Aquatic Botany 91 (2009) 151–156

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Physiological acclimation to light in *Chara intermedia* nodes

Miriam Gerhardt [a], Susanne Schneider [b], Wolfgang Brüggemann [a,*]

[a] University of Frankfurt, Institute for Ecology, Evolution and Diversity, Siesmayer Street 70, D-60323 Frankfurt, Germany
[b] Norwegian Institute of Water Research, Gaustadalléen 21, NO-0349 Oslo, Norway

ARTICLE INFO

Article history:
Received 1 October 2008
Received in revised form 4 May 2009
Accepted 6 May 2009
Available online 18 May 2009

Keywords:
Chara intermedia
Light acclimation
Photosynthesis
Physiology
Morphology
Pigment ratios

ABSTRACT

In this study we investigated the ability of *Chara intermedia* to acclimate to different irradiances (i.e. "low-light" (LL): 20–30 μmol photons m^{-2} s^{-1} and "high-light" (HL): 180–200 μmol photons m^{-2} s^{-1}) and light qualities (white, yellow and green), using morphological, photosynthesis, chlorophyll fluorescence and pigment analysis.

Relative growth rates increased with increasing irradiance from 0.016 ± 0.003 (LL) to 0.024 ± 0.005 (HL) g g^{-1} d^{-1} fresh weight and were independent of light quality. A growth-based branch orientation towards high-light functioning as a mechanism to protect the plant from excessive light was confirmed. It was shown that the receptor responsible for the morphological reaction is sensitive to blue-light.

C. intermedia showed higher oxygen evolution (up to 10.5 (HL) vs. 4.5 (LL) nmol O_2 mg Chl^{-1} s^{-1}), photochemical and energy-dependent Chl fluorescence quenching and a lower *Fv*/*Fm* after acclimation to HL. With respect to *qP*, the acclimation of the photosynthetic apparatus depended on light quality and needed the blue part of the spectrum for full development. In addition, pigment composition was influenced by light and the Chl a/Car and Antheraxanthin (A) + Zeaxanthin (Z)/Violaxanthin (V) + A + Z (DES) ratios revealed the expected acclimation behaviour in favour of carotenoid protection under HL (i.e. decrease of Chl a/Car from 3.41 ± 0.48 to 2.30 ± 0.35 and increase of DES from 0.39 ± 0.05 to 0.87 ± 0.03), while the Chl a/Chl b ratios were not significantly affected. Furthermore it was shown that morphological light acclimation mechanisms influence the extent of the physiological modifications.

1. Introduction

Charophytes (Charales, Charophyceae) are submerged green algae with a macroscopic thallus and an equisetum-like growth form, attached to the substratum by rhizoids. Charales can be found both in brackish and in fresh water habitats (Krause, 1997) and colonise depths from close to the water surface down to 30 m (Schwarz et al., 1996) with irradiances of as low as 14.5 μmol m^{-2} s^{-1} or less (Steinman et al., 1997). Underwater light climate is highly variable because of sun position, weather conditions, water colour, turbidity, depth and wave form (Schneider et al., 2006; Schubert et al., 2001). Reported acclimation strategies of other algae, including marine Charales, to different light climate conditions include both mechanisms for effective light use at deep, low light stands as well as protective mechanisms against excessive light (Sorrell et al., 2001; Schwarz et al., 2002; Küster et al., 2004). Light acclimation also includes chromatic acclimation. In several unicellular plankton algae of the Chlorophyta, it has been shown that blue-light enhances photosynthetic activity at various levels (summarized in Mouget et al., 2004).

Chara intermedia is a species of oligotrophic freshwater stands. Especially in deep clear lakes near the Alps *C. intermedia* is very dominant and forms dense algae beds. Its thallus reaches a length of up to 80 cm (Krause, 1997). It has been shown earlier (Schneider et al., 2006) that *C. intermedia* (as well as *Chara hispida*) shows a morphological light acclimation by differential growth responses: under low-light, its lateral branches are spread horizontally outwards and under high-light they grow vertically upwards towards the light. This has been explained in terms of optimal light use under conditions of growth in dense algae beds, because light can penetrate into deeper areas of an algae bed. It is also assumed that the upward orientation of the youngest (top) branches could be a protection against excessive light. In the present contribution, two questions will be addressed:

1: Does *C. intermedia* react both morphologically and physiologically to different irradiances?and
2: Does light quality influence the acclimation responses (i.e. are blue-light receptors or phytochromes responsible)?

* Corresponding author. Tel.: +49 69 79824745; fax: +49 69 79824822.
E-mail address: w.brueggemann@bio.uni-frankfurt.de (W. Brüggemann).

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doi:10.1016/j.aquabot.2009.05.002

2. Materials and methods

2.1. Site descriptions

Specimens of *C. intermedia* were collected from the Lauterbach River, Germany (47°33′N, 11°09′E; 650 m above sea level). The Lauterbach River is an oligotrophic groundwater-fed river located east of Oberau, Bavaria. At the stand, the algae form dense beds which float with the streaming water and are exposed to full sunlight for part of the day.

2.2. Experimental setup

All experiments were performed in a climate chamber running at 17 °C and 12 h light period, provided by incandescent light ("Fluora" 58 W, Osram, Germany, and white light, 58 W, Philips, Netherlands, at 1:1 ratio). Different irradiances (i.e. "low-light" (LL): 20–30 μmol photons m^{-2} s^{-1}, resembling the light conditions at the lower depth range of the occurrence of the Characeae, and "high-light" (HL): 180–200 μmol photons m^{-2} s^{-1}, equivalent to average daylight intensities in approximately 1–2 m depth of a clear English lake, (Pearsall and Ullyott, 1934)) were realised by adjusting the distance of the growth beakers from the lamps and/or using black nylon gaze (mesh width 1 mm, Farilia) as a diffuse neutral filter. Light intensities were measured with a LICOR LI-190 quantum sensor (LICOR, Lincoln, Nebraska, USA) at the exact position of each alga by moving the beakers or aquarium, respectively, and assuming that absorbance by the water layer above the plant was negligible. To ensure equal starting conditions for all experiments, charophytes were cut leaving intact the top two–three nodes and the head; for measurements the oldest (basal) node is referred to as node one and higher numbers indicate higher/younger nodes. Plants were planted upright either in a dense bed in an aquarium at a water column height of 30 cm, or as individual plants in glass beakers containing 500 mL per plant with a water column height of 15 cm, using quartz sand as the substrate and filled with local tap water (pH: 7.4–7.7, conductivity: 395–520 μS cm^{-1}, water hardness (Ca^{2+} + Mg^{2+}): 2–3 mM, nitrate: 0.2–0.3 mM) as medium. The initial (m_i on day d_i) and final (mf on day d_f) plant fresh weights (fw) were determined after carefully drying the plants on tissue paper. The relative growth rates (RGR) were calculated as $\ln(m_f/m_i)/(d_f - d_i)$ in g (fw) g^{-1} (fw) d^{-1}. To differentiate between light acclimation effects mediated by blue-light receptors or the phytochrome system, some growth experiments were carried out under yellow and green-light, which was realised by filtering the light from the incandescent tubes either through LEE filter foil 767 Oklahoma Yellow, cutting off light below 460 nm, i.e. in the absorption range of the blue-light receptors, or through foil 124 Dark Green, with significant transmission only between 450 and 550 nm and above 720 nm, i.e. outside the absorption range of the phytochrome system (Lee Filters, Andover, Hampshire, UK; for absorption spectra cf. www.leefilters.com). For analysing morphological changes under different light-qualities, the angles between branch tip and plant axis were measured before and after the cultivation.

2.3. Chlorophyll fluorescence and photosynthesis measurements

Chlorophyll fluorescence and gross oxygen evolution (taking into account the respiratory rate in the dark) were measured at 19 °C on individual nodes with intact side branches with 10 mM $KHCO_3$ (pH 8.0) to ensure CO_2-supply in a self-made temperated cuvette (Volume: 3.5 mL) from stainless steel with a Plexiglas lid, which could be illuminated from the top by a four-arm fibre optic and which was equipped with a magnetic stirrer and an oxygen electrode from the side (YSI Model 5300, Biological Oxygen Monitor, Scientific Division, Yellow Springs Instrument Co., USA). The nodes were put into the cuvette in their natural orientation, except for node three of high-light plants, which was spread open before closing the lid to ensure that measured fluorescence signals stemmed from the branches' upper surface. Irradiance was provided by attaching the fibre optic to the emitter-detector unit of the PAM 101 unit (Walz, Germany) and two KL 1500 white light sources for actinic and saturating flash-light (Schott, Germany). The Chl fluorescence quenching parameters were calculated as described earlier after 15 min of pre-darkening (dark-acclimated state) or 15 min of actinic light (light-acclimated steady-state), respectively (Brüggemann, 1992). With *Fm* being maximum fluorescence in the dark-acclimated state prior to illumination with actinic light, *Fm′* the maximum fluorescence in the light-acclimated steady-state after 15 min of illumination and *Fm″* the linear extrapolated value of the maximum fluorescence values measured for 15 min at three min intervals in the dark after the irradiance period to the point of time, when the actinic light was switched off (for details cf. Brüggemann, 1992), the non-photochemical Stern-Volmer quenching coefficients are given as:

$$qE_{SV} = (Fm/Fm') - (Fm/Fm'')$$

$$qI_{SV} = (Fm/Fm'') - 1$$

(Krause and Jahns, 2003).

2.4. Pigment analysis

Samples taken for pigment analysis by HPLC under the growth light conditions were immediately transferred into liquid nitrogen and stored at −23 °C until extraction. The material was crushed to powder under liquid nitrogen with mortar and pestle in the presence of $CaCO_3$ and extracted with 0.5 mL acetone. After centrifugation and filtration (Rotilabo PTFE microfilter, 0.2 μm pore width, Carl Roth GmbH, Germany), the supernatant was analysed on a 100 RP-18 LiChrospher column (Jasco, Germany) by HPLC, using the gradient system of Färber et al. (1997) with 87% (v/v) acetonitril, 10% (v/v) methanol, 3% (v/v) Tris-HCl, 100 mM, pH 8.0 as eluent A and 20% (v/v) n-hexane, 80% (v/v) methanol, as eluent B. Peaks were detected at 440 nm and quantified using the factors given by Färber et al. (1997), corrected for a conversion factor for our detector sensitivity using a solution of known concentration of purified ß-carotin (Beyel, 2003). Deepoxidation states (DES) were calculated using the molar concentrations of Violaxanthin (V), Antheraxanthin (A) and Zeaxanthin (Z) according to DES = (A + Z)/(V + A + Z). Chl a and Chl b concentrations and Chl a/Car ratios were determined directly by absorption measurements of 80% (v/v) acetone extracts at 663, 645 and 500 nm according to Arnon (1949) and Schneider et al. (2006).

2.5. Statistical analyses

To determine differences within the data of growth, photosynthesis, and pigmentation of samples grown at different light conditions a one-way ANOVA with Tukey *post hoc* test was applied. For examining the data for normal distribution and homoscedasticity the Kolmogorov-Smirnov-test and the Levene test were applied, respectively. If the Levene test was significant, a Kruskal–Wallis test was applied instead of ANOVA. In case of a comparison of two groups a *t*-test was used. The used tests are marked in brackets. A significant difference was detected at the 0.05 level.

3. Results

3.1. Growth and light acclimation of photosynthesis to LL and HL

When measured one day after the sampling from the Lauterbach River, Chl fluorescence parameters of all nodes showed no significant differences (Table 1).