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THE IMPACT OF DIFFERENT WATER REGIME ON CHLOROPHYLL FLUORESCENCE OF *PYRUS PYRASTER* L. AND *SORBUS DOMESTICA* L.

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Abstract

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The water deficit is considered to be significant cause of photosynthesis defects. Measuring of chlorophyll fluorescence is one of the methods revealing defects in the photosynthetic apparatus. The experiment was established with two woody plant (*Pyrus pyraster* L. and *Sorbus domestica* L.) cultivated in two different regimes of the substrate saturation. The measurement of the modulated fluorescence of chlorophyll *a* was done by FMS1 fluorometer during three-week period between June and September (2012 and 2013). There were analysed selected parameters of chlorophyll fluorescence: F_v/F_m – maximum quantum efficiency of PSII, Φ_{PSII} – effective quantum yield of PSII and R_{FD} – chlorophyll fluorescence decrease ratio. According to the obtained results, *Pyrus pyraster* has probably higher potential for adaptation to water deficiency. There were recorded the significant decreases mainly in the values of parameter R_{FD} and Φ_{PSII} for *Sorbus domestica* within duration of experiment with different water regime in both growing seasons 2012 and 2013. The results document a weak sensitivity of the parameter F_v/F_m on changes in the amount of available water in the substrate in both taxa.

Keywords: chlorophyll fluorescence, woody plants, drought, water deficit, xerophytic plants

INTRODUCTION

Recently there is high demand for search and use of the drought-adapted plants (species and cultivars), that would be planted in extreme conditions of the cultural landscape or urban environment. Water deficiency in the soil is one of the most significant stress-factors for the plants, which becomes apparent on physiological and morphological level. There are various methods for assessment of the plant responses to water deficiency. A measurement of the chlorophyll fluorescence is non-destructive method for screening of the plant response to stress factors, because activity of the photosynthetic apparatus can be significantly influenced by the stress factors. In the paper is presented study of the impact of water deficiency on chlorophyll fluorescence of *Pyrus pyraster* and *Sorbus domestica*. Both are light demanding tree species that grow also on stands influenced by water deficiency during the growing season (Brutsch and Rotach, 1993; Rittershoffer, 1998; Wilhelm, 1998; Paganová, 2003, 2008; Paganová and Jureková, 2011). *Sorbus domestica* prefers stands at lower altitudes with warm and rather arid climate (Paganová and Jureková, 2011). *Pyrus pyraster* has the wider ecological amplitude and occurs also at higher altitudes. In Slovakia *Pyrus pyraster* grows on stands with significantly different water regime – on regularly flooded locations, as well as on stands with negative water balance (Paganová and Jureková, 2011). According to our former research (Paganová and Jureková, 2011, 2012), adaptability of the studied tree species to water deficiency is different. *Pyrus pyraster* maintained balanced values of dry matter in both variants of water regime (Control and Stress). *Sorbus domestica* has lower potential for adaptability to water deficiency. Under conditions of the lower water content in the substrate (40% of full water capacity) significantly reduced the total dry matter in organs (Paganová *et al*., 2014).

Several parameters that characterize the performance of the plant photosynthesis (F_v/F_m – maximum quantum efficiency of PSII, Φ_{PSII} – effective quantum yield of PSII and R_{FD} – chlorophyll fluorescence decrease ratio) were selected for evaluation of water stress impact on model plants. The maximum quantum efficiency of PSII (F_v/F_m) represents effectiveness of light utilization under standard conditions of CO_2 fixation and the quantum yield of photochemical processes (Björkman and Demming, 1987). F_v/F_m is considered as the main marker of a plant's response to stress (Björkman and Demming, 1987; Váňová and Kummerová, 2006; Tomeková, 2010) and is the most important trait used for assessment of the photosynthetic status of plants in ecophysiological research (Čáňová *et al.,* 2012). Lichtenthaler (2000) considered that R_{FD} is a crucial parameter in reaction to environmental changes and also it is the „index of vitality" of the photosynthetic apparatus. Φ_{PSII} – the effective quantum yield of PSII represents a real yield of active reaction center of photosystem II when processing the absorbed solar energy (Schreiber, 2004).

The lack of water may adversely affect the photosynthetic activity of studied tree species (*Pyrus pyraster* and *Sorbus domestica*). Within quantitative evaluation of the experimental data the significant differences in variants with different levels of the substrate saturation were tested.

MATERIAL AND METHODS

The two-years old seedlings of *Pyrus pyraster* L. and *Sorbus domestica* L. were planted in 2 liter's size pots (in April) with the substrate Klasmann TS3 Standard, substrate based on the withe peat, enriched with the clay (20 $kg.m^{-3}$; pH 5.5–6.5 + fertilized NPK 14:10:18) under the polypropylene cover with 40% shading with natural daylight lighting. Any additional lighting or special light regimes were not used under covering.

Plants were regularly watered for one month (necessary for rooting) on 60% the full substrate saturation. After this time plants were divided to two variants with different substrate saturation. Control variant was maintained at 60% of the full water capacity and stress variant at 40% of the full water capacity. The water content in the substrate was determined by gravimetric method. Plants were irrigated to the required weight every two days. Experimental plants were grown in different irrigation regime to the half of September for 151 days in the year 2012 and for 154 days in the year 2013. Three plants were assessed in the each variant of the substrate saturation (3 plants in the control and 3 plants in the stress). There were used plants of the same origin, with the similar growth parameters. The plants came from half-sib progeny. Three leaves were labeled for measurement on each experimental plant (one from the lowest part, one from the middle and one from the top part of the plant) Chlorophyll fluorescence was carried out on the same labeled leaves in the morning hours by the fluorometer HANSATECH FMS 1 and for data analysis was used software MODFLUOR. The 21 days period of measurements was set for each taxon in both years. The plants were adapted for 30 minutes in the dark before measurement of chlorophyll fluorescence. The following characteristics of measurement protocol of the chlorophyll fluorescence were used: one second light pulses of red light with an intensity of 895 µmol. $m^{-2}.s^{-1}$, the intensity of actinic light 34 µmol. $m^{-2}.s^{-1}$ and the saturation light pulse 10 000 µmol. $m^{-2}.s^{-1}$. The following chlorophyll fluorescence parameters were measured: F_v/F_m – maximum quantum efficiency of PSII, Φ_{PSII} – effective quantum yield of PSII and R_{fd} – chlorophyll fluorescence decrease ratio (calculated as $R_{FD} = F_{d/}F_s$, were $F_d = F_m - F_s$), (Hlízová, 2008), where F_d is the fluorescence decrease, F_s is the steady state fluorescence and F_m is the maximal fluorescence. In both growing seasons there were used the same methods for plants treatments and measuring as well. There was tested whether the significant changes of the chlorophyll fluorescence will be found within duration of the experiment under different water regime for analyzed tree species. The null hypothesis state that the values of the parameters of chlorophyll fluorescence do not differ during the experiment. According to the course of experimental data recorded within the time series, three periods were selected for further analysis (1st, 42nd and 84th day of the duration of experiment) with significant deviations of the measured parameters. In mathematical and statistical analysis of data was used one-way ANOVA and Tukey's-test, $P < 0.05$. The quantitative data for all studied parameters have a normal distribution (Shapiro Wilk's test at significance level $\alpha = 0.001$) and meet the assumption of homogeneity (Levene's test at significance level $\alpha = 0.05$). A statistical assessment of the data was conducted using software Statgraphics Centurion XV (StatPoint Technologies, USA, XV (license number: 7805000000722).

RESULTS AND DISCUSSION

Based on the results of analysis of variance was adopted null hypothesis for almost all evaluated parameters of chlorophyll fluorescence for *Pyrus pyraster* in 2012 in variants with different levels of saturation of the substrate. The hypothesis of equality of mean values was rejected for the parameter maximum photochemical efficiency of photosystem PSII (F_v/F_m) for *Pyrus pyraster* in the control treatment. For *Sorbus domestica* null hypothesis was accepted only for the parameter F_v/F_m – maximal photochemical efficiency of photosystem PSII and in both variants. The hypothesis of equality of mean values for the parameters was rejected for R_{FD} – chlorophyll fluorescence decrease ratio and Φ_{PSII} – effective quantum yield of PSII in

I: *The maximum temperatures in the years 2012 and 2013 during the experiment*

	Month					
2012	**IV.**	**V.**	**VI.**	**VII.**	**VIII.**	**IX.**
Maximum temperature [°C]	30.1	31.40	34.60	36.00	35.10	31.10
	Month					
2013	**IV.**	**V.**	**VI.**	**VII.**	**VIII.**	**IX.**
Maximum temperature [°C]	28.06	27.57	33.71	35.93	37.96	26.98

Source: Wheather station, Department of Biometeorology and Hydrology, Horticulture and Landscape Engineering Faculty, Slovak University of Agriculture in Nitra

II: *The mean values of the analyzed parameters of chlorophyll fluorescence and 95% Tukey's-test for studied taxa (Pyrus pyraster/Sorbus domestica) and for two variants of the substrate saturation (Control/Stress). Values with the same letter are not significantly different*

2012	***Pyrus pyraster***						***Sorbus domestica***					
Duration of experiment	R_{FD}		F_v/F_m		Φ_{PSII}		R_{FD}		F_v/F_m		Φ_{PSII}	
	control	**stress**	**control**	**stress**	**control**	**stress**	**control**	**stress**	**control**	**stress**	**control**	**stress**
1st day	1.70 a	1.38 a	0.84 a	0.82 a	0.32 a	0.23 a	1.55 a	2.05 a	0.82 a	0.83 a	0.22 b	0.34 a
42nd day	1.66 a	1.53 a	0.85 a	0.84 a	0.29 a	0.23 a	1.88 b	1.88 ab	0.83 a	0.84 a	0.34 a	0.24 b
84th day	1.88 a	1.29 a	0.83 b	0.84 a	0.34 a	0.21 a	1.43 a	1.71 b	0.84 a	0.83 a	0.10 c	0.09 c
2013	***Pyrus pyraster***						***Sorbus domestica***					
Duration of experiment	R_{FD}		F_v/F_m		Φ_{PSII}		R_{FD}		F_v/F_m		Φ_{PSII}	
	control	**stress**	**control**	**stress**	**control**	**stress**	**control**	**stress**	**control**	**stress**	**control**	**stress**
1st day	1.80 a	2.08 a	0.80 a	0.85 a	0.20 a	0.32 a	1.98 a	2.01 a	0.82 a	0.81 a	0,18 a	0.29 c
42nd day	1.11 b	1.58 b	0.81 a	0.84 a	0.15 b	0.12 b	1.65 ab	1.43 b	0.83 a	0.81 a	0,20 a	0.23 b
84th day	1.19 b	1.41 b	0.84 a	0.84 a	0.13 b	0.13 b	1.35 b	1.14 b	0.83 a	0.83 a	0,17 a	0.12 a

both variants of the water regime. In the year 2013 the null hypothesis is accepted only for parameter F_v/F_m and the parameter Φ_{PSII} of *Sorbus domestica* in control variant. The null hypothesis is accepted only in parameter F_v/F_m in both variants of *Pyrus pyraster*.

The data show that during the growing seasons of 2012 and 2013 in both taxa are significant differences in chlorophyll fluorescence, depending on the level of the supply water in the substrate. These differences document the multiple comparisons of means, where Tukey's test was used (Tab. II).

The average values of maximum photochemical efficiency for *Pyrus pyraster* ranged 0.80–0.85 and for *Sorbus domestica* in the range 0.82–0.84 (Tab. I). This parameter is sensitive indicator of the photosynthetic performance with optimal values of around 0.83 for most plant species (Bjorkman and Deming, 1987; Johnson *et al*., 1993; Kalaji *et al*., 2012). In the dark-adapted plants, values of this parameter vary in the range 0.75–0.85 and the influence of stress factors reduces the value of this parameter (Hlízová, 2008). In relation to data of other authors, values of a maximum photochemical efficiency observed for both species in different water regime did not decrease below the significantly declared optimum. According to Percival *et al.* (2006) in young trees of drought tolerant species F_v/F_m values did not decreased despite 2 week cessation of watering, whilst in young trees of drought susceptible cultivars F_v/F_m values decreased up to 60% of the initial value of 0.77–0.80. The regulated water regime effect and lower water-supply have been not demonstrated significantly to the average values of this parameter for *Pyrus pyraster* and *Sorbus domestica* (Tab. II). The results document a weak sensitivity of parameter F_v/F_m to changes in the amount of available water in the substrate in both taxa.

Significant changes of R_{FD} (chlorophyll fluorescence decrease ratio) due to the different water regime in the year 2012 were recorded only for *Sorbus domestica* in both variants of the substrate saturation. In the control variant demonstrated a decrease of the value by 7% from 1.55 to 1.43 and in the stress variant by16.5% from 2.05 to 1.71. In the year 2013 a significant decrease in this parameter were observed after 84 days of treatment with different water regime in both taxa. For *Pyrus pyraster* value of R_{FD} in the control variant decreased by 33.8% from 1.80 to 1.19 and in the stress variant reduction of this parameter was by 32% from 2.08 to 1.41. For *Sorbus domestica* was more pronounced reduction in the value of R_{FD} in stress variant from 2.01 to 1.14 (by 43%) compared to the control variant, where the reduction of this parameter during the experiment was also evident (by 31.8%) from 1.98 to 1.35. Duration of different water regime significantly influenced values of the chlorophyll fluorescence decrease ratio (R_{FD}). The decrease of the R_{FD} values for *Pyrus pyraster* and *Sorbus domestica* is considered to be a response to long-term (84-day) duration of the reduced water content in the substrate.

Chlorophyll fluorescence decrease ratio (R_{FD}) is considered an index of plant vitality and decrease its value is expressed in response to various suboptimal conditions (Hlízová, 2008).

R_{FD} values are higher in sunny leaves (3–5.5 for a fully functional sunny leaves in direct light) than shaded leaves (1.2–2.7 for a fully functional green leaves in the shade), reflecting their higher photosynthetic activity (Lichtenthaler *et al.,* 2005). For *Pyrus pyraster* and *Sorbus domestica* average values of R_{FD} range between 1.11 and 2.08 (Tab. II) which correspond to shady leaves. During experiment the plants were placed under the polypropylene cover with shading at 40% of full light. Their leaves were therefore not exposed to direct illumination.

The quantum yield of Photosystem II photochemistry (Φ_{PSII}) measures the proportion of the light absorbed by chlorophyll associated with PSII and used in photochemistry (Genty *et al*., 1989). This provides a measure of the rate of linear electron transport which is an indication of the overall photosynthesis. Strong linear relationship has been observed between Φ_{PSII} and the efficiency of carbon fixation under laboratory conditions but some discrepancies between this parameter and the efficiency of carbon fixation may occur under stress conditions (Maxwell and Johnson, 2000).

In the year 2012 a significant changes of values of Φ_{PSII} recorded only for *Sorbus domestica* and in both variants of the regulated water regime. In control variant, the value of this parameter was reduced from 0.22 to 0.10 (54%) and in the stress variant has been more marked reduction of its values (74%) from 0.34 to 0.09. For *Pyrus pyraster* in the year 2012 did not show significant differences in the values of Φ_{PSII}.

In the year 2013 the values of the effective quantum yield of PSII (Φ_{PSII}) for *Pyrus pyraster* significantly decreased under the influence of the regulation of the water regime. Since the beginning to the 84th day of the experiment, the values of the parameter in the control variant decreased from 0.20 to 0.13 (35%) and in the stress variant decreased from 0.32 to 0.13 (59%). For *Sorbus domestica* in the control variant the values of Φ_{PSII} were not significantly reduced in the course of the experiment, the values ranged between 0.18 to 0.17 (Tab. II). For stress variant *Sorbus domestica* was decrease of values of Φ_{PSII} conclusive and significant from 0.29 to 0.12 (59%) (Tab. II).

The results document negative impact of the regulated irrigation regime on efficiency of the PSII photochemistry of the experimental plants, especially *Sorbus domestica*. In both growing seasons was observed significant effect of regulated water regime on the values of the effective quantum yield of PSII (Φ_{PSII}) for *Sorbus domestica*, the significant decrease in the values of this parameter was reflected in the stress variant, which was irrigated at 40% of full water capacity. The negative impact of the regulated irrigation on *Pyrus pyraster* was manifested only in the year 2013. Decrease of values of parameter Φ_{PSII} was in variants with different levels of saturation of the substrate very similar (around 30%) and could be supported by stronger heat stress during the reporting period (Tab. I).

Impact of drought on the decrease of Φ_{PSII} values has been similarly reported for *Quercus coccifera* L. (Peguero-pina *et al*., 2008), *Quercus pubescens* Willd. (Gallé *et al*., 2007), *Fagus sylvatica* L. (Gallé and Feller, 2007), *Spiraea japonica* L. 'Little Princess' and *Cornus stolonifera* Michx. 'Kelseyi' (Šajbidorová, 2013).

CONCLUSION

Water deficiency in the soil is one of the most significant stress-factors for plants. There are various methods for assessment of the plant responses to water deficiency. A measurement of the chlorophyll fluorescence is method for screening of plant responses to stress-factors, because activity of the photosynthetic apparatus can be significantly influenced by the stress-factors. In the paper is presented study of the water deficiency impact on chlorophyll fluorescence of woody plants *Pyrus pyraster* and *Sorbus domestica*, light demanding tree species that grow also on stands influenced by water deficiency during the growing season. The experiment was set on plants in two different water regime (control variant was irrigated up to 60% of the full water capacity and stress variant up to 40% of the full water capacity). Chlorophyll fluorescence was carried out on three plants from each variant of the substrate saturation by fluorometer HANSATECH FMS 1 in 21 days period in two growing season 2012 and 2013. Following chlorophyll fluorescence parameters: F_v/F_m – maximum quantum efficiency of PSII, Φ_{PSII} – effective quantum yield of PSII and R_{FD} – chlorophyll fluorescence decrease ratio were recorded. According to obtained results, *Pyrus pyraster* has probably higher potential for adaptation to water deficiency. There was recorded the significant decrease mainly in the values of parameter R_{FD} and Φ_{PSII} for *Sorbus domestica* within duration of experiment with different water regime in both growing seasons 2012 and 2013. The results document a weak sensitivity of the parameter F_v/F_m on changes in the amount of available water in the substrate in both taxa.

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