Flora 206 (2011) 1085–1091

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.de/flora

The effect of flow turbulence on plant growth and several growth regulators in *Egeria densa* Planchon

Champika Ellawala Kankanamge, Takashi Asaeda*, Kiyoshi Kawamura

Institute for Environmental Science and Technology, Saitama University, 255, Shimo-okubo, Sakura, Saitama 338-8570, Japan

A R T I C L E   I N F O

Article history:
Received 23 February 2011
Accepted 17 June 2011

Keywords:
Turbulence
Aquatic macrophyte
Indole acetic acid
Cytokinin
Oxidative stress
Chlorophyll

A B S T R A C T

The effects of turbulence velocity on *Egeria densa* Planchon was studied for 12 weeks using mechanically oscillating grid-generated turbulence without mean flow. The root-mean-square of the turbulence velocity fluctuations (u') ranged from 1.62 ± 0.44 to $2.86 \pm 0.8\,\mathrm{cm\,s^{-1}}$ (high turbulence), 1.36 ± 0.2 to $1.86 \pm 0.78\,\mathrm{cm\,s^{-1}}$ (medium turbulence) and 0.67 ± 0.12 to $0.81 \pm 0.16\,\mathrm{cm\,s^{-1}}$ (low turbulence). The control was subjected to gentle manual mixing once a day. Shoot elongation was significantly reduced with increasing turbulence intensity, and the endogenous indole acetic acid (IAA) concentration was significantly decreased with increasing turbulence intensity and exposure time. The plants exposed to high turbulence showed a 64.6% decrease in endogenous IAA concentration compared to the control, while it was decreased only 26.9% in plants exposed to low turbulence. IAA and cytokinin catabolism was increased, and there was an increase in the hydrogen peroxide concentration of the tissues, which triggered peroxidase activity. The total chlorophyll and chlorophyll a content decreased with the time of exposure. Although the flow turbulence negatively affected plant growth and metabolism, all of the plants survived for the experimental period.

Introduction

Water movement is a primary factor that regulates the growth and distribution of aquatic macrophytes in natural ecosystems (Madsen et al., 2001). Submerged macrophytes in flowing waters experience drag and lift forces that are caused by the flow velocity (Madsen et al., 2001; Sand-Jensen, 2008). In shallow lakes, wind-induced currents and waves create forces on the macrophytes, and the magnitude of these forces depends on depth and fetch (Schutten et al., 2004). Hence, macrophytes in both lentic and lotic ecosystems experience mechanical stresses that are induced in various scales by water movement (Schutten et al., 2004). Additionally, low current velocities and small-scale turbulence can increase nutrient uptake by reducing the boundary layer thickness (Madsen et al., 2001). Permanent exposure to flow and turbulence alters the morphological features and growth pattern of the resident plant life (Puijalon and Bornette, 2006). Plants subjected to mechanical stress often have altered growth patterns, such as retarded shoot elongation (Asaeda et al., 2007; Puijalon and Bornette, 2006).

Water flow has both a spatial and a temporal dimension. Although the spatial variability can be described by comparing the average velocity of two locations, the temporal variability at any point leads to rapid fluctuations of the velocity with the passage of turbulent eddies (Sand-Jensen and Pedersen, 1999). Turbulence is normally associated with high flow velocities, which occur when laminar flow breaks down; therefore, plants would experience greater and more varied mechanical stresses. Turbulence intensity is a measure of the strength of the turbulent fluctuations (Gordon et al., 2004), increases with the mean flow velocity, and is often expressed as a proportion to mean velocity in the mean direction of flow (Sand-Jensen and Pedersen, 1999). In reality, turbulent eddies break down into isotropic, and thus turbulent fluctuations occur in all directions at any point in the stream. A common assumption is that “turbulence intensities are the same in both horizontal and vertical directions since they arise from the same sets of the eddies” (Gordon et al., 2004, p. 135). Aquatic vegetation affects the mean velocity of flow, the turbulence intensity and diffusion. In addition, aquatic plants convert mean kinetic energy into turbulent kinetic energy at the scale of stems and branches (Nepf, 1999). Turbulence can be simulated in the laboratory using oscillating grids that generate different turbulence levels (De Silva and Fernando, 1994). Such design reproduces the effect of turbulence. These phenomena are of major interest in waterlogged wetlands and shallow lakes where wind-induced turbulence and waves (in shallow lakes) are key agents that affect the bottom boundary (Pujol et al., 2010).

Abbreviations: IAA, indole acetic acid; CK, cytokinin; ROS, reactive oxygen species; DW, dry weight; FW, fresh weight; POD, peroxidase; IAAO, indole acetic acid oxidase; CKX, cytokinin oxidase; iP, isopentyladenine.

* Corresponding author. Tel.: +81 48 858 3563; fax: +81 48 858 9574.
E-mail address: asaeda@mail.saitama-u.ac.jp (T. Asaeda).

0367-2530/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.flora.2011.07.014

The mechanical stimulus received by the plants at the cell wall, due to the different mechanical forces they experience, may cause changes in intra-cellular pressure and relay this information to the cell. This process is called mechanical stress signal transduction. In studies performed on bryophytes, stretch-activated Ca^{2+} channel opening has been hypothesized to be involved in this signal transduction by increasing cytosolic Ca^{2+} (Chehab et al., 2009; Knight et al., 1992). Phytohormone variation in response to mechanical stress has been well-studied for bryophytes but not for aquatic macrophytes. Though the role of the phytohormone ethylene in mechanical stimulation responses has been studied for decades, its involvement in the transduction of mechanical stress has been questioned later (Biro and Jaffe, 1984; Chehab et al., 2009). Additionally, mechanical stress has been observed to be involved in alterations of various hormones other than ethylene, including indole acetic acid (IAA) and cytokinin (CK) in land plants (Chehab et al., 2009; Victor and Vanderhoef, 1975). Another major response to a wide range of stress is the excessive production of reactive oxygen species (ROS); e.g. Kovtun et al. (2000) and Pasternak et al. (2005a). An over-accumulation of ROS may damage proteins, DNA and lipids of the cells. To protect against this potential damage, plants have an efficient antioxidant defense system that is activated upon changes in ROS production and scavenges the generated ROS (Chehab et al., 2009; Mittler, 2002). However, few studies have focused on the correlation between changes in hormones and enzymes in macrophytes exposed to water movement (Ke and Li, 2006).

IAA and CK are linked to cell division. Because most studies have shown that plants exposed to mechanical stress reduce their growth rate, we expect that mechanical stress would reduce the concentration of these hormones or catabolize these hormones at a higher rate.

Egeria densa Planchon is a submerged freshwater macrophyte that is commonly found in both lentic and lotic ecosystems worldwide. This plant typically grows into the bottom sediment of lakes at a depth of 1–2 m. In the natural environment, *E. densa* has a relatively high growth rate at optimum environmental conditions and forms dense canopies (Bini and Thomaz, 2005; Yarrow et al., 2009).

The objective of this study was to observe the visible responses of *E. densa* to different turbulence levels and to study the alterations of growth hormones and plant stress levels. This study focused on the changes in the growth hormones IAA and CK, their catabolism and oxidative stress responses.

Materials and methods

The experiments were conducted using 6-L (15.7 cm × 15.7 cm × 24.5 cm) microcosms with a water depth of 17.0 cm (this depth was available for plant growth, which was calculated after excluding the sediment layer thickness and grid movement area) for 12 weeks under controlled laboratory conditions. Although the microcosm was relatively small for the generation of turbulence, the study had to be done at this particular scale for handling to be possible using DC motors. Fig. 1 shows a cross-section of one experimental tank at the beginning of the experiment. The motors were located on an isolated support above each tank. One experimental setup consisted of four microcosms: three microcosms had different turbulence conditions, and the other was a control. The control was subjected only to gentle manual mixing once a day, to reduce limitations of photosynthesis due to the settlement of suspended matters on leaves. The experiment was conducted in triplicate using the same experimental setups under similar environment conditions. The temperature was maintained at 23 ± 2 °C in a room with fluorescent lighting. The pH of the media ranged from 6.8 to 7.2. The light intensity ranged from 240 to 270 $\mu mol\,m^{-2}\,s^{-1}$, and all of the microcosms were subjected to a 12 h/12 h light/dark period.

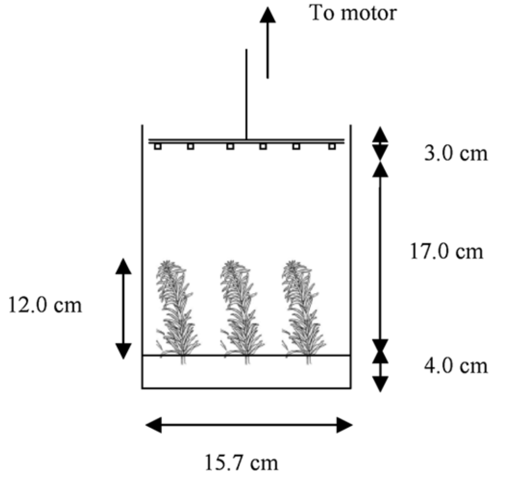

Fig. 1. Cross-section of an experimental tank at the beginning of the experiment. Initial plant height was 12.0 cm and the space available for the plant growth was 17.0 cm. The grid is shown on top of the tank and grid movement range was 3.0 cm. The thickness of the substrate was 4.0 cm.

Turbulence generation and quantification

Turbulence was generated using vertically oscillating horizontal grids at three oscillating frequencies: 1, 2 and 4 Hz. The grid spacing (M) was 2.5 cm, and the grid was made of 5-mm square Plexiglas rods, with a resulting solidity of 38% (De Silva and Fernando, 1994). The stroke length (S) was 3 cm, and the grids were oscillated from the top of the tank; the grid position varied between 0.5 and 3.5 cm below the water surface. Turbulence was generated behind the grid by the development of jet and wake structures, corresponding to the open areas and grid bars, respectively (De Silva and Fernando, 1994).

The horizontal velocity profile of the tanks was measured using a two-dimensional current meter (SF-5712, Tokyo-keisoku Corporation, Tokyo, Japan). The water velocity was measured at nine different points, which were symmetrically distributed over the area. In addition, the velocity profile was measured at four depths (6, 9, 12 and 15 cm from the top of the tank) for each of the nine points. All of the nine measurements were averaged to calculate the turbulence velocity at each depth in each tank. Because oscillating grids generate nearly isotropic and homogeneous turbulence, the vertical component should be the same as the measured horizontal component and was thus not measured (De Silva and Fernando, 1994). The time constant of the current meter (response time) was 20 Hz, and the measurements were performed at 10 Hz for 1 min. The current meter was calibrated in a constructed laboratory channel, by generating known velocities in the range of 1–10 $cm\,s^{-1}$ and a calibration graph was developed. The voltage signal was converted to velocity using the calibration graph after the data were extracted with the GL200_800-APS software Version 1.01 (Graphtec Corporation, Yokohama, Japan).

Plant material and growth conditions

Egeria densa was cultured for approximately 1 month in the laboratory at a controlled temperature of 23 ± 2 °C. A peaty sediment was collected from a nearby pond (Akigasei Park, Saitama, Japan) and passed through a 1-mm sieve to remove debris and floating matter, and a 4-cm thick layer of sediment was added to each tank. The sediment was used to add micronutrients to the growth media