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Research paper

Distinct growth light and gibberellin regimes alter leaf anatomy and reveal their influence on leaf optical properties

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ABSTRACT

Light capture by leaves is a key component for plant surveillance, and it has been accepted that blue and red lights are captured by palisade cells. However, the importance of green light absorption remains to be fully understood. Herein, wild-type tobacco plants treated with distinct gibberellin (GA) regimes [increased by gibberellic acid (GA_3)] supplementation and reduced by paclobutrazol [PAC, (GA biosynthesis inhibitor) treatment] and light intensity (full sunlight and low light – 91.5% shading) to induce variations in leaf thickness, photosynthetic pigment content and leaf anatomy were used to evaluate leaf optical properties. Gibberellin supplementation promotes an etiolated phenotype with thinner leaves. On the other hand, the use of PAC induces the growth of smaller plants with a dark green phenotype. GA_3-treated plants showed greater leaf area, thinner leaves and reduced pigment content expressed on a leaf area basis, but not on a dry weight basis, independently of light level. PAC-treated plants showed increased pigment content and two layers of palisade parenchyma cells in full sunlight-grown plants, but similar leaf thickness when compared to the controls. PAC-treated plants grown in the low light environment showed a dense spongy parenchyma and increased leaf thickness. All plants, including low light-grown individuals, displayed similar and high absorption of blue and red lights. GA_3-treated plants presented increased green light reflectance and transmittance, and reduced absorbance. The opposite behavior was observed for PAC-treated plants. Green light is more absorbed by leaves with high pigment content (on a leaf area basis), but leaf thickness is the major component that drives green light absorption. Taken together, these results indicate that blue and red light are very efficiently absorbed, despite low chlorophyll content by adaxial structures of the leaf (palisade parenchyma), while green light is proposed to penetrate deeper in leaf cells and is suggested as driving light reactions in the spongy parenchyma.

1. Introduction

Plants perceive the surrounding environment in very specific and sensitive ways, which can induce phenotypic changes in the individual, at biochemical, physiological or morphological levels (Wada et al., 2005). Such changes show fundamental importance for plant surveillance in heterogeneous and unstable environments (Gratani, 2014; Pintado et al., 1997; Warpeha and Montgomery, 2016). Light perception is considered one of major environmental factors controlling leaf morphology (Bar and Ori, 2014; Galvão and Fankhauser, 2015; Wit et al., 2016), while plant adjustments are often associated with changes in hormone levels. In addition, light absorbance is necessary for photosynthesis (Croce and van Amerongen, 2014; Eberhard et al., 2008).

Plants grown in high irradiance environments exhibit phenotypic alterations, such as greater leaf area, higher chlorophyll and carotenoid concentrations per leaf area unit and thicker leaves, as well as more elongated palisade parenchyma cells, that lead to acclimatization and better performances in these environments (Givnish, 1988; Pearcy, 2007). On the other hand, shade-grown plants display thinner leaves, lower photoprotection, lower carotenoid content in the thylakoid antennae, thinner cell walls and greater light transmittance through the leaves (Niinemets, 2007).

Leaf optical properties can be altered due to light availability responses *via* structural leaf modifications and concentration of photosynthetic pigments (Carriello et al., 2003; Hatier and Gould, 2007; Sanches and Válio, 2006) in both shady or high-irradiance

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environments or by endogenous manipulation of the gibberellin (GA) metabolism (Hedden and Sponsel, 2015; Yamaguchi, 2008). Incident light on leaf surfaces may be reflected, absorbed or transmitted, and leaf absorbance spectrum is a key component in driving photochemical reactions. In addition, changes in the position of chloroplasts (Davis et al., 2011; Lepetit and Dietzel, 2015), cell size and shape (Macedo et al., 2011; Poorter et al., 2013), the presence of trichomes and epicuticular wax characteristics (Aasamaa and Aphalo, 2016; Macedo et al., 2011) may also influence the photosynthetically active radiation fraction (PAR; 400–700 nm) effectively absorbed by palisade and spongy parenchyma chloroplasts. The absorbance of blue and red lights and their importance to photosynthesis is well known (Macedo et al., 2011; Muneer et al., 2014; Ouzounis et al., 2015), based on typical chlorophyll absorbance spectra (*a* and *b*), although little is discussed regarding other PAR spectra regions (Folta and Maruhnich, 2007; Wang and Folta, 2013).

Leaf absorption spectra show a distinct pattern than those of photosynthetic pigments (chlorophyll *a* (Chl*a*), *b* (Chl*b*) and carotenoids (carotenes and xanthophylls) (Car)) extracted in organic solvents, *e.g.* acetone or dimethyl sulfoxide (Lichtenthaler and Wellburn, 1983; Lichtenthaler, 1987). However, some mutations that drive regions without chloroplasts (variegations) generate some white or near transparent leaf portions in many species. White portions of the leaf indicate lower influence of structural portions (*e.g.* waxes, cell wall, starch, proteins, organelles other than chloroplasts) of the cell on PAR light (visible) absorption (Fourty et al., 1996; Wang et al., 2004). This points to a major chloroplast control role in leaf absorption spectra (Terashima et al., 2009). According to this hypothesis, the number, size, ultrastructural thylakoid architecture and concentration of photosynthetic pigments in chloroplasts may exert a major influence on light absorption. Even so, leaf thickness alone increases the optic path (détour effect) (Terashima et al., 2009) and the probability for light to encounter chloroplasts, leading to the increase in absorbance can be an important physiological feature for plant surveillance and growth in low-light environments.

Variations in the absorbance spectrum in the blue and red regions may be more strongly related to the action of leaf palisade photosynthetic pigments (Chl*a*, Chl*b* and Car) than to absorbance by the entire leaf (Hatier and Gould, 2007). In contrast, both pigment content and leaf thickness are important regarding differences in the absorption in the green region, that is less absorbed by chlorophylls (Terashima et al., 2009). The wavelengths weakly absorbed by chlorophylls, such as green light, can drive a quantum yield at a similar or higher order than blue or red lights (Terashima et al., 2009). The lower absorption coefficient of green light by chlorophylls and its effectiveness to drive photosynthesis open the proposition that structural changes in palisade and spongy parenchyma cells are crucial in maximizing the efficiency of light capture (Carter and Knapp, 2001; Hatier and Gould, 2007; Sun et al., 1998; Terashima et al., 2009). In addition, changes in cell morphology have been highlighted as one of the most important components of plant surveillance (Brodersen et al., 2008; Brodersen and Vogelmann, 2010) in distinct light environments.

Based on the influence of leaf pigment content and leaf thickness on leaf optical responses (reflectance, absorbance and transmittance) in the PAR region of the spectra, pigment content, leaf optical properties, anatomical particularities and leaf thickness were evaluated in *Nicotiana tabacum* L. leaves grown in the shade and in a high-irradiance environment while varying gibberellin levels. Gibberellin supplementation promotes an etiolated phenotype with thinner leaves. On the other hand, the use of paclobutrazol (a gibberellin biosynthesis inhibitor) induces the growth of smaller plants with a dark green phenotype (Hedden and Sponsel, 2015; Ribeiro et al., 2012; Yamaguchi, 2008). This experimental strategy allowed for the manipulation of leaf thickness, photosynthetic pigment content and cell morphology, in order to evaluate how these features control leaf optical properties. It has been hypothesized that the increase in leaf thickness as well as in pigment content in leaves increase light absorbance mainly to enhanced green light absorption.

2. Material and methods

2.1. Growth conditions and experimental design

Nicotiana tabacum L. (cv. HAV 425) plants in the exponential growth phase were cultivated in a greenhouse at full (100%) sunlight irradiation and lower irradiance (low light – 8.5% of sunlight) in 5 L pots. A group has their leaves sprayed with gibberellic acid (GA_3) at different concentrations, while paclobutrazol (PAC) was applied in the soil. All plants grow for 20 days in these conditions. Spraying was conducted every 2 days totaling 5 applications using reverse osmosis water for the controls (Cont), 10 mM GA_3 (GA10) and 100 mM GA_3 (GA100). PAC 50 mg L^{-1} (PAC) was applied according to Ribeiro et al. (2012), as well as combined with GA_3 as follows: GA_3 10 mM + PAC (GA10P) and GA_3 100 mM + PAC (GA100P), forming a 2 × 6 factorial scheme (light and GA_3) with 12 treatments, with 6 repetitions each.

2.2. Plants growth analysis

Leaf area was evaluated each 5 days by a non-destructive analysis based on leaf length and width using the allometric equation proposed by Antunes et al. (2017). Leaf area was estimated following the equation[$LA = k*L*W$], where LA is leaf area, k is a (0.70014) correction factor, L is maximum leaf length and W is maximum leaf width.

2.3. Determination of leaf photosynthetic pigments content

Chlorophylls and carotenoids from 2 cm^2 leaf blade segments from the 4th or 5th expanded leaf (> 5 cm length) from the top of the plant were extracted with 10 mL of 80% acetone saturated with $CaCO_3$ for 18–20 h at room temperature in the dark. The absorbance spectra were measured on a Shimadzu UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan). Chlorophyll and carotenoid concentrations were estimated as reported by Lichtenthaler (1987) and note expressed by area unit and dry weight (DW).

2.4. Optical leaf properties

Reflectance (R) and transmittance (T) were measured directly using two coupled spectroradiometers (ASD Inc; FieldSpec, Colorado, USA), collimated and calibrated with a standard barium sulfate dish as the 100% reflectance reference.

A high irradiance light beam (upper 2000 mmol/m^2 s^{-1}) was coupled to one of the probes and positioned on the adaxial surface of the leaf and a second probe coupled to another spectroradiometer was positioned on the abaxial surface, in order to simultaneously measure leaf reflectance and transmittance, respectively. Absorbance (A) was estimated by the equation[A=1 − (R+ T)]. Key wavelengths of blue (435 nm), green (550 nm) and red (674 nm) were selected for the evaluation of possible correlations with pigments extracted from plant leaves.

2.5. Light microscopy analysis

Leaf blade segments (2 cm^2) similar to those used for the pigment analysis were immersed in Karnovsky's fixative solution (Karnovsky, 1965) and stored at 4 °C until processing. Subsequently, the fixed slides were dehydrated in ethanol [50,70, 80, 90 and 100% (three times)] and infiltrated with methyl-methacrylate (Leica Historesin) (Kraus and Arduin, 1997; Souza et al., 2016). Block sectioning was performed on a rotation microtome (Eikonal, São Paulo, Brazil) (8 mm) and the sections were then dyed with Toluidine Blue in acetate buffer pH 4.7 (O'Brien et al., 1964; Souza et al., 2016). The images were obtained

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