Environmental and Experimental Botany 136 (2017) 50–58

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Research Paper

Temporal dynamics in photosynthetic activity of *Spirodela polyrhiza* turions during dormancy release and germination

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ARTICLE INFO

Article history:
Received 1 December 2016
Received in revised form 10 January 2017
Accepted 10 January 2017
Available online 16 January 2017

Keywords:
Spirodela polyrhiza
Turion
Dormancy and after-ripening
Chlorophyll fluorescence
O_2-evolution
Photosynthetic pigment content

ABSTRACT

Turions (vegetative propagules) of aquatic plants functionally resemble seeds but in fact they are overwintering buds having the ability to photosynthesize. They spend long periods in dormant state in the dim and cold bottom layer of natural waters during winter of temperate regions. In this study photosynthetic acclimation of *Spirodela polyrhiza* (L.) Schleiden (giant duckweed) turions to contrasting environments was assessed in laboratory experiments by means of chlorophyll fluorescence induction and O_2-evolution methods and photosynthetic pigment content.

Photosynthetic performance of turions had been monitored for 5 weeks in dark and cold (after-ripening) and then for 48 h under continuous irradiation at room temperature (germination induction).

Photosynthetic activity of turions displayed biphasic acclimation during after-ripening. A gradual decrease in O_2-evolution, variable fluorescence and relative fluorescence decrease was observed during the first 3 weeks with a parallel increase in chlorophyll fluorescence parameters indicative of excitation energy dissipation. Most interestingly later, while turions were still kept in dark and cold, their photosynthetic activity increased again to the level of newly-formed turions. This temporal pattern suggests that changes in photosynthetic performance might be regulated in close connection with dormancy release of turions.

After transferring after-ripened turions to warm and illuminated conditions their photosynthetic acclimation took place rapidly. All parameters reflecting photosynthetic efficiency of germinating turions reached or even exceeded the respective levels of newly formed control turions within 24–48 h.

1. Introduction

Dormancy is a crucial life stage in survival of plants under adverse environmental conditions (e.g. dry and cold periods). Seeds and buds could tolerate extreme conditions and, depending on complex regulation by internal and external factors, are ready to produce descendant plants or continue growing when favourable environmental conditions return (Thomas and Vince-Prue, 1997; Finch-Savage and Leubner-Metzger, 2006; Klimešová and Klimeš, 2007).

Turions of aquatic plants represent an interesting dormancy strategy. These modified vegetative organs are produced asexually like normal buds but functionally they resemble seeds lacking the ability for further growth, instead, they produce progeny plants (Appenroth et al., 1996; Wang et al., 2014). In temperate regions turion-like vegetative propagules are usually formed during late summer and autumn and having higher density than water they sink down and overwinter on the bottom and buried by sediment.

Abbrebviations: ABA, abscisic acid; Car, carotenoid; Chl-*a*, chlorophyll-a; Chl-*b*, chlorophyll-b; Chl-*a*+*b*, total chlorophyll content; Chl-*a*/*b*, chlorophyll-a to b ratio; I-D intermediate, bending or plateauing of the fluorescence signal during fast chlorophyll fluorescence induction; F_o, ground fluorescence signal; Fm, maximum fluorescence signal; F_t, steady state fluorescence signal; F_v/F_m, maximal quantum yield of PSII photochemistry; PN, net photosynthesis; PPFD, photosynthetic photon flux density; PQ, plastoquinone; PSII, photosystem II; Q_A, primary quinone acceptor; R_D, dark respiration; R_{fd}, chlorophyll fluorescence decrease ratio; S_m, normalized complementary area over the fast chlorophyll fluorescence transient curve; Y(II) or $\Delta F/F_m$', actual quantum yield of PSII photochemistry in light adapted sample; Y (NO), quantum yield of non-regulated non-photochemical quenching; Y(NPQ), quantum yield of regulated non-photochemical quenching; VAZ, Z/VAZ, A/VAZ, V/VAZ - total viola, , anthera- and zeaxanthin pool and relative proportions of its components.

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http://dx.doi.org/10.1016/j.envexpbot.2017.01.007
0098-8472/

(Adamec, 2008; Appenroth et al., 2013). This way they avoid freezing on water surface though the hypoxic, dark and cold environment inhibit their normal metabolism (Adamec, 2008). Turions maintain very low metabolic activity and thereby preserve their nutrient and carbohydrate reserves for new sprouts (Adamec, 2010).

Among aquatic plants turions of *Spirodela polyrhiza* (L.) Schleiden (giant duckweed) are the most studied. Turions of *S. polyrhiza* provide a unique model system for plant physiology research (Appenroth et al., 1996). Because of their small size and easy production by application of abscisic acid (ABA) (Perry and Byrne, 1969; Smart and Trewavas, 1983; Wang et al., 2014) or nutrient limitation (Appenroth et al., 1989, 1996) they have been widely used in studying morphogenesis, endo- and exogenous regulators of dormancy and germination (Appenroth and Bergfeld, 1993).

Germinating *S. polyrhiza* turions form an external gas bubble to reach the water surface. This bubble was originally assumed to be O_2 as the result of photosynthesis (Jacobs, 1947; Beer, 1985). Formation of gas bubble in complete darkness, however, might indicate that it consists of CO_2 produced by germination-induced metabolization of carbohydrates (Newton et al., 1978). A similar 'CO_2-propelled' lifting mechanism was hypothesized by Adamec (2003) in *Aldrovanda vesiculosa* L. turions.

Light-grown (i.e. non-etiolated), newly formed turions are green and even they retain photosynthetic pigment content during after-ripening (Adamec, 2011). This suggests that turions has photosynthesizing capability and preserve it during the dormancy (Czopek, 1967; Beer, 1985; Adamec, 2011). Photosynthetic properties of turions are, however, less discussed in the literature most probably because photosynthesis supposedly has smaller importance in their survival and early phases of germination. Most PSII reaction centers in *S. polyrhiza* turions are not functional (Susplugas et al., 2000) and net photosynthesis in turions of various aquatic plant species was reported to be close to zero or even negative after their formation (Czopek, 1967; Adamec, 2011). Being storage organs they rely on their carbohydrate reserves during dormancy (Ley et al., 1997; Adamec, 2008). Thus photosynthetic pigments might rather be preserved to avoid *de novo* synthesis during germination (Adamec, 2011). Rapid light acclimation, on the other hand, might play important role in energy and carbohydrate supply for germination after surfacing of turions thus explaining why turions preserve their photosynthetic pigments. From this point of view turions might display a similar strategy of photosynthetic acclimation to that of overwintering terrestrial evergreen plants.

Maintaining evergreen foliage during winters is a common strategy amongst woody species in boreal forests (Öquist and Huner, 2003). Overwintering photosynthetic tissues are exposed to combined effects of low temperature and temporarily high irradiance exceeding sink capacity of enzymatic reactions in photosynthesis (Huner et al., 1993). In order to avoid photooxidative damages these plants usually down-regulate transfer of absorbed light energy to photosynthetic processes. Decreased antenna sizes, increasing number of non-functioning reaction centers and de-epoxidation of violaxanthin to anthera- and zeaxanthin (xanthophyll-cycle) help in dissipation of excess excitation energy (Demmig-Adams et al., 1999; Öquist and Huner, 2003; Verhoeven, 2014). Recovery from this cold-acclimated state takes place shortly upon warming. The increase in PSII efficiency is biphasic starting with a rapid (several hours) phase and followed by a slower (several days) one including *de novo* synthesis of proteins involved in photosynthesis (Verhoeven et al., 1996; Öquist and Huner, 2003). A similar pattern of down-regulation in the winter period and a consequent recovery of photosynthetic traits during germination can be assumed in case of turions.

Regulation of dormancy prevent turions from premature germination until the habitat becomes suitable again (Adamec, 2003). Newly formed turions are in the state of innate dormancy that is they cannot germinate even under favourable environmental conditions (e.g. during late summer) due to endogenous regulators. To break this state and enter the imposed dormancy state −when only external factors block the germination- turions require after-ripening under defined combination of environmental conditions (e.g. temperature, light intensity, photoperiod). When imposed dormancy is broken by the appropriate signal germination starts quickly. In case of *S. polyrhiza* turions germination is triggered by red light and maximal responsivity can be reached by 4 weeks of after-ripening in dark and cold (Appenroth et al., 1996). Turions of free-floating plants gain buoyancy and emerge to the water surface within days where they restart the life cycle (Appenroth et al., 1989; Adamec, 2011; Oláh et al., 2016). Contrasting conditions of the dark and cold water bottom and the warmer, sunlit water surface inevitably require plastic modulation of photosynthetic traits which can be co-regulated by other factors of turion dormancy.

The aim of the present study was to assess changes in photosynthetic performance of *S. polyrhiza* turions in consecutive developmental stages starting from their detachment from mother frond through after-ripening in cold and dark and finished with germination under irradiation at room temperature. Our work focused on the following questions: i) whether turions maintain the photosynthetic capacity under after-ripening conditions or gradually release it; and ii) by what mechanisms and how fast photosynthesis of turions adapts to irradiation after several weeks-long stay in cold and dark.

2. Materials and methods

2.1. Stock culturing, after-ripening and germination of turions

Turions of *S. polyrhiza* clone 5501 were obtained from axenic cultures maintained at the Dpt. Botany, University of Debrecen, Hungary (Oláh et al., 2015). Growing fronds of the clone were maintained in sterile Steinberg's medium (pH 5.5) under constant irradiation (PPFD: $80 \pm 10\,\mu mol\,m^{-2}\,s^{-1}$, GE Polylux F30W/830) and temperature ($22 \pm 2\,^{\circ}C$) and were forced to produce light-grown turions by phosphate limitation (Appenroth et al., 1996; Oláh et al., 2016). Detached and sunken turions were collected, transferred to freshly prepared sterile Steinberg's medium (pH 5.5), and stored under culturing conditions until after-ripening treatments. These turions were regarded and hereafter referred to as 'control' state representing newly formed turions with full innate dormancy (0 week of after-ripening).

After-ripening of turions was conducted by transferring them in pure Steinberg's medium to a cool and dark room ($6\,^{\circ}C$). The maximum length of after-ripening was set to 5 weeks in order to make sure that innate dormancy of turions was completely diminished (Appenroth et al., 1989). During after-ripening period turions were sampled weekly (samples hereafter referred to as 1, 2, 3, 4 and 5 w, respectively) for physiological measurements. After 5 weeks of after-ripening turions were placed on filter paper soaked with Steinberg's medium in sealed Petri-dishes and transferred to germination-inducing conditions identical to those of stock culturing (PPFD: $80 \pm 10\,\mu mol\,m^{-2}\,s^{-1}$ constant warm white irradiation, $22 \pm 2\,^{\circ}C$) and were let germinate for 48 h. The length of germination period was limited to 48 h in this study since after 2 days newly formed fronds start to protrude (Appenroth et al., 1996; Oláh et al., 2016) and thus might affect either determination of biomass or influence the assessed photosynthetic parameters. During germination period turions were sampled for physiological measurements 4, 8, 24 and 48 h after transferring them to