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The effect of light quality on leaf production and development of *in vitro*-cultured plants of *Alternanthera brasiliana* Kuntze

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ABSTRACT

We investigated the influence of light quality on the leaf development of *Alternanthera brasiliana* Kuntze (Amaranthaceae) grown *in vitro*. Growth parameters including specific leaf mass, thickness, and leaf density were lowest in plants grown under red light. Blue light induced the largest number of leaves/plant, and the largest thickness and area of the leafblade. Green and red lights induced the smallest leaf areas. The thickness of the abaxial-face epidermis and spongy parenchyma of the plants was significantly reduced in plants grown under red light. The thickness of the palisade parenchyma and upper epidermis were significantly increased in plants grown under blue light, compared to the other fluorescent-light treatments. The specific spectral band also influenced the differentiation of mesophyll cells. In the dark and under red light, the mesophyll was homogenous; and in the dark and under green light, the leaves were more compact. Under blue light, the cells displayed the characteristic palisade morphology. The results showed that the increase of a specific parenchyma type was related to a specific spectral band. All spectral-quality treatments reduced the numbers of stomata and trichomes. The results for green light were in some respects similar to those for red light, and in other respects similar to those for blue light, probably because phytochromes and cryptochromes are green-light receptors. This study indicated that *Alternanthera* plants have strong morphological plasticity induced by light. The results suggest that high-quality *Alternanthera* can be achieved by culturing the plants *in vitro* under a combination of blue and red light.

1. Introduction

Plants have sophisticated photosensitive mechanisms to capture light energy for photosynthesis (Walters, 2005; Jiao et al., 2007). Light intensity and quality are important factors for plant growth and development (Fukuda et al., 2008). Specifically, changes in light quality strongly affect several plant anatomical, physiological, morphological, and biochemical parameters (Haliapas et al., 2008).

Photomorphogenesis involves three main families of information-transducing photoreceptors: phytochromes, blue-light receptors, and UV-B photoreceptor(s) (Jiao et al., 2007). However, the molecular nature of the UV-B (280–320 nm) photoreceptor(s) is still elusive (Chen et al., 2004). Some reports also substantiate many green-light (GL)-mediated responses in plants, which has given rise to speculation about the occurrence of a zeaxanthin-based compound as a green-light receptor (Folta and Maruhnich, 2007).

Phytochrome is a soluble pigmented protein that can exist in two spectrally distinct, photointerconvertible forms: Pr, a red-absorbing form, and Pfr, a far-red-absorbing form. The absorption maximum of the phytochrome 'Pr' form is close to that of the chlorophylls (red light), but the 'Pfr' form absorbs at a longer wavelength (far-red light) (Mathews, 2010). Phytochrome responses have been subdivided into different classes based on the radiation energy of light that is required to obtain the response (Casal et al., 1997). These include low-fluence responses (LFRs), very low-fluence responses (VLFRs), and high-irradiance responses (HIRs). In a typical VLFR, plants respond to between 0.1 and 1 μmol/m^2 of

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light, whereas the LFRs are typically between 1 and 1000 μmol/m^2 of light. In contrast to VLFR and LFR, which require transient exposures of lesser or greater duration, respectively, high-irradiance responses (HIRs) require continuous long-term irradiation, and they are dependent on wavelength (Strasser et al., 2010). Phytochrome photoreceptors play direct roles in germination, seedling establishment, flowering, dormancy, nyctinasty, stomatal development, plant architecture, and shade avoidance (reviewed by Franklin and Quail, 2010).

Along with phytochromes, another type of photoreceptors, blue-light receptors such as cryptochromes and phototropins were found (Strasser et al., 2010). The cryptochrome family is involved in light-signal transduction regulating phototropism, de-etiolation, chloroplast movements, and light-induced stomatal opening. In addition, this class of photoreceptor is important for photoperiod-dependent flowering induction and in resetting the circadian oscillator (Kinoshita et al., 2001).

Phototropins allow optimal photosynthesis, including phototropism, chloroplast movements, and stomatal opening. This last light response is also controlled by other photosensory systems, including a UV-B and a blue-green light receptor (Briggs and Christie, 2002). Recently, additional blue-light photoreceptors called ZEITLUPE have been characterized (Imaizumi et al., 2003).

Alternanthera brasiliana (L.) Kuntze (Amaranthaceae) is used for medicinal purposes in southern Brazil (Delaporte et al., 2001). The pharmacological features of this species have been investigated by several groups because of its analgesic, lymphocyte antiproliferative, and anti-inflammatory properties, anti-edemogenic activity, and activity against the herpes simplex virus (De Souza et al., 1998; Macedo et al., 1999; Delaporte et al., 2001; Brochado et al., 2003; Lagrota et al., 2006). Its morphology, anatomy, and chemical composition have also been studied by several groups (Delaporte et al., 2002; Duarte and Debur, 2004; Pereira et al., 2008; Macedo et al., 1999; Brochado et al., 2003).

A previous study of *A. brasiliana* indicated that a specific spectral band of light radiation can alter not only the accumulation of biomass in plantlets cultured by standard methods, but also optimize the analgesic activity of extracts from the plantlets in comparison to those obtained from *ex vitro*-developed material (Macedo et al., 2004). Extensive information is available on the photoregulation of plant development under red/far-red, blue (Christophe et al., 2006; Leicht and Silander, 2006), and UV light (Nogués et al., 1998), obtained from plants grown *ex vitro*. However, very little is known about the specific effect of light quality on plant physiology and on growth regulation under *in vitro* culture conditions (Kim et al., 2004; Muleo and Morini, 2006). Assuming that different bands of the radiation spectrum can influence structural aspects of plants in otherwise standard culture conditions, we evaluated the effect of alterations in the spectral quality of light on the growth *in vitro* and the leaf anatomy of *A. brasiliana*.

2. Materials and methods

2.1. Plant material and tissue culture

Greenhouse-grown plants of *A. brasiliana* (L.) Kuntze were identified by the Systematics Department of the Rio de Janeiro Botanical Garden, and registered under voucher RB 310.939, A.F. Macedo 01. Surface-sterilized seeds were sown into sterile medium (Murashige and Skoog, 1962) supplemented with 30 g/L sucrose and vitamins, without growth regulators (MS0) (Macedo et al., 1999). After 50 days, nodal explants of the germinated plantlets were subcultured in MS. Finally, randomized nodal explants were cultured in MS0 medium and grown under different light conditions or in the dark. For each treatment, 40 explants were used, and each

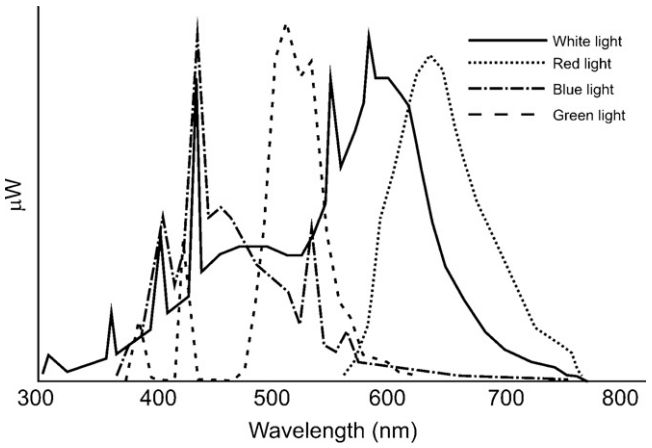

Fig. 1. Spectral power distributions of the light sources utilized (data provided by the Sylvania supplier).

experiment was repeated three times. Light-quality experiments were performed in growth chambers (Controlled Environments) equipped with Sylvania Cool 60 F20T12 fluorescent tubes (approximately 20 μmol m^{-2} s^{-1} photosynthetically active radiation), to provide different light qualities: red light (RL), green light (GL), white light (WL), and blue light (BL) (Fig. 1). WL and darkness (D) conditions were used as control treatments to assess the effect of light on plantlets in the same medium formulation. Spectral outputs from the lamps were recorded with a calibrated spectroradiometer (LI-COR 1800, Lincoln, NE, USA) placed horizontally in the cabinets used for the experiments, with the sensor covered with the glass lid of the vessel. For all treatments, a 16-h photoperiod was used, and the cultures were maintained at 25 ± 1 °C.

2.2. Assessment of plant development

After 60 days, the effects of each treatment were evaluated according to the following criteria—1: number of leaves/plant, 2: blade area of the third-node leaf, 3: dry and fresh leaf mass, 4: leaf weight ratio (RPF) = (leaf dry weight/plant dry weight) × 100. Leaf-blade area was calculated from fresh, individual leaves that were flattened between two glass plates and scanned (Hewlett-Packard ScanJet 5100C, Greeley, CO, USA) using calibration software (Sigma Scan Version 4.0, SPSS Inc., Chicago, IL, USA). The same leaves and plants were then individually oven-dried in aluminum vessels at 40 °C to constant mass, and weighed. To measure fresh weight, the material was removed from the culture flasks and immediately weighed, to prevent dehydration. The specific leaf mass (SLM) was calculated as mass per unit area (Witkowski and Lamont, 1991); the leaf thickness (LTh), as the sum of the thickness of the abaxial and adaxial faces of the epidermis and of the mesophyll; and leaf density (LD) as SLM/LTh (Witkowski and Lamont, 1991).

2.3. Harvesting, microtomization, and staining

After 60 days of *in vitro* culture, leaves were harvested from the third node, starting from the apex, from plants grown under different bands of the radiant spectrum or in the dark. The middle third of the leaf segments was fixed with glutaraldehyde and formaldehyde according to the method of Karnovsky (1965). After fixation, the plant material was embedded in methacrylate resin (hydroxyethylmethacrylate, Leica Historesin Embedding Kit), and cross-sectioned with a rotating microtome set for 7 μm thickness. The sections were stained with 0.05% Toluidine Blue (O'Brien et