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ACCLIMATION OF PEDUNCULATE OAK SEEDLINGS TO DIFFERENT LIGHT CONDITIONS IN THE FIRST MONTHS AFTER GERMINATION

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Abstract

The first months are the most sensitive phase of oak seedling development. Light conditions have an important role in this sense, both from the physiological and management viewpoint. We investigated the response of pedunculate oak seedlings to three growth light intensities (100, 550 and 2000 μmol cm^{-2}s^{-1}) during development of the first and second growth flush. The low and high light intensities of PAR are supposed to mimic the effects of solar radiation under extreme natural conditions (closed canopy and open field). The response of seedlings to different light intensities was evaluated by determining the photochemical activity of photosystem II, leaf chlorophyll concentration and epidermal flavonoid accumulation for both growth flushes. At the end of the experiment (after 4.5 months) the effects of different treatments on growth parameters were also determined. We showed here that oak seedlings responded to varying light intensities by modifying their physiological and morphological traits of successive growth flushes. At medium light, seedlings had the highest PSII photochemical activity in the 2nd flush. High light induced very low photochemical efficiency of photosystem II in both growth flushes indicating the development of high non-photochemical quenching of chlorophyll fluorescence as part of photoprotective mechanism. In accordance with high photosynthetic yield at ML, the investment of photosynthates in growth, especially biomass allocation towards root system was confirmed. ML was optimal for seedling development in the first months. The results may contribute to a better understanding of oak seedling development and acclimation and could have importance for oak natural regeneration.

Keywords: growth flush, photochemical efficiency, morphological parameters.

INTRODUCTION

The early stages of oak seedling development are crucial for their establishment and competitiveness with other tree species and herbaceous plants [1]. Although many factors influence seedling growth, light conditions can be considered as one of the most important determinants of oak seedling development [2–4]. Morphological traits were widely used for characterization of oak seedlings development or growth in various studies on different species [4,5]. However, the underlying physiological mechanisms of the morphological changes are of essential importance for understanding acclimation to natural conditions and for application in oak regeneration management [6]. Multi-flush growth is a characteristic morphological trait for pedunculate oak, both in juvenile and adult individuals [6–8] and implies a rhythmic growth pattern that is under strong genetic control [9]. Although this growth pattern is found in pedunculate oak seedlings in a wide light gradient (from closed canopies with no more than 5% sunlight to full sunlight, in the open areas), it can be stimulated by silvicultural measures in order to increase pedunculate oak competitiveness against surrounding vegetation [1,7].

The aim of this study was to investigate how pedunculate oak seedlings respond in their early phase of development (first 4.5 months) and acclimate to three different light intensities of photosynthetically active radiation (PAR) by assessing: i) photochemical activity of photosystem II (PSII), leaf total chlorophyll (Chl) and epidermal flavonoid (EpFlav) contents during the first two growth flushes and ii) morphological parameters such as fresh weight, dry weight, shoot/root ratio, leaf area and specific leaf area.

MATERIALS AND METHODS

Plant material and experimental design

The acorn was collected in pedunculate oak forest in Morović in a stand that belongs to the Sava-Danube provenance (Serbia). The acorns of good quality and similar size were selected and planted in 12 plastic containers (each with one acorn) at 1–2 cm depth filled with Klasmann Potground H commercial substrate. The seedlings were grown in a growth chamber under controlled conditions: 14/10 h day/night photoperiod, 26 °C temperature and relative humidity of 40–50%.

From the start of the experiment, the seedlings were divided into three groups (four seedlings each) growing in the following light intensities: low light (~100 µmol cm^{-2} s^{-1}, LL); medium light (~550 µmol cm^{-2} s^{-1}, ML) and high light (~2000 µmol cm^{-2} s^{-1}, HL). The plants were grown for about 18 weeks.

Chlorophyll fluorescence measurements, leaf chlorophyll and epidermal flavonoid contents

The photosynthetic yield (YPSII) was derived from measuring modulate pulse chlorophyll fluorescence using Junior PAM portable chlorophyll fluorometer (Gadermann Instruments GmbH, Würzburg, Germany). Measurements of photochemical activity [Y(II)], were calculated using the WinControl software (v3.29; Heinz Walz GmbH, Effeltrich, Germany) as described by Van Kooten and Snel [10] and were performed continuously during 24 h by applying a saturating pulse (10,000 µmol $m^{-2}s^{-1}$) every 20 minutes. Changes in diurnal photosynthetic activity were followed on a mature leaf of each seedling on two development stages [after the formation of the 1st flush (6 weeks after planting) and the 2nd flush (10 weeks after planting)]. Total chlorophyll content (Chl), the content of leaf epidermal flavonoids (EpFlav) and the nitrogen balance index (NBI) were determined using Dualex FLAV (Force-A, Orsay, France; Cerović *et al.* [11]) on the basis of three leaves per plant 14 weeks after planting.

Morphological parameters

Fresh (FW) and dry weight (DW) of stem, roots and leaves were measured 18 weeks after planting. Dry weight data were used for calculation of the shoot/root ratio. Total leaf area per plant (LA) was measured using ImageJ software [12], while specific leaf area (SLA) was expressed as the ratio between leaf area and dry weight per plant.

Statistical analysis

Two-way ANOVA was used to determine the effects of light intensity and growth flush on NBI, Chl and EpFlav mean values. Fisher's LSD test was used for determination of differences between the treatments with a 0.05 significance threshold. The same test was used for determination of significant differences between the mean values of the analysed morphological parameters. The experimental data were analysed using software package Statistica 8.0.

RESULTS AND DISCUSSION

After 6 weeks when the first growth flush was formed, Y(II) was constant during the day with expected variations in the HL treatment. Upon darkening, Y(II) attained their maximal values by a similar rate within the first 80 minutes. Interestingly, ML and HL treatments brought about similar Y(II) throughout the day.

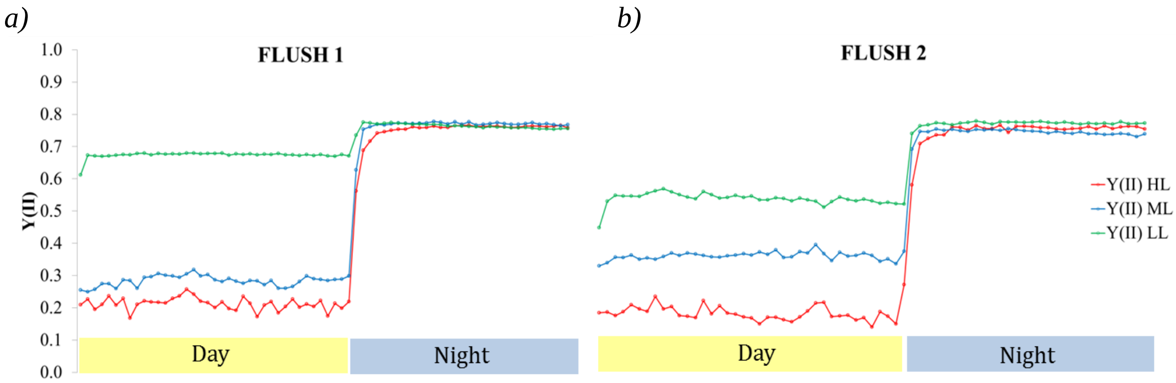

Figure 1 *Diurnal photochemical efficiency of photosystem II [Y(II)] of pedunculate oak seedlings a) 6 weeks (1^{st} flush) and b) 10 weeks (2^{nd} flush) after planting*

After 10 weeks, once the seedlings have developed another growth flush, Y(II) at LL was reduced, while at ML plants showed an increase in photochemical activity (Figure 1). The results show that oak seedlings acclimated in the 2^{nd} flush compared to the 1^{st} flush. By increasing light intensity, root growth was stimulated in the 18-week-period (by 57% in HL and 81% in ML conditions, compared to LL conditions). With increasing light intensity, oak seedlings also showed a tendency to increase the dry weight of leaves and stem, even though this was not confirmed by LSD test. The shoot/root ratio of all oak seedlings showed that the largest part of the biomass was allocated to root (57–64%). Previous research on one- or two-years-old seedlings showed that the shoot/root ratio is generally higher in low light conditions [3,13]. However, this was not confirmed in our study, because of the shorter investigated time span, as the differentiation in the shoot/root ratio between the treatments probably occurs somewhat later in the seedling development. As expected, with increasing light intensity, LA decreases due to higher leaf thickness, as indicated by SLA, that may be explained by acclimatization to high light and multiplication of palisade cells in order to increase CO_2 assimilation [14]. Such morphological plasticity was observed on cork oak seedlings under a light gradient [4]. The plants in excess light of the HL treatment, which was noticed for some leaves, showed photoinhibition based on observed chlorophyll damage (Figure 2a).

Table 1 *Morphological parameters of pedunculate oak seedlings 18 weeks after planting (different letters indicate significant differences on the basis of LSD test)*

Light intensity	LL	ML	HL
Leaves FW (g)	4.19 ± 0.44 a	6.25 ± 1.09 a	5.39 ± 0.77 a
Stem FW (g)	3.02 ± 0.27 a	5.37 ± 1.20 a	4.34 ± 0.36 a
Roots FW (g)	13.02 ± 2.00 a	20.84 ± 2.11 b	18.01 ± 1.30 ab
Plant FW (g)	20.23 ± 4.04 a	32.47 ± 8.35 b	27.74 ± 3.13 ab
Leaves DW (g)	1.63 ± 0.18 a	2.61 ± 0.43 a	2.44 ± 0.33 a
Stem DW (g)	1.31 ± 0.16 a	2.57 ± 0.61 a	2.09 ± 0.30 a
Roots DW (g)	4.85 ± 0.88 a	8.80 ± 1.02 b	7.63 ± 1.33 ab
Plant DW (g)	7.80 ± 1.80 a	13.98 ± 3.93 b	12.16 ± 2.09 ab
Leaves FW/DW	2.57 ± 0.02 a	2.39 ± 0.07 a	2.35 ± 0.62 a
Stem FW/DW	2.32 ± 0.07 a	2.13 ± 0.06 a	2.11 ± 0.11 a
Roots FW/DW	2.71 ± 0.08 a	2.39 ± 0.07 a	2.46 ± 0.30 a
Shoot/root ratio	0.64 ± 0.11 a	0.57 ± 0.08 a	0.64 ± 0.16 a
LA (m^2)	279.1 ± 18.8 ab	316.8 ± 48.7 b	189.8 ± 22.2 a
SLA ($cm^2 g^{-1}$DW)	36.8 ± 4.33 b	22.5 ± 1.01 a	16.31 ± 3.56 a

*Rows with significance differences between some of the treatments are marked grey.

As a result of increasing light intensity, flavonoids accumulated in the epidermal layer of leaves to fulfil their sun protective function (Figure 2). EpFlav are also indicators of higher stimulation of phenylpropanoids biosynthesis, on account of primary metabolites, which was followed by decreasing NBI. These results are in accordance with the changed trade-off strategy of plants.

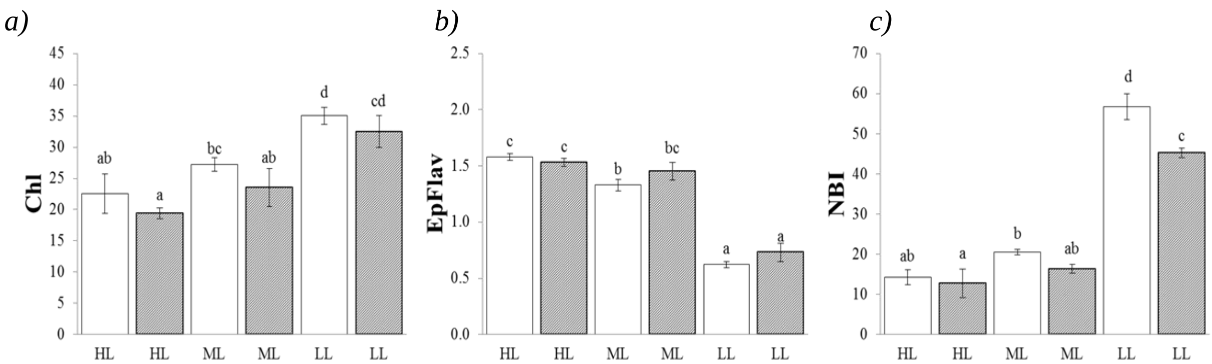

Figure 2 *a) Chlorophyl content (Chl), b) leaf epidermal flavonoid content (EpFlav) and c) nitrogen balance index (NBI) under high (HL), medium (ML) and low light (LL) conditions in first flush leaves (white bars) and second flush leaves (grey bars). Different letters indicate significant differences on the basis of LSD test*

Regardless of light conditions, pedunculate oak produces 2nd growth flush, which is in line with previous research [6,7]. The seedlings employ this 2nd flush to acclimate to the environmental conditions which was confirmed in our research [6].

CONCLUSION

Oak seedlings acclimated to the given environmental conditions by modifying some of the observed morphological and physiological traits in the successive growth flush. We found that optimal growth conditions in the first few months of oak seedling development are in the medium light treatment that corresponds to about 50% of the maximum daily sunlight. In these conditions, pedunculate oak seedlings produced highest total biomass while improving their photochemical efficiency in the second growth flush. In low light (closed canopy conditions), a reduction of photochemical efficiency was noted. Seedlings grown under high light showed a constant, but very low photochemical efficiency of photosystem II in both growth flushes. This, however, may partly be attributed to excessive light for 14 hours a day. The results may contribute to better understanding of oak seedlings development and acclimation that may have importance for oak natural regeneration.

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