunii* odm. Felopa. Mieszaniec *F. braunii* charakteryzował się największą redukcją plonu suchej masy oraz najniższą wartością wskaźnika *PI* w pierwszym i drugim odroście. Na podstawie średniej straty plonów w warunkach stresu badane gatunki traw uszeregowano pod względem odporności na krótkotrwałą suszę w następującej kolejności: *F. pratensis* > *F. braunii – L. multiflorum* > *F. arundinacea* > *L. perenne*. Stwierdzono istotną zależność pomiędzy plonem suchej masy i powierzchnią liściową oraz pomiędzy plonem a wskaźnikami fluorescencji chlorofilu.

Słowa kluczowe: *Festuca*, *Festulolium*, indeks *PI*, *Lolium*, niedobór wody w glebie, wskaźnik *Fv/Fm*

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