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±0.25 and 0.522±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±0.0100 and 0.427±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
условима, методом индукиране флуориметрије. Као супстрат за гајење садница коришћена је глиновита иловача, чију је доминантну текстуру 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 вељања и лентокапиларног капацитета). Фотосинтетичка ефикасност износила је 0,642±0,258 код контролнih садница, односно 0,522±0,0243 код изложених водном стресу, што је углавном било узроковано поремећајима пресађивањем као и појави хлорозе на листовима. Током експеримената, вредност \(F_v/F_m\) се смањила у обе групе садница (0,551±0,0100 код \(C\), односно 0,427±0,018 код \(WS\)). У оба мерења утврђене су статистички значајне разлике између стресиране и контролне групе садница у погледу испитаних параметара. Излагање садница храста лужњака краткотрајном водном стресу имало је значајан утицај на смањење фотосинтетичке ефикасности садница, али у мањој мерi 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-Nordenkampf 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 (Zanneto 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 $T=25\pm3^\circ C$, $RH=65\pm5\%$, 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, Oquist, Wås, 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 µmol·m$^{-2}$·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 form each plant on both measurement dates. The same intact leaves were subjected to measurements on 15th and on 30th 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

<table>
<thead>
<tr>
<th>Coarse sand</th>
<th>Fine sand</th>
<th>Silt</th>
<th>Clay</th>
<th>Total sand</th>
<th>Total clay</th>
<th>Textural class after Ferré</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>Clayey Loam</td>
</tr>
<tr>
<td>9.35</td>
<td>29.59</td>
<td>31.36</td>
<td>29.70</td>
<td>38.93</td>
<td>61.07</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Differential porosity of substrate

<table>
<thead>
<tr>
<th>Bulk density</th>
<th>Particle density</th>
<th>Total porosity</th>
<th>Differential porosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>g cm⁻³</td>
<td>g cm⁻³</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>0.81</td>
<td>2.55</td>
<td>68.20</td>
<td>20.44</td>
</tr>
</tbody>
</table>

Table 3. Capacity of holding the water

<table>
<thead>
<tr>
<th>Soil Moisture at</th>
<th>Amount of water per one pot at</th>
</tr>
</thead>
<tbody>
<tr>
<td>FWC</td>
<td>LCC WP</td>
</tr>
<tr>
<td>v %</td>
<td>v %</td>
</tr>
<tr>
<td>47.76</td>
<td>29.12 23.40</td>
</tr>
<tr>
<td>3.18</td>
<td>1.94 1.56</td>
</tr>
</tbody>
</table>

Legend: FWC - field water capacity; LCC - lentocapillary capacity; WP - Wilting point

3.2. Photosynthetic efficiency of photosystem II

At the beginning of experiment, the photosynthetic efficiency of photosystem II was 0.642±0.25 in treated group (WS) and 0.522±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 (Fv/Fm=0.83, Powels, 1984) (Table 4). During the experiment with drought simulation, Fv/Fm decreased in both WS and C group (0.551±0.010 and 0.427±0.018, respectively) (Table 5).

Similarly, the tendency of significant decreasing was noticed for the parameter T½, i.e. values of T½ 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).

<table>
<thead>
<tr>
<th>Параметар / Parameter</th>
<th>Третман / Treatment</th>
<th>$F$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_v/F_m$</td>
<td>Водни стрес / Water stress</td>
<td>0.642</td>
<td>0.025</td>
</tr>
<tr>
<td>$T_1/2$</td>
<td>Контрола / Control</td>
<td>451.730</td>
<td>26.019</td>
</tr>
</tbody>
</table>

Table 5. Photosynthetic efficiency of *Q. robur* seedlings one month from the beginning of experiment (30 days of treatment).

<table>
<thead>
<tr>
<th>Параметар / Parameter</th>
<th>Третман / Treatment</th>
<th>$F$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_v/F_m$</td>
<td>Водни стрес / Water stress</td>
<td>0.551</td>
<td>0.010</td>
</tr>
<tr>
<td>$T_1/2$</td>
<td>Контрола / Control</td>
<td>104.363</td>
<td>3.612</td>
</tr>
</tbody>
</table>

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, Powels, 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 $F_v/F_m$ (Powels, 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 thought 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 $T_\frac{1}{2}$). 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 $T_\frac{1}{2}$).

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 индукиране 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 са другим процесима ослобођања топлоте у хлоропластима. Због тога, флуоресценција хлорофила представља поуздан показа те 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 текстуру можно присутан прашак, затим глина па ситан песак. Укупна порозност супстрата је висока 68,2%, што је последица додавања значајних количина тресета. При овом 47,76% волумена земљишта је чинио пољски водни капацитет, док је дренажна порозност 20,44%. Водећа 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 не приступачна 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 венења 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±0,25 у C и 0,522±0,024 у WS групи на почетку експеримента, што је 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 уочени 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 ($T_{\text{1/2}}$, полувреме постигања 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 стресираних и контролних група 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...
добијених резултата и поређењем са претходно објављеним студијама о фотосинтетичком капацитету и водним односима урађеним на овој врсти, можемо закључити да је фотосинтетичка ефикасност садница храста лужњака била у већој мери под негативним утицајем обилног заливања него краткотрајног водног стреса. Такође, последице прилагођавања фотосинтетичког апаратата 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, храст лужњак мање толерантан на светлосне промене, него на промене водног режима станишта.