Chapter 10

Determination of Phototropism by UV-B Radiation

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Abstract

UV-B phototropism in etiolated *Arabidopsis* seedlings has only been shown recently and needs further exploration. Here we elaborate on how to generate a customized setup with a unilateral UV-B light source, the required plant materials, different growth substrates, and a framework for data analysis.

Key words UV-B, Phototropism, *Arabidopsis* seedlings, UV-B light sources, Growth substrates, Medium reflectance, Medium fluorescence

1 Introduction

The ultraviolet radiation (UV) part of the electromagnetic spectrum comprises three waveband ranges: UV-C (100–280 nm), UV-B (280–315 nm), and UV-A (315–400 nm). Only UV-B and UV-A reach the Earth's surface, considering that light with wavelengths below 290 nm declines to an undetectable level [1]. The level of UV-B is predominantly depending on atmospheric ozone absorption and factors like alternating altitude, latitude, surface albedo, clouds, aerosols, and solar angle [2]. Although UV-B radiation is only a minor fraction of the sunlight reaching the Earth's surface, it considerably affects plant morphology. Well-documented morphological adaptations include shorter petioles and shorter stems, yielding compact plants. Interestingly, phototropic responses to UV-B light were also reported in etiolated seedlings [3]. Many of the UV-B-induced morphological adaptations are ascribed to the UV-B-specific photoreceptor UV RESISTANCE LOCUS 8 (UVR8), but the underlying mechanisms by which UV-B radiation alters plant architecture are still poorly understood.

Phototropism is the growth of plants and their parts toward or away from a light source (tropism = directional growth). In positive

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phototropism, the directional movement allows plants to optimize the position of their photosynthetic tissues in accordance with the incoming light. There are two long-standing theoretical models on the mechanism of phototropic bending. The first model, proposed by Blaauw [4], states that differential growth is caused by unilateral photomorphogenic inhibition of growth. The alternative model of Cholodny [5] and Went [6], which is most widely accepted and well documented for blue light phototropism, stipulates that phototropic bending is caused by the lateral distribution of a growth hormone, inducing differential growth [7]. Phototropism has been linked to at least six photoreceptors, PHOT1, PHOT2, PHYA, PHYB, CRY1, and CRY2, with the two first mentioned playing the most prominent role. The bulk of the phototropism literature deals with blue and UV-A light [8]. Besides these spectral bands, plants also respond phototropically to shorter wave UV-B light (Fig. 1) [3, 9–11].

Phototropins are membrane-associated photoreceptors which trigger a downstream signal transduction cascade [12]. *Arabidopsis* has two phototropins, namely, phototropin1 (PHOT1) and phototropin2 (PHOT2). These are very similar in structure, amino acid sequence, and domain organization. Flavin mononucleotide (FMN) is the chromophore of phototropin and binds both LOV1 and LOV2 [13]. FMN is noncovalently associated with LOV in darkness, while in light conditions, FMN gets activated and binds LOV covalently through a conserved cysteine residue [13, 14]. Under conditions of moderate or high light, PHOT1 and PHOT2 act together to attain downstream signalling, whereas under low light conditions, PHOT1 acts as the plant's exclusive directional blue light and UV-A photoreceptor [15]. Along with the phototropic responses, phototropins also mediate other blue light responses that contribute to an increased photosynthetic efficiency. The strength of these responses corresponds with the light intensity; examples are stomatal opening, leaf blade expansion, and chloroplast positioning [15, 16]. In addition to phototropins, plants can perceive and distinguish UV-B in a direct way with the UV-B-specific photoreceptor UV RESPONSE LOCUS 8 (UVR8). UVR8 is a seven-bladed β-propeller protein consisting of 440 amino acids. Intrinsic tryptophans serve as UV-B chromophores [17]. In the absence of UV-B, the major fraction of UVR8 can be found in the cytoplasm as dimers owing to a nuclear export signal. When exposed to UV-B, UVR8 accumulates in the nucleus within minutes, monomerizes, and induces downstream signalling. The role of UVR8 and phototropins in hypocotyl bending toward UV-B has recently been elucidated in *Arabidopsis*. UVR8-mediated signalling can induce bending of etiolated *Arabidopsis* hypocotyls toward UV-B light, independently of phototropin signalling. Nevertheless, phototropins are the dominant receptors for UV-B-induced phototropism in seedlings with increasing importance at declining UV-B fluence rates. Similar to blue light

perception, UV-B perception by phototropins relies on phototropin kinase activity and NPH3 regulation [3, 18].

Since the phototropins and UVR8 pathways share a phenotypic overlap of the phototropic response in seedlings, a thorough characterization in blue and UV-B light is necessary. Here we describe how to use light sources and growth media to do this, focusing on the UV-B aspect.

2 Materials

1. Plant material: For UV-B phototropism experiments of *Arabidopsis* seedlings, it is recommended to include Col-0 (NASC, Nottingham, UK), *uvr8–6* (Prof. Roman Ulm from the University of Geneva), and *phot1-5 phot2-1* [19] as controls.
2. Agar medium, e.g., half-strength Murashige and Skoog (*see* **Note 1**): Add 2.15 g MS salt mixture for half-strength and 8 g plant tissue culture agar to ca. 900 mL of distilled water. Adjust to pH 5.7 with KOH, and top up to 1 L with water. Steam autoclave for 30 min at 15 psi. Pour in petri dishes and let solidify.
3. Soil: Any soil or UV-absorbing substrate with pH between 5 and 7 will do, e.g., Jiffy peat pellets (Jiffy Stange, Norway). Add tap water and let the pellet fully expand (*see* **Note 1** and Fig. 2).
4. Experimental room requirements: Constant temperature of 21 °C and infrared light (IR) equipped lighttight container of at least 20 × 20 × 20 cm (*see* **Note 2**).
5. UV-B light source (*see* **Notes 3** and **4**): Monochromator, narrowband UV-B light (tubular), broadband UV-B light (tubular, e.g., UVM57 lamp (Ultraviolet Products); requires combination with a cutoff or band-pass filter, e.g., NS297 (International Light Technologies)), or UV-B LED light.
6. A camera system (*see* **Note 5**): Webcam or digital single-lens reflex (DSLR) camera.
7. Computer (*see* **Note 6**) with software for time-lapse photography and image analysis.

3 Methods

3.1 Grow Your Etiolated Seedlings

3.1.1 Option 1: Medium

1. Surface-sterilize the seeds in a 5% NaOCl/0.05% Tween 20 solution for 15 min.
2. Remove the sterilization solution thoroughly by washing five times for 3 min with sterilized water.
3. Sow 15–20 seeds on the medium.
4. Stratify the seeds in darkness at 4 °C for 2 days.

5. Induce germination by exposing the seeds to white light for 6 h at 21 °C (*see* **Note 7**).
6. Transfer the petri dishes with seeds to the experimental setup (*see* Subheading 3.2).
7. Leave in darkness for 2 days at 21 °C, supplemented with IR light to track the growth with the camera system.
8. Turn on the UV-B lamp when seedlings are 2 days old and/or about 1 cm long.
9. Continue tracking for a day.

3.1.2 Option 2: Soil

1. Sow 15–20 seeds dispersed or in rows of 5 with equal distance to the light source in soil.
2. Stratify the seeds in darkness at 4 °C for 2 days.
3. Induce germination by exposing the seeds to white light for 6 h at 21 °C (*see* **Note 7**).
4. Transfer the pots with seeds to the experimental setup (*see* Subheading 3.2).
5. Leave in darkness for 2 days at 21 °C, supplemented with IR light to track the growth with the camera system.
6. Turn on the UV-B lamp when seedlings are 2 days old and/or about 1 cm long.
7. Continue tracking for a day.

3.2 Use of the Hardware Set

See Fig. 3 for a practical example of the setup.

1. Acclimatize a room at constant temperature of 21 °C.
2. Prevent plant exposure to any other light source besides UV-B light as much as possible.
3. Construct a lighttight container.
4. Make an opening in the lighttight box (e.g., 2.5 cm in diameter, the size of a filter).
5. Install infrared light on top for camera detection.
6. Mount your UV-B light source.
7. Install a UV filter when required.
8. Verify the resulting spectrum and light intensity with a spectroradiometer and/or radiometer.
9. Determine the distance from the light source for desired intensity (e.g., 0.002 μmol m^{-2} s^{-1} or 0.12 μmol m^{-2} s^{-1}) as described in Vanhaelewyn et al. [18].
10. Determine the UV-B exposed surface (*see* **Note 8**).
11. Install a camera system with focal plane of the pictures parallel with the incoming unilateral light.

12. When everything is in place and running, put in a temperature logger to estimate the stability and height of the temperature inside the container.

3.3 Experiment and Analysis

1. Track germination and initial growth of the etiolated plants with the camera system for desired required hypocotyl length.
2. Start the UV-B light exposure.
3. Have the system take pictures every 5–15 min.
4. Digitally analyze the images with an angle measurement tool (*see* **Note 9** and Fig. 1).
5. Generate output in excel: "angles of curvature" are 180°—measured angle α (*see* **Note 10**).
6. Calculate the averages and the standard error. Analyze at least ten plants.

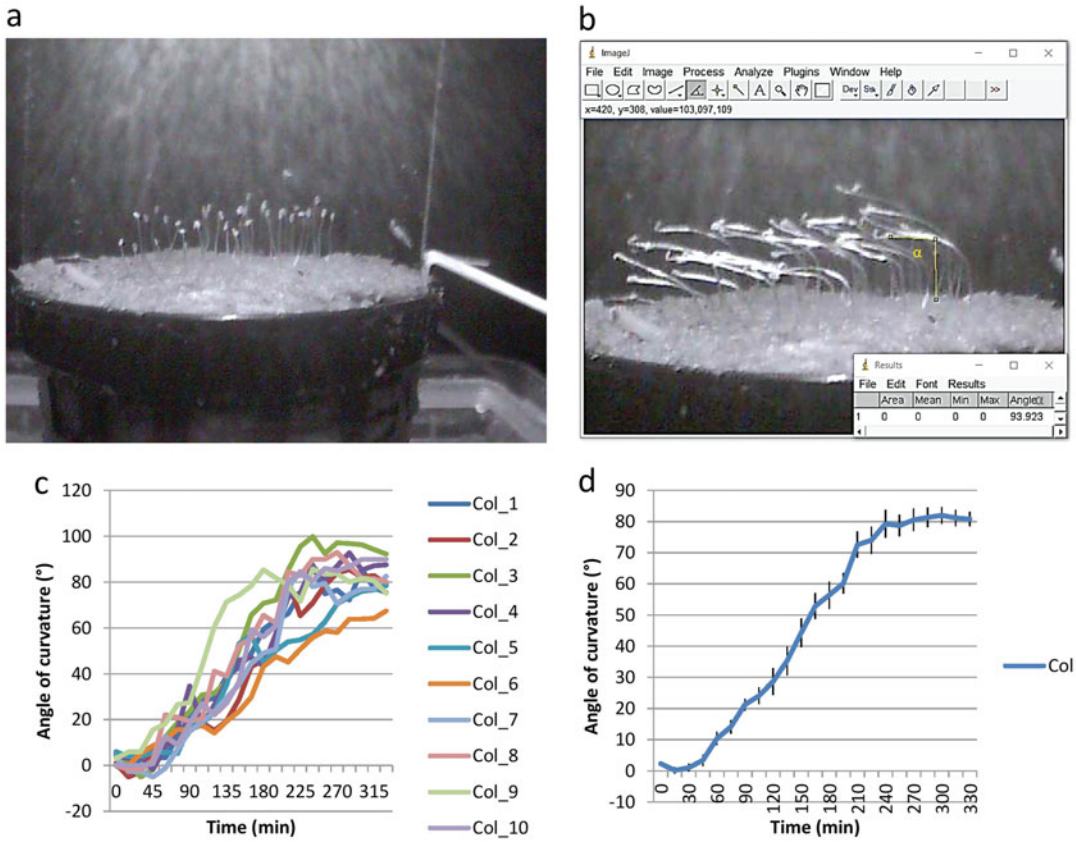

Fig. 1 Result and output of an experiment on *Arabidopsis* wild type (Col-0). (**a**) Two-day-old etiolated seedlings, tracked for germination with the IR camera system. (**b**) Measuring the angle α of unilateral UV-B exposed seedlings with the angle tool of ImageJ. (**c**) Kinetic analysis of the angles of curvature (180°–α) of ten wild-type plants, indicating the variation in one experiment. (**d**) Output of the averages of the angles of curvature of ten wild-type plants with standard errors

4 Notes

1. Phototropic response experiments can be performed on both agar medium and soil substrates. Agar medium-based experiments allow better standardization of the supplied nutrients or chemicals but are subject to reflectance of the medium upon UV illumination which is potentially problematic for studying photomorphogenesis (Fig. 2a, b). Therefore we advise to opt for soil or another UV-B-absorbing substrate. In addition, media and plants may also emit a small amount of blue fluorescent light when exposed to unilateral UV-B (Fig. 2c, d).
2. The lighttight container should contain IR light so that the camera system can record (*see* Fig. 3). The best choice is by

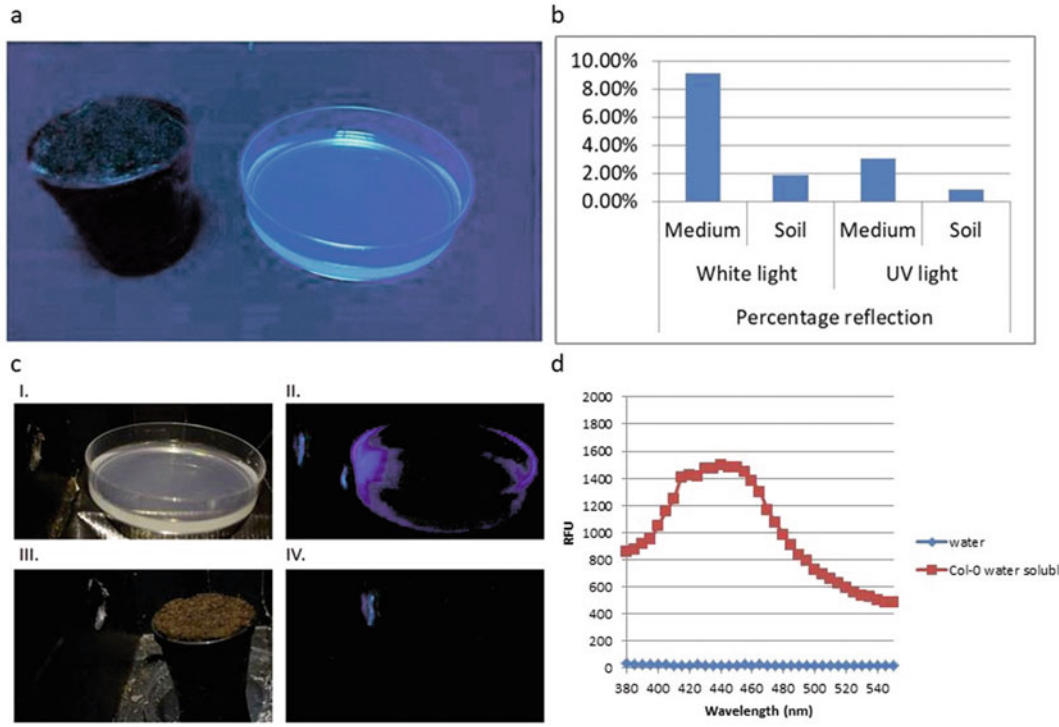

Fig. 2 Reflection and fluorescence of medium, soil and plants. (**a**) Visual representation of medium vs. soil growth substrates under narrowband UV-B with peak at 311 nm. (**b**) Percentage of reflection of white and UV-B light on the different substrates when exposed with UV-B narrowband with peak at 311 nm from the top and measured by a PAR or UV-B meter in a 45-degree angle. (**c**) Petri dish with agarose (I) and soil (III) under white light and when exposed to 302 nm monochromatic UV-B from the left-hand side (II and IV). (**d**) Low-level blue fluorescence in the water-soluble fraction of plants, quantified in relative fluorescence units (RFU). Three-day-old etiolated seedlings were grown on MS/2 + 1% sucrose; the plant material was homogenized with a ball mill in liquid N_2, including seed coats. Extracts: 200 plants +300 μL water, centrifuged for 2 min at 10621 × *g*, samples kept on ice and measured with a SpectraMax Gemini xs (Molecular Devices) with 310 nm excitation

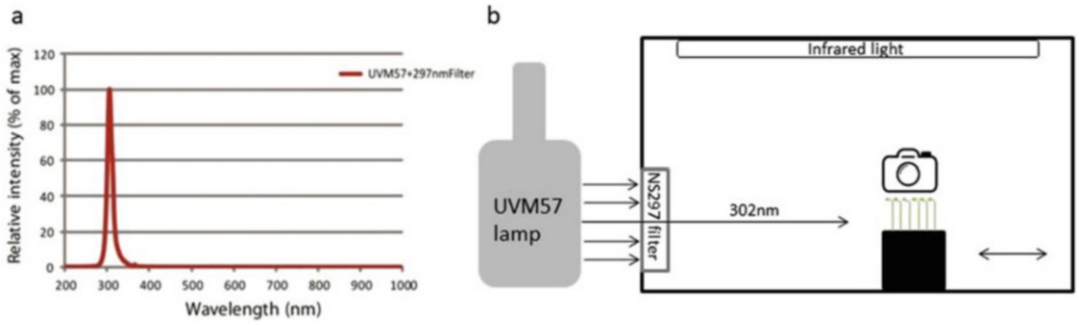

Fig. 3 Experimental setup and spectrum of the UV-B light source. (**a**) Full spectrum from 200 to 1000 nm from a broadband UVM-57 lamp in combination with an NS297 band-pass filter [3]. (**b**) Example of an experimental setup: an UVM57 lamp was attached to the side of a sealed container, emitting light through a 2.5 cm diameter hole with a NS297 band-pass filter attached at the back, allowing unilateral monochromatic 302 nm light to shine on *Arabidopsis* seedlings. The relative distance of the seedlings to the lamp can be changed to obtain the desired fluence rate. Supplemented infrared light from the top allows photography of the seedlings

installing an infrared LED strip at the top of the container since LEDs produce little to no heat; moreover LED strips are flexible and easy to install. An alternative is to record in the UV-B spectrum, but most cameras don't record UV-B which makes this option expensive and unfavorable.

3. When working with UV-B light sources, it is imperative to take protective measures to avoid exposure of skin or eyes to the light. These include UV opaque clothing, gloves, and a UV blocking face shield.
4. Monochromators are very expensive, and the associated light sources (e.g., xenon arc lamp) produce a lot of heat. UV-B LED lights produce the least heat but are rather uncommon (and still expensive) to date, yet the availability is likely to increase the coming years as they are applied in treatment for psoriasis. Therefore we advise to work with narrowband UV-B lights or broadband lights outside of the container in combination with a band-pass filter built-in in the wall of the container (Fig. 3b). Broadband UV-B can also be used, but most lamps need a cutoff filter (e.g., two layers of cellulose acetate (Jürgen Rachow, Hamburg, Germany)) to remove UV-C coming from the light source. Varying light intensities are mostly achieved by changing the distance of the light source to the sample seedlings.
5. Many high-resolution DSLR cameras can be converted into a camera that detects IR light. We recommend this to be done by specialized companies. However, in order to save on costs, a DSLR camera system can be replaced by a webcam system; moreover webcams are usually smaller and are easier to fit in the setup. It is best to check beforehand whether the webcam

IR filter can easily be removed—or is built in into the lens of the camera—and consequently not removable. In addition, when the simultaneous use of multiple webcams is desired, you might run into hardware recognition problems if choosing the same type of camera and drivers and may need software which recognizes the cameras as separate cameras or different types of cameras.

6. There are no special requirements as there is little computational power required from the computer. An example of a webcam tracking software is Active WebCam (PY Software).
7. The light pulse can be generated by a lamp containing sufficient red light. This can be incandescent light or fluorescent (e.g., cool white) light at a photosynthetically active radiation (PAR) level of 50–150 μmol m^{-2} s^{-1}.
8. The vertical field of exposure can be determined quantitatively by a UV-B radiometer or simply qualitatively by using regular printing paper which contains optical brighteners that light up under UV-B exposure.
9. It is worthwhile mentioning that automated analysis software to measure hypocotyl growth and form is available, but the major issue is that infrared pictures have very little contrast which makes automated analysis rather complicated. The use of software such as HYPOtrace can be considered [20]. The angle of curvature is defined as the angle between the tangential through the upper end of the hypocotyl and the vertical, with 0° being completely vertical, 90° being horizontal directed toward the light source, and −90° being horizontally directed away from the light source. By taking pictures every 5 min, a bending kinetics can be generated.
10. In rare cases, hypocotyls bend away from the light or in a random fashion. When a hypocotyl bends away from the light, the angle is calculated as –(180°–α).

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