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Growth and photosynthesis of *Lemna minor* L. exposed to different light conditions and sucrose supplies

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Abstract – Duckweed (*Lemna minor* L.) is a model plant suitable for investigation into plant physiology, biochemistry and ecotoxicology. Depending on the type of the experiment, duckweed is cultivated on different nutrient media under various chamber conditions. In our work, duckweed was cultivated on Pirson-Seidel's nutrient solution supplemented with 5, 7.5 or 10 g L^{-1} sucrose under cool white (CW) or Gro-Lux (GL) light sources. When different light sources and sucrose supplies are compared, a significant stimulative effect of GL light on duckweed grown on 7.5 and 10 g L^{-1} sucrose was seen to start on day 9. Considering photosynthetic performance the results showed that there were no significant differences in maximum quantum yield of PSII (F_v/F_m) after 7 and 16 days of exposure, regardless of light source and sucrose supply. Effective quantum yield of PSII (Φ_{PSII}) decreased only after 16 days of exposure to 5 g L^{-1} sucrose under CW light. The higher growth rate and photosynthetic performance in plants exposed to GL light is a consequence of its spectral distribution resembling the action spectrum of photosynthesis. Furthermore, enhanced growth noticed in plants cultivated on higher sucrose contents (7.5 and 10 g L^{-1}) indicated the promotive effect of sucrose in plants grown under low light conditions.

Key words: chlorophyll fluorescence, growth rate, light, *Lemna minor*, photosynthesis, photosystem II, sucrose

Abbreviations: Chl – chlorophyll, **CW** – Cool White light source, **GL** – GroLux light source, **GR** – growth rate, **PPFD** – photosynthetic photon flux density, $\mathbf{F_v/F_m}$ – maximum quantum yield of PSII, $\mathbf{\Phi_{PSII}}$ – effective quantum yield of PSII, **PSII** – photosystem II, **PS** – Pirson and Seidel's nutrient solution

Introduction

Duckweed (*Lemna minor* L.) is a small, free-floating freshwater macrophyte. Due to its small size, high multiplication rate, vegetative propagation, high sensitivity and easy handling, duckweed has been commonly used as model plant in evaluating the effects of a wide

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range of substances (WANG 1990, LEWIS 1995, NAUMANN et al. 2007, ZHANG et al. 2010). Moreover, Lemna bioassay has been recommended by the EPA, ASTM, OECD, SIS and ISO (EBERIUS 2001) in order to make it possible to use duckweed for reproducible toxicological studies in different laboratories (NAUMANN et al. 2007). As well as for ecotoxicological studies, duckweed is an excellent plant model for molecular (MARDANOV et al. 2008), biochemical (SHERAMETI et al. 2002) and physiological studies (KRAJNČIČ and DEVIDÉ 1980, KONDO 1988, CHI et al. 2012), as well as for phytoremediation of polluted waters (MKANDVIRE and DUDEL 2007), environmental biomonitoring (WANG and WILLIAMS 1988) and biotechnology (GYUNTER et al. 2008). Furthermore, it has also been used to evaluate the effects of nutrient deficiency (RAPPARINI et al. 2002), extreme temperatures, radiofrequency radiation (GERM and GABERŠČIK 1999, TKALEC et al. 2007) and different light conditions (ARTETXE et al. 2002, SOMSRI et al. 2010).

According to the concept of an experiment, duckweed is cultivated on different nutrient media, e.g. Bristol's, Hunter's, Hoagland's and Schenk and Hildebrandt's (WANG 1990, LEWIS 1995, ZHANG et al. 2010). Besides mineral elements, some nutrient media contain organic compounds, usually amino acids and carbohydrates (WANG 1990, FRICK 1994, TKALEC et al. 2007). Depending on the concentration, organic substances can stimulate plant growth but also can cause osmotic stress and down-regulation of photosynthesis (HDIDER and DESJARDINS 1994, BALEN et al. 2012, and references therein). Plant growth is dependent not only on the composition of nutrient media but also on chamber conditions – temperature, photoperiod and light quality. Artificial light sources differ from sunlight in both spectral characteristics and intensity. In growth chambers, usually cool white (CW) or day-light fluorescent tubes with a wide range of light intensities (40–180 $\mu mol_{PHOTONS}\ m^{-2}\ s^{-1}$) are used. Some of the commercially available light sources are not the appropriate for photosynthesis due to light intensities and spectral characteristics that are incompatible with the absorption spectrum of photosynthetic pigments.

The aim of this work was to evaluate the impact of sucrose supply and light conditions on the growth rate and photosynthetic efficiency of *Lemna minor* L. Duckweed was grown on Pirson-Seidel's (PS) nutrient solution (PIRSON and SEIDEL 1950) under cool white (CW) or Gro-Lux (GL) fluorescent light sources that have different spectral energy distributions. The light energy of CW sources is mainly distributed in the blue, green and yellow parts of the spectrum while GL sources emit most of energy at the blue and red which are the spectral regions most effective in photosynthesis.

PS medium originally contains 10 $g\ L^{-1}$ of sucrose (PIRSON and SEIDEL 1950). In our experiment, growth and photosynthetic efficiency in duckweed cultivated on media supplemented with lower sucrose concentrations (5 and 7.5 $g\ L^{-1}$) were also tested. According to the recommendation of HUEBERT and SHAY (1993), we previously tested the duration of exponential growth on PS medium. Since the exponential growth during 16 days was confirmed, this period was taken for the maximum duration of the experiment.

Material and methods

Plant material and growth conditions

Duckweed (*Lemna minor* L.) was collected from the Botanical Garden, Faculty of Science, University of Zagreb in 1996, and sterilised according to KRAJNČIČ and DEVIDÉ (1980).

Axenic stock cultures were maintained on Pirson-Seidel (PS) nutrient solution (PIRSON and SEIDEL 1950) containing 3.95 mmol L^{-1} KNO_3, 5.46 mmol L^{-1} $CaCl_2 \times 2\ H_2O$, 1.47 mmol L^{-1} KH_2PO_4, 1.21 mmol L^{-1} $MgSO_4 \times 7\ H_2O$, 49 µmol L^{-1} $Na_2EDTA \times 2\ H_2O$, 20 µmol L^{-1} Fe-citrate, 1.5 µmol L^{-1} $MnCl_2 \times 4\ H_2O$, 8.1 µmol L^{-1} H_3BO_3, 29.2 mmol L^{-1} sucrose and 0.66 mmol L^{-1} asparagine. The pH value of nutrient solution was adjusted to 4.55 using KOH. Plants were grown at 24 ± 2 °C under an illumination provided by CW fluorescent light (F36W/33-640, Sylvania), PPFD = 45 µmol $_{PHOTONS}$ m^{-2} s^{-1} at plant level and a light:dark cycle of 16:8 hours. Cultures were grown under static conditions and subcultured biweekly.

Experimental conditions

Experimental cultures were started by inoculating a healthy duckweed colony consisting of three or four fronds into 100 mL Erlenmeyer flasks containing 60 mL of PS nutrient solution supplemented with sucrose in concentrations of 10 g L^{-1} (29.2 mmol L^{-1}), 7.5 g L^{-1} (21.9 mmol L^{-1}) or 5 g L^{-1} (14.6 mmol L^{-1}). The experimental plants were grown under CW fluorescent lights (F36W/33-640, Sylvania), PPFD = 50±5 µmol $_{PHOTONS}$ m^{-2} s^{-1} or GroLux (GL) (F36W/GRO, Sylvania), PPFD = 50±5 µmol $_{PHOTONS}$ m^{-2} s^{-1}. Experimental plants were exposed to the same photoperiod and temperature as stock cultures.

Growth assessment

Growth rate (GR) on the basis of frond number was calculated on days 2, 5, 7, 9, 12, 14 and 16, according to the equation:

$$GR_i = \frac{\ln N_{t_i} - \ln N_{t_0}}{t_i - t_0}$$

GR_i is the growth rate per day; N_{t_0} is the frond number at day t_0 (the beginning of the experiment); N_{t_i} is the frond number at day t_i (i = 2, 5, 7, 9, 12, 14, 16); $t_i - t_0$ is the time period between t_i and t_0, expressed in days (ISO 20079, 2004).

The frond number doubling time (T) was calculated for days 2, 5, 7, 9, 12, 14 and 16, using the equation (ISO 20079, 2004):

$$T_i = \frac{\ln 2}{GR_i}$$

T_i is the frond number doubling time for day i (i = 2, 5, 7, 9, 12, 14, 16).

Each result was expressed as an average of 20 replicates (10 replicates from two independent experiments) ± standard error (SE).

***In vivo* measurement of chlorophyll fluorescence**

Duckweed photosynthetic efficiency was assessed by measuring *in vivo* chlorophyll fluorescence on day 7 and day 16 of the experiment. Chlorophyll fluorescence was measured from the upper face of dark-adapted fronds by pulse-modulated chlorophyll fluorometer (Qubit Systems Inc., Canada). Minimal fluorescence (F_0) was measured using a weak red modulated light, resulting in 0.2 – 0.25 mV F_0 signal for dark-adapted plants. Then, a satu-

rating light pulse (PPFD = 3760 μmol $_{PHOTONS}$ m^{-2} s^{-1}, t = 0.7 s) was applied to induce maximal fluorescence (F_m). The difference between F_m and F_0 was used for calculation of variable fluorescence (F_v). After quenching of maximal Chl fluorescence in the dark, plants were illuminated by continuous actinic light (PPFD = 90 μmol $_{PHOTONS}$ m^{-2} s^{-1}). Steady-state fluorescence (F) and maximum fluorescence (F'_m) of the illuminated samples were measured. Saturating pulses were applied at 40 s intervals. After 5 min of irradiation by actinic light, fluorescence signal reached the steady-state. Finally, F_0' value was determined after turning off the actinic light. All the values determined during the measurement (F_0, F_m, F'_m, F and F_0') allowed the calculation of PSII maximum quantum yield (F_v/F_m) and PSII effective quantum yield (Φ_{PSII}) according to MAXWELL and JOHNSON (2000).

The results were given as the average of four replicates ± standard error (SE).

Data analysis

Data were analysed by one-way analysis of variance (ANOVA) and subsequent Newman-Keul's test. Differences between treatments were considered statistically significant at $P < 0.05$. All statistical analyses were performed using the STATISTICA 7.1 (StatSoft, Inc., USA) software package.

Results

At the beginning of the experiment (after 2 days of exposure), the light quality and sucrose content did not affect duckweed frond number, which resulted in similar growth rates in all treatment groups. After 5 and 7 days of exposure to CW light, the duckweed growth rates did not change with variation of sucrose content. The same was noticed in plants exposed to GL light. However, when different light sources were compared, a stimulative effect of GL light was noticed in plants grown on 7.5 and 10 g L^{-1} sucrose. The growth rates of

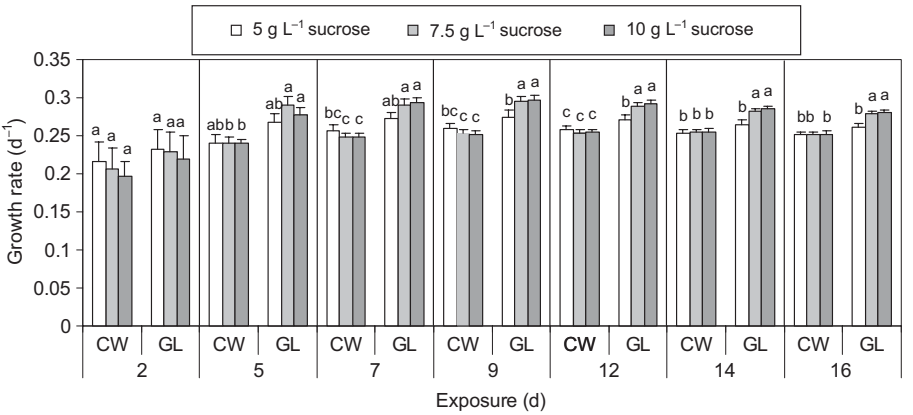

Fig. 1. Growth rates (GR) of *L. minor* during 16 days of cultivation on PS nutrient solution containing different sucrose supply under different light sources are shown as the mean of 20 replicates ± SE. Mean values were compared within each exposure time and were considered statistically significant at $P < 0.05$. Significantly different values for each treatment period (days) are marked with different letters.

plants grown on 5 g L^{-1} sucrose under GL light did not differ from those obtained under CW light. Starting with day 9, the growth rates increased at higher sucrose supply (7.5 and 10 g L^{-1}) but only in plants exposed to GL light. However, in plants grown on 5 g L^{-1} sucrose under GL light the growth rates were low and similar to those obtained under CW light at all sucrose supplies tested. The exception was growth rate observed after 12-days exposure to GL light, when even the lowest sucrose supply (5 g L^{-1}) allowed significant increase of growth rate in comparison to that obtained in plants exposed to CW light (Fig. 1).

At the end of the experiment (on day 16), cultivation on 5 g L^{-1} sucrose resulted in similar frond-number doubling times (2.77 and 2.65) under both tested light sources (CW and GL, respectively; Tab. 1). In plants exposed to CW light, addition of higher sucrose supplies did not change the doubling time. However, in plants grown under GL light, increase of sucrose supply (7.5 and 10 g L^{-1}) shortened the frond-number doubling time (6.0 and 6.3%, respectively). The stimulative effect of GL light on duckweed grown on higher sucrose supplies started as early as day 7 (data not shown), and continued until the end of the experiment. On day 16 the doubling time of plants grown on 7.5 and 10 g L^{-1} sucrose under GL light shortened by 9.7 and 10.1%, respectively, in comparison to plants exposed to CW light.

Tab. 1. Doubling time (T) after 16 days of cultivation of *Lemna minor* L. on PS nutrient solution containing different sucrose supply under different light sources. Each value represents the mean of 20 replicates ± SE. Comparisons between mean values were considered statistically significant at $P < 0.05$. Significantly different values are marked with different letters.

	5 g L^{-1} sucrose	7.5 g L^{-1}sucrose	10 g L^{-1}sucrose
CW light	$2.770^{a} \pm 0.041$	$2.763^{a} \pm 0.038$	$2.765^{a} \pm 0.047$
GL light	$2.653^{a} \pm 0.047$	$2.493^{b} \pm 0.025$	$2.485^{b} \pm 0.033$

Chlorophyll fluorescence was measured on days 7 and 16. On both days, the values of maximum quantum yield of PSII (F_v/F_m) ranged from 0.67 to 0.72 and showed no significant influence of sucrose supply and light source (Tab. 2). Like F_v/F_m, effective quantum yield of PSII (Φ_{PSII}) also showed no significant influence of sucrose supply and light source on day 7. However, on day 16 it decreased in plants grown on 5 g L^{-1} sucrose under CW light, the decrease being significant in comparison to plants grown on 7.5 and 10 g L^{-1} sucrose under GL lights.

Discussion

In natural conditions, duckweed prefers nutrient-reach waters, where the conditons of its exposure to light ranges from full sunlight to dense shade. Like many other species, it possesses a highly plastic photosynthetic apparatus allowing quick adaptation to changes in the light environment (Demmig-Adams and Adams 1992). In order to interpret the results properly, one has to take into consideration the effects of chamber conditions as well as the composition of the nutrient medium on the physiological processes of experimental plant.

The influence of light spectral distribution and intensity on plant growth rate and photosynthesis was proved a long time ago. The plants achieve optimal growth and productivity when exposed to light sources ensuring adequate spectral distribution. The

Tab. 2. Maximum quantum yield of PSII (F_v/F_m) and effective quantum yield of PSII (Φ_{PSII}) in *Lemna minor* L. after 7 and 16 days of cultivation on PS nutrient solution containing different sucrose supply under different light sources. Values represent means of four replicates ± SE which were compared within each exposure time and considered statistically significant at $P < 0.05$. Significantly different values are marked with different letters.

Light source	Sucrose content	Day 7		Day 16	
		F_v/F_m	Φ_{PSII}	F_v/F_m	Φ_{PSII}
CW	5 g L^{-1}	$0.671^{a} \pm 0.007$	$0.572^{a} \pm 0.008$	$0.698^{a} \pm 0.002$	$0.586^{b} \pm 0.005$
	7.5 g L^{-1}	$0.688^{a} \pm 0.004$	$0.600^{a} \pm 0.014$	$0.693^{a} \pm 0.002$	$0.607^{ab} \pm 0.017$
	10 g L^{-1}s	$0.693^{a} \pm 0.033$	$0.567^{a} \pm 0.031$	$0.707^{a} \pm 0.009$	$0.616^{ab} \pm 0.005$
GL	5 g L^{-1}	$0.689^{a} \pm 0.004$	$0.581^{a} \pm 0.010$	$0.705^{a} \pm 0.002$	$0.620^{ab} \pm 0.006$
	7.5 g L^{-1}	$0.687^{a} \pm 0.007$	$0.600^{a} \pm 0.014$	$0.709^{a} \pm 0.006$	$0.633^{a} \pm 0.014$
	10 g L^{-1}	$0.695^{a} \pm 0.003$	$0.622^{a} \pm 0.006$	$0.720^{a} \pm 0.011$	$0.637^{a} \pm 0.003$

photosynthetic pigments (chlorophylls and carotenoids) absorb light most strongly in blue and red regions of the spectrum, which coincides with the action spectrum of photosynthesis. So, it was not surprising that in our experiment plant growth was more pronounced in plants exposed to GL light, the spectral distribution of which corresponds more to the action spectrum of photosynthesis than CW light. Since plants as autotrophic organisms have the ability to convert light into chemical energy and produce organic compounds, nutrient media containing essential macro- and micronutrients should suffice for their growth. However, the light intensity used in this experiment (50 ± 5 µmol $_{PHOTONS}$ m^{-2} s^{-1}) was insufficient to ensure optimal photosynthetic rate. ISO and OECD protocols suggest performing 7-day Lemna assay on media containing mineral elements only, using light intensities of 85–125 µmol $_{PHOTONS}$ m^{-2} s^{-1} (ISO 20079, 2004; OECD 221, 2006).

During the first week of the experiment, the growth rates of plants exposed to the same light source (CW or GL) did not depend on the sucrose content of the nutrient medium. However, in a comparison of the effect of different light sources on plants supplied with the same amount of sucrose, the stimulative effect of GL light became evident. Hence, for example, after 5 day cultivation on 7.5 and 10 g L^{-1} sucrose, GL light revealed higher growth rates than CW light. This trend started on day 5 and continued until the end of the experiment. The results confirmed the stimulative effect of GL light on the growth of *L. minor* cultivated on higher sucrose supplies under low light conditions. At low-light conditions, 5 g L^{-1} sucrose did not satisfy the growth requirements under both, CW and GL light. During the second week of the experiment, plants grown on 5 g L^{-1} sucrose under GL light showed significantly lower growth rates than those obtained on higher sucrose supplies. However, under CW light the growth rate remained low even at 7.5 and 10 g L^{-1} sucrose.

ISO and OECD protocols (ISO 20079, 2004; OECD 221, 2006) recommend a doubling time of up to 2.5 days. In our experiment the doubling time of duckweed grown on 7.5 and 10.0 g L^{-1} under GL light, which showed the most pronounced growth, amounted 2.49 and 2.48 days, respectively. However, the doubling time of all other treatments exceeded 2.5 (Tab. 1) indicating suboptimal growth conditions.

Photosynthetic performance of duckweed grown under CW and GL light was evaluated using chlorophyll fluorescence. The F_v/F_m ratio is often used as a parameter reflecting the maximum (i. e. potential) quantum efficiency of PSII in dark-adapted plants (WU et al. 2003). The F_v/F_m values obtained in this experiment varied between 0.67–0.72, and as such they reached the lower limit of optimal F_v/F_m, for most plant species ranging between 0.7–0.83 (RITCHIE 2006). Therefore, F_v/F_m values obtained were still within the optimal F_v/F_m range. As opposed to F_v/F_m, effective quantum yield of PSII (Φ_{PSII}) is considered an indicator of actual photochemical efficiency of PSII in plants exposed to light (WU et al. 2003). In this experiment, Φ_{PSII} attained values of 0.57–0.64 which is in accordance with the normal Φ_{PSII} range, varying between 0.4–0.6, as proposed by RITCHIE (2006). Our results showed that there were no significant differences in duckweed F_v/F_m and Φ_{PSII} after 7 and 16 days of exposure to CW and GL light, regardless of the sucrose content. The only exception was Φ_{PSII} obtained in plants grown on 7.5 and 10 g L^{-1} sucrose after 16 days of exposure to GL light, which showed the highest values. Φ_{PSII} of the remaining treatment groups showed a downward trend, with the lowest value obtained in plants grown on 5 g L^{-1} sucrose under CW light (Tab. 2). As plants acclimated to low light conditions maximize photon energy capture due to more efficient light-harvesting complex (ANDERSON et al. 1988), they highly depend on the light source. In our experiment the effect of inappropriate spectral characteristics of CW light source was most obvious in plants grown on medium supplied with the lowest energy source in the form of sucrose, where the minimum value of Φ_{PSII} was noticed. The most efficient photosynthetic performance of the plants grown on 7.5 and 10 g L^{-1} sucrose under GL light was probably due to the spectral distribution of GL light which favours photosynthesis more than CW light. Plants grown on nutrient media containing sucrose depend on light intensity less than plants grown on media containing mineral nutrients only. It has been shown previously that sucrose supply decreased photosynthetic performance, probably because it provides enough energy for other metabolic activities thus supporting heterotrophic and photomixotrophic growth (FRICK 1994, JO et al. 2009 and references therein). This is in agreement with the slight increase of photosynthetic performance in duckweed grown under low light intensity (50 μmol $_{PHOTONS}$ m^{-2} s^{-1}) on media containing mineral nutrients only (ARTETXE et al. 2002) when compared with values we obtained on medium containing sucrose.

According to the results obtained, it may be concluded that the higher growth rate and photosynthetic performance in plants exposed to GL light were related to its spectral energy distribution favouring photosynthesis. Since plants cultivated on higher sucrose content (7.5 and 10 g L^{-1}) showed better growth than those on 5 g L^{-1} sucrose, it indicated the promotive effect of sucrose in plants grown under low light conditions.

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