Aquatic Botany 136 (2017) 39–42

Contents lists available at ScienceDirect

Aquatic Botany

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

Short communication

Photosynthetic activity detected in the seed epidermis of *Thalassia testudinum*

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ARTICLE INFO

Article history:
Received 2 October 2015
Received in revised form 6 June 2016
Accepted 13 September 2016
Available online 15 September 2016

Keywords:
Seed
Photosynthesis
Thalassia testudinum
Seagrass

ABSTRACT

Seagrasses are marine angiosperms that have evolved from terrestrial ancestors. Photosynthetic activity on seed has been studied before in *Posidonia* genus. *T. testudinum* and *P. oceanica* share some evolutionary aspects as they live in more stable environments. As in *P. oceanica*, *T. testudinim* seed is also green-coloured suggesting the presence of photosynthetic pigments. Here, the photosynthetic activity in the *T. testudinum* seed was examined. Measurements of photosynthetic production, pigment content and maximum PSII photochemical efficiency (Fv: Fm) were determined. Epifluorescence microscopy analysis was used to obtain evidence of photosynthetic structures within the seed. This study revealed photosynthetic activity in seed epidermis which increased linearly with increasing irradiance. *T. testudinum* seed displayed a higher Photosynthetic efficiency (α), and lower light saturated photosynthesis (P. max), saturation irradiance (Ek), and compensation irradiance (Ec) compared to adult leaves. Epifluorescence microscopy images of the seeds showed an epidermis composed of relatively small, regular cells, tending towards a rectangular shape, containing abundant chloroplasts. The photobiological behaviour results in the *T. testudinum* seed being strongly adapted to colonize environments with reduced light availability. Photosynthetic activity in *T. testudinum* seed shed some light on seagrass dispersal and expansion mechanisms.

1. Introduction

Seagrasses are fully submerged aquatic plants and possess numerous adaptations that promote their growth and survival in permanently flooded sediments (Cummings and Zimmerman, 2003). Adaptive morphological features include arenchyma that transports photosynthetically-generated oxygen from the shoot to below-ground tissues (Smith et al., 1984); flowers specifically adapted for underwater pollination (Ackerman, 1986); leaves that lack stomata and are covered by a thin, porous cuticle that permits gas and solute transfer; and an epidermis rich in chloroplasts and a largely unpigmented mesophyll (Hemminga and Duarte, 2000).

Some seagrasses are short-lived, with fast growth and high seed production relying on long-lived seeds, while other species largely rely on asexual reproduction for population maintenance (Rasheed, 1999; Waycott et al., 2006). The seagrass beds in the Caribbean are subject to major meteorological events such as hurricanes and tropical storms (Reading, 1990; Creed et al., 2003). A reproductive strategy involving clonal growth and production of long-lived, locally dispersed seeds, may provide an evolutionary advantage to plants growing in environments subject to temporally unpredictable disturbances (Rasheed et al., 2004; Williams, 1975).

The herbaceous flowering plant known as turtle-grass, *Thalassia testudinum* Banks ex Köning is found in extensive submarine beds throughout the Caribbean and the Gulf of Mexico (Humm, 1956; Thorne, 1954). *Thalassia* plant are dioecious, the male and female flowers being produced by different clones (Cox and Tomlinson, 1988). The mature fruit of *T. testudinum* is an elliptical to globose capsule, 20–25 mm wide by 15–20 mm high, with a beak of 3–7 mm. The fruit floats on the surface in the water column and contains between one and four negatively buoyant seeds that lack dormancy (Lacap et al., 2002). After dehiscence, the seed due to negative buoyancy, sinks to the bottom, where primary roots and a leaf system rapidly develop. The seed of *T. testudinum* is green-coloured (Fig. 1), suggesting the presence of photosynthetic pigments. This morphological feature has been related before with photosyn-

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http://dx.doi.org/10.1016/j.aquabot.2016.09.004
0304-3770/

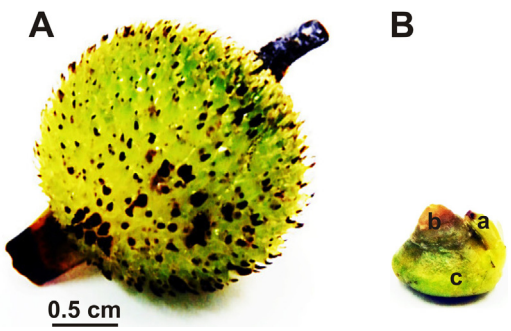

Fig. 1. Appearance of *Thalassia testudinum* fruit and seed collected. A: Mature fruit. B: Different areas of the seed (scale bar = 0.5 cm), a: Plumule; b: Chalaza; c: Cotyledon.

thetic activity in seeds of the *Posidonia oceanica* (Celdran and Marín, 2011). Photosynthetic activity of *P. oceanica* seeds enhanced seedling growth of leaves and roots, was found functional and more active than leaves during early seedling growth (Celdrán and Marín, 2013). Last studies discovered photosynthetic activity in seed of two more species of the *Posidonia* genus, (*P. australis* and *P. sinuosa*), (Celdran et al., 2015). The green-coloured seed of *T. testudinum* could indicate photosynthesis activity in a genus different than *Posidonia.*

The aim of this study was to determine whether *T. testudinum* seed has photosynthetic activity through the determination of seed photosynthetic production, pigment content and maximum PSII photochemical efficiency (Fv: Fm). Furthermore, epifluorescence microscopy analysis was utilised to obtain evidences of photosynthetic structures.

2. Materials and methods

2.1. Seed collection site

T. testudinum fruits deposited along the shoreline of Benito Juarez, Quintana Roo, Mexico, were collected during October 2013. *T. testudinum* flowering usually takes place typically in June however late flowering has been reported previously (Durako and Moffler, 1987; Moffler et al., 1981). Seeds were extracted from not open mature fruits immediately after collection. Only seeds that showed no sign of herbivory or mechanical damage were selected. Seed leaf plumules were covered with black plastic to avoid a possible interaction with seed epidermis photosynthetic production.

2.2. Photosynthetic production and maximum PSII photochemical efficiency (Fv: Fm)

To determine photosynthetic production, the methodology of Cayabyab and Enríquez (2007) was followed with slight modifications for seed, rather than leaf, photosynthetic analysis. Seeds (n = 6) were placed individually in 12 mL borosilicate vases within water-jacked chamber (DW3, Hansatech Instruments Ltd., Norfolk, UK). Seeds were orientated with the basal area pointed to the source light thus ensuring that the epidermis of the cotyledon was illuminated. The tip of the instrument's optical fiber was placed 10 mm from, and perpendicular to, the seeds' surface. Incubations were run immediately after seed extraction from the fruit.

Oxygen evolution was measured as Net Production and then converted in Gross Production (GP) by adding respiration values. Oxygen values were normalized to seed area, incubation time (1 h) and the results expressed as μmol O_2 cm^{-2} h^{-1}. The software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, 1997–2016) was used to obtain the seed areas from pictures of plastic papers previously placed surrounded every seed.

A Clark-type O_2 electrodes (Hansatech) was used within a circulating bath with a controlled temperature system (RTE-100/RTE 101LP; Neslab Instruments Inc., Portsmouth, NH, USA) maintaining filtered seawater at 28 °C. The photosynthesis–irradiance curves (P vs I) were determined by exposing seeds 30 min to nine irradiance levels: 5.6, 9.8, 19.4, 40.5, 85, 160, 356.3, 755.8 and 1380 μmol quanta m^{-2} s^{-1}, while respiration was determined in dark conditions. Irradiance doubled at each irradiance level until a saturated level (approximately 1000 μmol quanta m^{-2} s^{-1}) was obtained. The electrodes were calibrated with air and N_2 saturated filtered seawater. Oxygen levels within the incubation chambers were maintained at 20–80% saturation by bubbling with N_2 gas. Chamber was flushed with N_2 and air before each light incubations and respiration incubation respectively. GP of the *T. testudinum* seeds were plotted against the varying irradiance levels. Light saturated photosynthesis (P. max) was determined from the average maximum values above saturating irradiance from the P vs I curves. Photosynthetic efficiency (α) was obtained from the initial slope of the light response curve by linear least-squares regression analysis, the compensation irradiance (Ec) was determined from the intercept on the abscissa and the saturation irradiance (Ek) was estimated as the ratio: P. max/α. Immediately after incubations, seeds were placed into Petri dishes with water flushed with air and kept in dark 10 min to ensure relaxation of photosynthetic structures. Maximum PSII photochemical efficiency (Fv: Fm) was determined on dark-acclimated seeds by an underwater pulse amplitude modulated fluorometer (Diving-PAM; Walz).

2.3. Pigment content and epifluorescence microscopy

Pigment content in seeds was determined spectrophotometrically at 447, 664 and 750 nm with an Ocean Optics USB4000 spectrophotometer (Ocean Optics, Dunedin, USA) using a xenon light source (PX-2, Ocean Optics, Dunedin, USA) after manual extraction of a homogenized suspension from every seed mashed with 80% acetone (Dennison, 1990). The acetone extracts (4 mL) were stored at 4 °C in the dark for 12 h and centrifuged. Chl *a* and Chl *b* concentrations were quantified using the equations of Jeffrey and Humphrey (1975).

With respect to epifluorescence microscopy, when the cell of the *T. testudinum* seed epidermis was irradiated with a fluorescent light source, an auto-fluorescence process was pictured; no stain was used. To detect chloroplasts in the seed epidermis, seeds were cut into fine slices with a blade. Cells were observed by epifluorescence microscopy under a Zeiss Axioskop 40 microscope (Carl Zeiss, Göttingen, Germany) with 63× objectives using two filters, one blue cyan filter (filter set 02) and one green filter (filter set 09). Data were acquired using a Canon Power Shot A640 camera (Canon Inc., Japan) and Axio Vision software (Carl Zeiss). To determine seed biomass (dry weight), ten seeds were placed in an oven at 60 °C for 48 h. After drying, the samples were put inside a desiccator and weighed using an analytical balance (E-11140; PineBrook, NJ, USA) with 0.1 mg precision.

2.4. Data analysis

Analyses of variance, one-way ANOVA, were used to examine significant differences in (GP) among irradiances. Prior to one-way ANOVA, data were tested for normality using Kolmogorov–Smirnov tests and homogeneity of variance using Cochran test. Significance level for all tests was set to p = 0.05. If the data were not parametric, then a Kruskal–Wallis test was applied. After one-way ANOVA