Plant Physiol. (1989)89, 732–734
0032-0889/89/89/0732/03/$01.00/0

Received for publication August 29, 1988
and in revised form October 26, 1988

Communication

Endogenous Abscisic Acid Content Correlates with Photon Fluence Rate and Induced Leaf Morphology in *Hippuris vulgaris*[1]

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ABSTRACT

This research focused on studying how light and endogenous abscisic acid regulate leaf development in *Hippuris vulgaris*, a species of heterophyllic aquatic plant. Amounts of photosynthetically active radiation greater than 300 micromoles per square meter per second caused submerged *H. vulgaris* shoots to produce aerial-type leaves. Abscisic acid was not detected in shoots grown under noninducing light quantities (100 micromoles per square meter per second), but was present at 13.4 nanograms per gram fresh weight in shoot tips after plants were exposed to 1 photoperiod of inducing light (500 micromoles per square meter per second). This supports a role for abscisic acid in the high light-induced heterophylly in *H. vulgaris*, and provides additional support for the general hypothesis that abscisic acid regulates leaf development in heterophyllic aquatic plants. No relationship was observed here between postphotoperiodic light treatments of various red/far red ratios and heterophylly in *H. vulgaris*.

Hippuris vulgaris is a heterophyllic aquatic plant and can grow in either an underwater or aerial environment. Underwater, its shoots produce long thin leaves with a relatively large surface area and no stomates ('submerged-type leaves'); above water it produces short thick leaves with a smaller surface area and numerous stomates ('aerial-type leaves') (13). These leaf characteristics have adaptive significance in their respective environments since they affect water loss, photosynthetic capacity, and nutrient uptake. The transition between the two types of leaves normally occurs at the water surface, but the physiological mechanisms that control leaf development in this and other heterophyllic aquatic species are not well understood (17).

During the past 10 years, two lines of evidence have emerged that suggest a role for ABA in regulating leaf type in heterophyllic aquatic plants. First, endogenous ABA levels rise rapidly in submerged-type *H. vulgaris* shoots that are subjected to water stress, as would happen when a shoot tip begins to grow up through the water surface (9). Second, exogenous ABA induces the production of aerial-type leaves on underwater shoots in this and other species of heterophyllic aquatic plants (2, 3, 7, 10, 11, 14, 18, 19).

Under certain conditions in the natural environment (*e.g.* during the summer months at northern latitudes) aerial-type leaves are produced on *H. vulgaris* shoots even when they are as much as a meter below the water surface (6). Since these shoots are completely surrounded by water this response cannot be attributed to osmotic stress. Rather, it is thought to be regulated by light, possibly as a phytochrome response (4). If so, then endogenous ABA levels might be expected to correlate with some characteristic of the light environment. The research presented here reports on the relationship of light quantity and quality to endogenous ABA and the control of leaf morphology in *H. vulgaris*.

MATERIALS AND METHODS

Collection and growth of *Hippuris vulgaris* L. shoots, criteria for determination of aerial- and submerged-type leaves, and quantification and verification of endogenous ABA were as described previously (9). Briefly, the ABA extraction method was based on that of Lewis and Visscher (12) and involved the use of chemical partitioning in conjunction with elution through a Sep-Pak C18 column (Waters Associates, Milford, MA) to extract ABA from plant tissue. The extracted samples were methylated with diazomethane and quantified by means of GC-ECD.[2] The ABA peak was verified by (a) coelution with authentic ABA, (b) coelution of the UV-isomerized sample with UV-isomerized authentic ABA, and (c) ELISA assays performed on selected samples. Recovery of ABA from plant extracts was ca. 40%, and the limit of detection by GC-ECD was about 2 ng/g fresh weight. ABA extractions were performed on a minimum of three independent tissue samples to obtain the average values that are reported. Leaf type was determined using a dissecting microscope.

Growth of shoots under different light conditions was accomplished by placing submerged shoots in flasks under General Electric Co. MVR1000\VBU Multi-vapor high intensity lamps set to a 14 photoperiod. Photon fluence rate was varied between 300 and 1000 μmol m^{-2} s^{-1} by adjusting the plant level and location. Low fluence rate controls were grown either in a growth chamber or under identical conditions as above but with a sheet of gray Plexiglas (AIN Plastics, Berkeley, CA) placed between the light source and plants so that the fluence rate at plant level was 100 μmol m^{-2} s^{-1}. R/

[1] Supported in part by National Aeronautics and Space Administration Graduate Student Researcher's Program grant NGT-50017.

[2] Abbreviations: ECD, electron capture detector; R/FR, red/far red.

FR ratios of the various light sources are listed with the "Results." Flasks containing plants were placed in a running water bath to maintain plant temperature at about 22°C.

Postphotoperiodic light treatments were performed by exposing high fluence rate treated plants to various R/FR ratios of light for 10 min immediately following the high light photoperiod. Red irradiation (R/FR ratios greater than 2) was from one or a combination of the following light sources and filters: (a) a microscope tungsten light filtered through a Corning 650 nm band-pass filter, (b) General Electric F20T12R red fluorescent lights (GE Supply Co., Emeryville, CA), or (c) regular fluorescent lights filtered through red Plexiglas. Specific R/FR ratios and light intensities that were used are stated with the "Results." Far red irradiation (R/FR ratio of 0.013) was obtained by using a theater-style par lamp with a 100 W tungsten light bulb and Roscolux No. 85 (deep blue) and No. 19 (fire) acetate filters (Stagecraft Studios, Berkeley, CA).

PAR was measured using a Lambda LI-185 Quantum Radiometer/Photometer (LI-COR Inc., Lincoln, NE). R/FR ratios were determined using a QSB170B Digital Scalar Irradiance Meter (Biospherical Instruments, Inc., San Diego, CA) custom built and calibrated for 660 nm and 730 nm light.

RESULTS

Light Quantity and ABA

Submerged *Hippuris vulgaris* shoots were grown under PAR ranging from 100 to 1000 μmol m^{-2} s^{-1} to determine whether leaf type was affected by light quantity. These experiments revealed that shoots exposed to PAR levels of 300 μmol m^{-2} s^{-1} and higher produced aerial-type leaves, even though the shoots remained underwater (Table I). Submerged shoots grown under PAR of 100 μmol m^{-2} s^{-1} produced only submerged-type leaves.

Endogenous ABA was measured in submerged-type shoots exposed to light conditions that were either inducing (500 μmol m^{-2} s^{-1}) or noninducing (100 μmol m^{-2} s^{-1}) for the production of aerial-type leaves. ABA was not detected in submerged shoots grown under noninducing fluence rates, but was present at a level of 13.7 ng/g fresh weight 24 h after the beginning of one aerial-type leaf inducing photoperiod (Table II). Furthermore, ABA was detected only in 0.5 cm portions of shoot tips, but not in 0.5 cm segments taken from the middle portions of shoots. ABA was present at 9.7 ± 1.6 ng/g fresh weight after three inducing photoperiods, by which time the first aerial-type leaves were observable at the shoot tip.

In several experiments the growth medium was analyzed for ABA to determine whether significant amounts of ABA might be leaking from plants in the medium. No ABA was detected in the medium from either low or high fluence rate treated plants.

Table I. *Effect of Light Intensity on Leaf Morphology*

Submerged *H. vulgaris* shoots were grown for 1 week under light of various intensities and R/FR ratios (14 h photoperiods), and the morphology of new-growth leaves was determined using a dissecting microscope.

Fluence rate	R/FR Ratio	New Growth Leaf Morphology
μmol m^{-2} s^{-1}		
100[a]	1.4	Submerged
100[b]	1.6	Submerged
300	0.81	Aerial
500	1.9	Aerial
1000	1.5	Aerial

[a] Mixed fluorescent and incandescent lights. [b] Same light source as for 500 μmol m^{-2} s^{-1} but filtered through a neutral density filter to obtain the lower light intensity.

Table II. *Light Intensity and Endogenous ABA*

ABA levels were measured in 0.5 cm portions of submerged-type *H. vulgaris* tissue taken from either the tips or mid-sections of shoots 24 h after the beginning of one 14 h photoperiod. The light source was the same for both fluence rates, but a sheet of gray Plexiglas was mounted between the light source and plants to obtain the lower fluence rate. The R/FR ratios of the 100 and 500 μmol m^{-2} s^{-1} fluence rates were 1.6 and 1.9, respectively.

Photon Fluence Rate	Engogenous ABA Level	
	Midsections	Tips
μmol m^{-2} s^{-1}	ng/g fresh wt	
100	Not detected	Not detected
500	Not detected	13.4 ± 6.3

Light Quality

According to a previous report (4), the production of high fluence rate induced aerial-type leaves on *H. vulgaris* could be suppressed by exposing plants to post-photoperiodic red light (R/FR of 35 or higher). In contrast to that finding, no effect of postphotoperiodic light treatments on leaf morphology was observed here (Table III).

DISCUSSION

Many species of heterophyllic aquatic plants show a transition from submerged-type leaves to aerial-type leaves when they grow up through the water surface (17). This observation, along with evidence suggesting that ABA regulates leaf development in these species, led to the following hypothesis (2): As a shoot tip reaches the water surface it begins to dry out. This causes a rise in endogenous ABA (by unknown mechanism), which in turn induces the production of aerial-type leaves. This hypothesis has been supported both by experiments using exogenous ABA (2, 3, 7, 10, 11, 14, 18, 19) and by measurements of endogenous ABA in *H. vulgaris* subjected to osmotic stress (9).

However, the transition between submerged-type and aerial-type leaves does not always occur at the water surface. In *H. vulgaris*, aerial-type leaves are sometimes induced even though the shoot tips are up to 1 m below the water surface (6). This phenomenon cannot be explained as a response to osmotic stress, since such shoots are still completely surrounded by water. One environmental factor that varies with water depth is light quantity, or fluence rate. The research here revealed a correlation between light quantity and the production of aerial-type leaves in *H. vulgaris*. This response

Table III. *Postphotoperiodic Treatments and Leaf Type*

Submerged *H. vulgaris* shoots were grown under 500 μmol m^{-2} s^{-1} PAR photoperiods with 10 min postphotoperiodic light of various R/FR ratios. Morphology of the new-growth leaves, determined after 1 week, was all of the aerial type

Treatment	Photoperiod		Postphotoperiod	
	PAR	R/FR	PAR	R/FR
Control	500	1.5		
Control	500	2.4		
Far red	500	2.4	0.05	0.013
Red	500	2.4	13	7
Red	500	2.4	0.3	12
Red	500	2.4	0.2	71

was associated with a rise in endogenous ABA, as is the response to osmotic stress (9). Also, ABA was detected only in the tips of shoots (where leaves are differentiating and new leaf primordia are being formed) but not in shoot segments taken from the middle portions of shoots. Shoots exposed to high fluence rate photoperiods in turn produced aerial-type leaves that were visible within 3 d.

ABA levels declined somewhat in shoot tips after 3 high light photoperiods. The cause of this is unknown, but a similar decline in ABA was also observed in *H. vulgaris* shoots exposed to osmotic stress (9). Perhaps the presence of free ABA leads to an increase in its own turnover.

High light could trigger increased ABA levels within the plant (and, in turn, cause a change in leaf development) as a result of either (a) increased carotenoid biosynthesis (8, 16), which could increase the pool of ABA precursors (5); or (b) some factor related to the osmotic concentration of cells. For example, in a review of factors affecting heterophylly, Allsopp (1) concluded that the internal concentration of cell solutes, either organic or inorganic, is the decisive factor in determining leaf type in heterophyllic aquatic plants. If this is the case, then a common mechanism between light-induced and osmotic stress-induced heterophylly can be suggested. Osmotic stress causes an increase in cell solute concentration because water moves out of cells while solutes stay the same, whereas a high fluence rate, through photosynthesis, increases cell solutes but the water content stays the same. In both cases, the osmotic potential of the cell would become more negative, leading (theoretically) to an increase in endogenous ABA, which would in turn lead to the production of aerial-type leaves. Further research into the relationship of cellular osmotic potential to ABA biosynthesis is required in order to substantiate this hypothesis. Although current evidence suggests that ABA biosynthesis is controlled by loss of turgor (15), the possibility has not been ruled out that cells possess some mechanism by which they can sense solute concentration and respond to water deficits (20).

A possible reason why it might be advantageous for a plant to produce aerial-type leaves on submerged shoots under high light conditions could relate to net carbon gain. Aerial-type leaves have a thicker mesophyll layer than submerged-type leaves and would presumably be better able to harvest and store the energy from higher fluence rates.

Finally, an earlier study (4) reported evidence that leaf type in *H. vulgaris* could be controlled as a phytochrome response. An attempt was made here to confirm some of those results using postphotoperiodic irradiations of various R/FR ratios. However, under the conditions used in this study, no effect of postphotoperiodic irradiations on the control of leaf morphology in *H. vulgaris* was observed.

ACKNOWLEDGMENTS

The author thanks Dr. Lewis Feldman for considerable support and advice, and Dr. Bruce Bonner for helpful discussions.

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