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## PHOTOSYNTHETIC EFFICIENCY OF PEDUNCULATE OAK SEEDLINGS UNDER SIMULATED WATER STRESS

**Abstract:** Photosynthetic performance of seedlings of *Quercus robur* exposed to short-term water stress in the laboratory conditions was assessed through the method of induced fluorometry. The substrate for seedlings was clayey loam, with the dominant texture fraction made of silt, followed by clay and fine sand, with total porosity 68.2%. Seedlings were separated in two groups: control (C) (soil water regime in pots was maintained at the level of field water capacity) and treated (water-stressed, WS) (soil water regime was maintained in the range of wilting point and lentocapillary capacity). The photosynthetic efficiency was  $0.642 \pm 0.25$  and  $0.522 \pm 0.024$  (WS and C, respectively), which was mostly due to transplantation disturbances and sporadic leaf chlorosis. During the experiment  $F_v/F_m$  decreased in both groups ( $0.551 \pm 0.0100$  and  $0.427 \pm 0.018$  in C and WS, respectively). Our results showed significant differences between stressed and control group, in regard to both observed parameters ( $F_v/F_m$  and  $T_{1/2}$ ). Photosynthetic efficiency of pedunculate oak seedlings was significantly affected by short-term water stress, but to a lesser extent than by sufficient watering.

**Keywords:** *Quercus robur* L., 3-year-old seedlings, photosynthetic efficiency, water shortage

### ФОТОСИНТЕТИЧКА ЕФИКАСНОСТ САДНИЦА ХРАСТА ЛУЖЊАКА У УСЛОВИМА ВОДНОГ СТРЕСА

**Извод:** У раду је анализирана фотосинтетичка ефикасност садница *Quercus robur*, изложених краткотрајном водном стресу у лабораторијским

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условима, методом индуковане флуориметрије. Као супстрат за гајење садница коришћена је глиновита иловача, чију је доминантну текстурну фракцију чинио прах, затим глина па ситан песак; тоталне порозности 68,2%. Саднице су биле подељене у две групе: *C* (контролна група, код којих је водни режим супстрата у саксијама одржаван на нивоу пољског водног капацитета) и *WS* (група изложена водном стресу, код којих је водни режим супстрата одржаван између тачке већења и лентокапиларног капацитета). Фотосинтетичка ефикасност износила је  $0,642 \pm 0,258$  код контролних садница, односно  $0,522 \pm 0,0243$  код изложених водном стресу, што је углавном било узроковано поремећајима изазваним пресађивањем као и појави хлорозе на листовима. Током експеримента, вредност  $F_v/F_m$  се смањила у обе групе садница ( $0,551 \pm 0,0100$  код *C*, односно  $0,427 \pm 0,018$  код *WS*). У оба мерења утврђене су статистички значајне разлике између стресиране и контролне групе садница у погледу испитиваних параметара. Излагање садница храста лужњака краткотрајном водном стресу имало је значајан утицај на смањење фотосинтетичке ефикасности садница, али у мањој мери него излагање садница обилном заливању.

**Кључне речи:** *Quercus robur L.*, трогодишње саднице, фотосинтетичка ефикасност, водни стрес

## 1. INTRODUCTION

Climatic changes can affect forest ecosystems through the changes in frequency, intensity and duration of fires, drought, dynamics of introduced species, insect outbreaks, epidemics fungal diseases, hurricanes, wind- and snow damages or landslides (Dale *et al.*, 2001). Any of these disturbances has different effect to forest ecosystem, although the damages caused by insects and pathogens are the most severe (Dale *et al.*, 2001).

In particular, climatic changes lead to the changes in distribution, reproduction, development and mortality of insect species, as well as to mutual changes between insects and their host plants. Environmental factors have crucial effect on photosynthetic production of host plant, and consequently on its nutritive status. Therefore, to determine the effect of climatic changes on plants, as well as forest ecosystem in its complexity, it is necessary to observe changes in plant's photosynthetic performance.

Slatyer (1967) emphasizes two main effects of drought to photosynthesis: (a) indirect effect of water deficit to stomatal closure and reduction in spreading of carbon-dioxide throughout the leaf and (b) direct effect of water deficit to biochemical reactions involved in photosynthetic process. Generally, all of processes important for photosynthetic production (diffusion, photochemistry, chemistry, and transport) could be affected by the negative effect of water stress.

The aim of our study was to determine the effect of simulated water stress on photosynthetic efficiency of *Quercus robur* seedlings using the method of induced fluorometry. This method is based on the fact that the level of emission of fluorescence by chlorophyll molecule is associated with the proportion of energy of absorbed light used in

photosynthesis, but also with other processes connected with the heat realizing in chloroplasts (Krause, Weis, 1991). Therefore, chlorophyll fluorescence is a good predictor of efficiency of photosynthetic apparatus in situ, and also a measure of response to various stresses with negative effect to photosynthesis (Bohlar-Nordenkamp *et al.*, 1989, Schreiber *et al.*, 1995).

Natural distribution of Pedunculate oak in Europe is throughout British Isles to 450 m, and Europe to 1,000 m, from southern Scandinavia east to the Caucasus and south reaching the Mediterranean in Italy and the Adriatic coast; west to the Pyrenees and on north and west coast of Spain (Zanetto *et al.*, 1994). It occurs in a range of contrasting habitats in lowlands throughout and is dominant on basic loams and clays. It is also abundant in parks, deer-parks, gardens and woods.

## 2. MATERIAL AND METHODS

### 2.1. Plant material

We used 3+0 years old seedlings of Pedunculate oak, obtained from the nursery of Srbijašume in Rogot. These seedlings were taken from the nursery during the March, before the vegetation season have started, and transported to Belgrade with the turf. After arrival, the soil was cleaned from the roots, and seedlings were transplanted into plastic pots of 12 L. Transplantation was performed by adding of 6 kg of previously prepared substrate into each pot. Seedlings were regularly and equally watered during next two months.

The experiment with drought simulation began in the middle of August, when all seedlings were marked and separated into two groups which were differently treated in regard to soil water content. Firstly, pots were weighted to obtain the value of water content in each of them. Then, each pot was supplied with certain amount of water, to achieve and maintain certain soil water regime. In first group of seedlings, soil water regime was maintained at the level of field water capacity (control group, C). In second group of seedlings, soil water regime was maintained in the range of wilting point and lentocapillary capacity (water-stressed group, WS). The measurements were done twice, at the beginning of September (after 15 days of treatment) and two weeks later (after 30 days of treatment). The experiment was performed in the laboratory conditions  $T=25\pm 3^{\circ}\text{C}$ ,  $RH=65\pm 5\%$ , L: natural photoperiod.

### 2.2. Type of soil and soil water regime

Substrate which was used in our experiment is a mixture of soil collected from cambic horizon of eutric cambisol and peat (vol 1:1). Particle size composition of soil was determined by international pipette-B method using sodium pyrophosphate as a peptising agent, and textural class was determined using triangle after Ferre (Hadžić *et al.*, 1997).

The substrate bulk density was determined in steel rings, whereas the particle density was determined by gravimetric method after Gračanin (Bošnjak, 1997). For determination of the total porosity, the appropriate equations were used. Field water capacity of substrate was determined in the steel ring, using the undisturbed sample from the pot after a several watering and compression of substrate to natural consistency. Lentocapillary capacity and capacity for unavailable water in disturbed sample of substrate were determined in the pressure membrane after Richard (Bošnjak *et al.*, 1997).

### 2.3. Estimation of photosynthetic efficiency

Steady state fluorescence was determined with a Plant Stress Meter (BioMonitor S.C.I. AB, Sweden) by method of induced fluorometry (Powels, 1984, Öquist, Wass, 1988). Photosynthetic function was assessed by the rate of basic fluorescence, i.e. ratio of variable to maximal fluorescence ( $F_v/F_m = (F_m - F_o)/F_m$ , where  $F_o$  and  $F_m$  are initial and maximal fluorescence of dark-adapted leaves). Each leaf was illuminated with saturating low light ( $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) for 2 s, after having been in darkness for at least 20 min. We analyzed 17 water stressed plants and 17 control plants, by measuring three leaves from each plant on both measurement dates. The same intact leaves were subjected to measurements on 15<sup>th</sup> and on 30<sup>th</sup> day of treatment. The following parameters were analyzed: the ratio of variable and maximal fluorescence ( $F_v/F_m$ ) and a half-time of reaching the maximum fluorescence ( $T_{1/2}$ , i.e. the measure of the speed of photochemical reactions in photosystem II).

### 2.4. Statistics

Mean and standard errors (*SE*) for estimated parameters were determined, and analysis of variance was applied for determination of differences between groups.

## 3. RESULTS

### 3.1 Physical characteristics of substrate

According to texture, this kind of substrate is clayey loam (Table 1), with the dominant texture fraction made of silt, followed by clay and fine sand. Total porosity of substrate is relatively high (Table 2), due to the adding of significant amount of peat. 47.76% of soil volume is for the account of field water capacity (Table 3), and 20.44% is for the account of drainage porosity.

Easily available water for plants is represented with 18.64% and hardly available water is represented with 5.73% of substrate volume, respectively. The highest percent of field water capacity is the portion unavailable to plants, due to the high content of clay and adding of amount of peat as well.

**Table 1.** Particle size composition and textural class of substrate**Табела 1.** Текстулни састав супстрата

Coarse sand Крупан песак	Fine sand Ситан песак	Silt Прах	Clay Глина	Total sand Укупан песак	Total clay Укупна глина	Textural class after Ferré Текстулна класа по Ferré-у
%	%	%	%	%	%	
9.35	29.59	31.36	29.70	38.93	61.07	Clayey Loam

**Table 2.** Differential porosity of substrate**Табела 2.** Диференцијална порозност супстрата

Bulk density Запремин. густина	Particle density Специфична густина	Total porosity Укупна порозност	Differential porosity Диференцијална порозност			
			Pores / pore >8 $\mu$	Pores / pore 1-8 $\mu$	Pores / pore 0.2-1 $\mu$	Pores / pore <0.2 $\mu$
$g \cdot cm^{-3}$	$g \cdot cm^{-3}$	%	%	%	%	%
0.81	2.55	68.20	20.44	18.64	5.73	23.40

**Table 3.** Capacity of holding the water**Табела 3.** Капацитети примања и задржавања воде

Soil Moisture at Влажност земљишта при			Amount of water per one pot at Количина воде у саксији при		
FWC	LCC	WP	FWC	LCC	WP
v %	v %	v %	$dm^3$	$dm^3$	$dm^3$
47.76	29.12	23.40	3.18	1.94	1.56

**Legend / Легенда:** FWC - field water capacity / пољски водни капацитет, LCC - lentocapillary capacity / лентокапиларни капацитет, WP - Wilting point / влажност већења

### 3.2. Photosynthetic efficiency of photosystem II

At the beginning of experiment, the photosynthetic efficiency of photosystem II was  $0.642 \pm 0.25$  in treated group (*WS*) and  $0.522 \pm 0.024$  in untreated group of seedlings (*C*), respectively, which is significantly lower in comparison to the values usual for well watered, non stressed plants ( $F_v/F_m = 0.83$ , Powels, 1984) (Table 4). During the experiment with drought simulation,  $F_v/F_m$  decreased in both *WS* and *C* group ( $0.551 \pm 0.010$  and  $0.427 \pm 0.018$ , respectively) (Table 5).

Similarly, the tendency of significant decreasing was noticed for the parameter  $T_{1/2}$ , i.e. values of  $T_{1/2}$  were approx. four times lower in both *WS* and *C* seedlings at the end of experiment (Tables 4 and 5).

**Table 4.** Photosynthetic efficiency of *Q. robur* seedlings at the beginning of experiment (15 days of treatment).**Табела 4.** Фотосинтетичка ефикасност садница *Q. robur* на почетку експеримента (после 15 дана третмана)

Параметар Parameter	Третман / Treatment				F	p
	Водни стрес / Water stress		Контрола / Control			
	$\bar{X}$	$S_E$	$\bar{X}$	$S_E$		
$F_v/F_m$	0.642	0.025	0.522	0.024	11.62	0.0011
$T_{1/2}$	451.730	26.019	286.325	38.854	12.11	0.0008

**Table 5.** Photosynthetic efficiency of *Q. robur* seedlings one month from the beginning of experiment (30 days of treatment).**Табела 5.** Фотосинтетичка ефикасност садница *Q. Robur* после месец дана од почетка експеримента (након 30 дана третмана)

Параметар Parameter	Третман / Treatment				F	p
	Водни стрес / Water stress		Контрола / Control			
	$\bar{X}$	$S_E$	$\bar{X}$	$S_E$		
$F_v/F_m$	0.551	0.010	0.427	0.018	35.34	0.0000
$T_{1/2}$	104.363	3.612	77.784	8.542	8.21	0.0051

#### 4. DISCUSSION

Photochemical quantum yield of PSII ( $F_v/F_m$ ) displayed low values at the very beginning of experiment in both groups of seedlings, indicating stressful conditions prior to further treatments. This might be attributed to transplantation disturbances (possible injuries of roots), and also to adaptation to different light conditions in comparison with nursery. This conclusion was made from the observation that both groups showed certain misalignment from the theoretical optimal value (0.83, Powells, 1984), regardless of water supply. Sporadic occurrence of chlorotic areas on leaves of almost all seedlings also contributed to somewhat unfavorable state of photosystem II at that point.

Drought simulation caused significant decrease of photosynthetic efficiency, but unexpectedly, the same tendency of decreasing  $F_v/F_m$  was observed in the group of seedlings which were watered regularly.

Another parameter,  $T_{1/2}$  is a predictor of the rate of photochemical reactions within the photosystem II. According to literature, it should be changed in opposite course than  $F_v/F_m$  (Powells, 1984). However, our results showed the same tendency, i.e. decreasing of the rate of photochemical reactions. It is the contradictory finding, indicating that the rate of biochemical reactions is higher at the same time when the less energy is used in photosynthesis. This could be explained with the disturbances in the chlorophyll content and

structure apparent though the leaf chlorosis. Additional measurements of the biochemical status of photosynthetic pigments would be needed to confirm this point.

The general low performance of photosynthetic efficiency of *Q. rubra* seedlings is probably related to low chlorophyll content rather than water shortage. All seedlings suffered from sporadic chlorosis which was caused by the disturbances in pigment apparatus and exposition to full irradiance conditions. This resulted in the onset of long-lasting non-photochemical fluorescence quenching and further decrease in  $F_v/F_m$  (Butler, 1978). However, the additional analysis would be needed to support these predictions.

Our results showed significant differences between stressed and control group, in regard to both observed parameters ( $F_v/F_m$  and  $T_{1/2}$ ). When the effect of water shortage was compared in stressed and control group after 15 days of treatment, it was approved that  $F_v/F_m$  decreased significantly in both groups (for 14.2% and 18.2% in *WS* and *C* group, respectively). These data indicate that water shortage hasn't the crucial effect to decrease of photosynthetic efficiency. These results could be supported with the previous studies on the same species. In the comprehensive study on the effects of water and light supplies on three oak species (*Quercus robur*, *Q. petraea* and *Q. rubra*), pedunculate oak was the least sensitive to water shortage (Wagner, Dreyer, 1997). Furthermore, this species showed decline in all photosynthetic parameters, including  $F_v/F_m$ , in the conditions of waterlogging. Other studies also demonstrated that *Q. robur* is, among other oak species, particularly efficient in water usage, even in the conditions of severe water stress (Molchanov, 2009, Rosenquist, 2010). From our results, it could be concluded that the photosynthetic efficiency of seedlings of Pedunculate oak was more affected by sufficient watering than by short-term water stress.

In particular, drought stress was not the promoter for the decreases of  $F_v/F_m$  in Pedunculate oak (Dreyer *et al.*, 1991, Dreyer, 1994, Gardiner, Hodges, 1996). This is because during water stress photosynthesis is limited mainly by stomatal closure (Epron, Dreyer, 1992), and decreases of  $F_v/F_m$  occur only when diurnal assimilation rates are already reduced to nil (Epron *et al.*, 1993). However, this reduction is rarely expected, since the seedlings of Pedunculate oak have great stomatal conductance, high net assimilation rate and photosynthetic capacity (Epron *et al.*, 1993). Also, the short-term drought never resulted in reductions of maximal photosynthetic capacity to the extent of those recorded with waterlogging (Chaves, 1991, Epron, Dreyer, 1993). On the other hand, this species showed great sensitivity to the changes of light environment, preferring shade than full light conditions (Epron, Dreyer, 1992). These findings align *Q. robur* as less light stress-tolerant and more water stress-tolerant species.

## 5. CONCLUSIONS

Low photosynthetic performance of *Q. robur* seedlings exposed to water shortage and control seedlings was due to previous disturbances of seedlings, and especially to appearance of leaf chlorosis.



Water shortage had significant effect to the lower performance of photosystem II in treated seedlings, which was approved by significant differences between stressed and control group in regard to both observed parameters ( $F_v/F_m$  and  $T^{1/2}$ ).

Prolonged water shortage caused further decreasing of photosynthetic efficiency of seedlings. To a lesser extent, the effect of decreasing of  $F_v/F_m$  values over time was observed in control group as well.

Furthermore, it was demonstrated that photosynthetic efficiency of seedlings of Pedunculate oak was more affected by sufficient watering than short-time water shortage.

Additional biochemical measurements on pigment status are needed to confirm the conclusion that the photosynthetic efficiency was affected by the chloroplast disturbances.

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## ФОТОСИНТЕТИЧКА ЕФИКАСНОСТ САДНИЦА ХРАСТА ЛУЖЊАКА У УСЛОВИМА ВОДНОГ СТРЕСА

### Резиме

У овом раду је анализирана фотосинтетичка ефикасност садница храста лужњака (*Quercus robur L.*) изложених краткотрајном водном стресу у лабораторијским условима, уз коришћење методе индуковане флуориметрије. Ова метода се базира на чињеници да је ниво емисије флуоресценције од стране молекула хлорофила повезан са пропорцијом светлосне енергије коришћене у фотосинтези, али такође и са другим процесима ослобађања топлоте у хлоропластима. Због тога, флуоресценција хлорофила представља поуздан показатељ ефикасности фотосинтетичког апарата *in situ*, а такође и меру одговора на различите врсте стреса са негативним ефектом на фотосинтезу.

Анализом физичких карактеристика супстрата коришћеног за пресађивање садница, утврђено је да по текстурном саставу супстрат припада глиновитим иловачама. Доминантну текстуру фракцију је чинио прах, затим глина па ситан песак. Укупна порозност припремљеног супстрата је висока 68,2%, што је последица додавања значајних количина тресета. При овој 47,76% волумена земљишта је чинио пољски водни капацитет, а дренажну порозност 20,44%. Биљкама лако приступачна вода заузимала је 18,64% волумена супстрата, а теже приступачна 5,73%. Доста висок садржај глине, а такође и додавање значајних количина тресета супстрату условили су да највећи део пољског водног капацитета чини неприступачна вода.

Саднице су подељене у две групе, при чему је у првој групи (контрола, *C*) водни режим земљишта одржаван на нивоу пољског капацитета, док је у другој групи (водни стрес, *WS*) водни режим земљишта одржаван на нивоу између тачке већења и лентокапиларног капацитета. Мерења параметара фотосинтетичке ефикасности су урађена 2 пута, на почетку септембра (после 15 дана симулирања водног стреса) и половином септембра (30 дана после почетка симулирања водног стреса).

Фотосинтетичка ефикасност ( $F_v/F_m$ ) износила је  $0,642 \pm 0,25$  у *C* и  $0,522 \pm 0,024$  у *WS* групи на почетку експеримента, што су значајно ниже вредности у односу на теоретски оптимум код не-стресираних биљака при повољном водном режиму ( $F_v/F_m = 0,83$ ). Ово одступање се може приписати поремећајима изазваним пресађивањем садница (могућим оштећењима кореновог система), а нарочито хлоротичним променама које су уочене на готово свим биљкама. Током експеримента, забележен је пад фотосинтетичке ефикасности садница, и то у обе групе (за 14,2% код *WS* и за 18,2% код *C*). Слична тенденција је забележена и у променама другог параметра ( $T_{1/2}$  полувреме постизања максималне флуоресценције), за који је било очекивано да има сасвим супротну динамику. Овај налаз, такође, указује на поремећаје у садржају и структури хлоропласта, али захтева и додатна мерења да би ова претпоставка била потврђена.

Поређењем вредности параметара фотосинтетичке ефикасности код стресиране и контролне групе утврђене су статистички значајне разлике у погледу испитиваних параметара ( $F_v/F_m$  и  $T_{1/2}$ ), и то у оба мерења (15-ог и 30-ог дана од почетка третмана). Анализом

добijenih rezultata i poređenem sa prethodno objavljenim studijama o fotosintetičkom kapacitetu i vodnim odnosima urađenim na ovoj vrsti, možemo zaključiti da je fotosintetička efikasnost sadnica hrasta lужњака bila u већој мери под негативним утицајем обилног заливања него краткотрајног водног стреса. Такође, последице прилагођавања фотосинтетичког апарата новим светлосним условима (различитим од оних у расаднику) су уочљиве кроз хлоротичне промене на листовима готово свих садница. Оба ова закључка потврђују да је у условима деловања различитих стресних фактора, хрст лужњак мање толерантан на светлосне промене, него на промене водног режима станишта.

