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Photosynthetic response of *in vitro* guayule plants in low and high lights and the role of non-photochemical quenching in plant acclimation

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ABSTRACT

Guayule (*Parthenium argentatum* L.) is a hypoallergenic latex-producing rather recalcitrant crop. During *in vitro* regeneration, the growth and the photosynthetic response of guayule is strongly affected by light intensities. We have used chlorophyll a (Chl-a) fluorescence to study the photosynthetic responses of *in vitro* grown guayule plants under low light (100 μmol m^{-2} s^{-1}) and high light (1250 μmol m^{-2} s^{-1}). In high light (HL), the shoot length was reduced and fresh and dry weights were enhanced, contrary to low light (LL) plant response. Total chlorophyll (Chl) and carotenoid contents based on fresh weight or leaf area were reduced by about 50% in HL compared to LL. Although maximum efficiency (F_v/F_m) of photosystem II (PSII) in the dark, electron transport rate (ETR-I), and quantum yield of photosystem I (PSI) were unaffected, the electron transport rate (ETR-II), quantum yield of PSII and non-photochemical quenching (NPQ) were ~78–88% higher in HL than LL. There were no significant differences observed in malondialdehyde (MDA) content during regeneration of plants in either HL or LL. The higher NPQ in HL grown plants than LL grown plants suggests that NPQ plays an important role in photoprotection during acclimation of guayule plants when exposed to HL.

1. Introduction

Guayule (*Parthenium argentatum*) is a shrub native to the Chihuahuan desert of Texas and Mexico. It is an alternate specialty rubber crop and recalcitrant species, which provides hypoallergenic natural rubber suitable for medical applications. It is currently under research and development for rubber production (Dong et al., 2006; van Beilen and Poirier, 2007) and commercially cultivated in Arizona, USA.

Light is one of the main factors that substantially affect plant growth and photosynthetic rate (Kangasjarvi et al., 2012). In nature, light varies in both intensity and spectral composition, which compels plants to survive by keeping the balance between light absorption required for photosynthesis and photoprotection (Wagner et al., 2006). Light energy is mainly utilized by plant pigments (chlorophyll and carotenoids), and protein complexes photosystem II (PSII) and photosystem I (PSI). When the amount of light energy absorbed by chlorophyll (Chl) exceeds the capacity of the photochemical process, singlet Chl (^{1}Chl*) molecules remain in an excited state long enough to cause their transition to reactive triplet Chl (^{3}Chl*) molecules (Barber and Andersson, 1992). These may then react with oxygen in the chloroplast, creating singlet oxygen radicals, which further react with membrane lipids and proteins resulting in photoinhibition and/or irreversible photo-oxidative damage (reviewed in Logan et al., 2006; Krieger-Liszkay et al., 2008; Triantaphylidès and Havaux, 2009).

Plants have evolved different mechanisms to protect the chloroplast from photo-oxidative damage (reviewed in Krieger-Liszkay et al., 2008; Takahashi and Badger, 2011). These include enzymatic and non-enzymatic antioxidants (glutathione, ascorbate, ascorbate peroxidase, superoxide dismutase, *etc.*), as well as scavengers of excess light energy (carotenoids). Any process that inhibits the use of absorbed light energy relative to its rate of influx will cause 'excitation pressure' on the photosystem, that is, more light

Abbreviations: Chl, chlorophyll; ETR, electron transport rate; F_0, minimum fluorescence yield in dark adapted leaf; F_0', the minimal fluorescence yield in light adapted leaf; F_m, maximum fluorescence yield in dark adapted leaf; F_m', maximum fluorescence yield in light adapted leaf; F_v/F_m, maximum quantum yield of PSII; NPQ, non-photochemical quenching; MDA, malondialdehyde; PSI, Photosystem I; PSII, photosystem II; q_p, photochemical quenching.

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energy is absorbed than can be processed by photochemistry so the reaction centers become reduced or 'closed' (Huner et al., 1998).

Chl-a fluorescence kinetics is an informative tool for studying the effects of different environmental stresses on photosynthesis (Kalaji et al., 2012). It is one of the main methods used to investigate the function of PSII and its reaction to changes in the environment and growth conditions (Desotgiu et al., 2012). Among the various parameters, non-photochemical quenching (NPQ) has commonly been used to measure the dissipation of excess excitation energy as heat. Heat dissipation occurrs in the antennae (Belgio et al., 2012) or in the reaction center (Wilhelm and Selmar, 2011). The basic principle of NPQ operation is the same in all chloroplast-containing species but differences occur in its mechanistic regulation (Kaňa et al., 2012). Light-induced formation of NPQ is activated by a pH decrease in the thylakoid lumen, which further activates the violaxanthin de-epoxidase to convert violaxanthin to zeaxanthin (Jahns and Holzwarth, 2012). The protonation of the PsbS protein is also an essential element of NPQ mechanism in higher plants (Li et al., 2004). Generally, in excess light, the rate of NPQ increases, but high light (HL) stress may also reduce NPQ, which is indicative of irreversible damage to the PSII reaction center and/or antennae.

The *in vitro* regeneration of plants is a crucial technique in crop development, but is affected by light intensities. Here we have used low and high light (100 and 1250 $\mu mol\, m^{-2}\, s^{-1}$) to discern the acclimatory response in guayule during its *in vitro* regeneration. Since Chl-a fluorescence is a good marker for estimating the photosynthetic rate under varying light intensity, the outcome of this study should be helpful in understanding high light acclimatory mechanisms and photosynthetic regulation in recalcitrant *in vitro* grown plants.

2. Materials and methods

2.1. Plant material and growth conditions

P. argentatum (guayule) shoot-regenerated cultures were used as an experimental material. Guayule seeds (*P. argentatum* cv. PanAridus-2, (Panaridus, Casa Grande, Arizona, USA)) were sterilized by treating with 70% ethanol for 30 s, then with 0.5% NaOCl + Tween 20 for 3 min, and then washed 5–6 times with sterilized autoclaved water. Seeds were germinated in glass jars on 0.5× MS (MS basal salts) medium with 15 g/L sucrose, 100 mg/L inositol, 0.5 mg/L Thiamine-HCl, 8 g/L agar and pH 7.0 under low light ($100\, \mu mol\, m^{-2}\, s^{-1}$) and at $27 \pm 1\,^{\circ}C$ temp. Cultures were maintained through shoot apex culture and rooted on medium (0.5× MS basal salts) containing 15 g/L sucrose, 100 mg/L inositol, 0.5 mg/L Thiamine-HCl, 0.1 mg/L Indole-3-butyric acid, 8 g/L agar and pH 7.0. Shoots were grown for fifteen days to develop roots and subsequently plants were either maintained at $100\, \mu mol\, m^{-2}\, s^{-1}$ (LL) or transferred to $1250\, \mu mol\, m^{-2}\, s^{-1}$ (HL) for acclimation. The LL and HL levels chosen based on previous published reports for the different plant species. The solid state (SSL) lamps (DesignInnova, New Delhi, India) were used as the light source. According to vendor's photobiosim, natural sun light was mimicked for the plant cultures using specific dominant wavelengths modulated in the analog domain after eliminating the IR, UV and EMI light spectra. Specific wavelength generating semiconductor multichips were used to mix up the 30% light from the complete spectrum with the 70% of dominant wavelengths in the PAR region (*i.e.* 400–470 nm and 625–700 nm). The Solid state light (SSL) is a super set of light that makes use of semiconductor LEDs, Polymer LEDs (PLED), and Organic LEDs (OLED), as illumination sources instead of plasma (used in fluorescent lamps), gas or electric filaments. All experimental measurements were conducted after 15 days of the plants' transfer to light (LL and HL).

2.2. Chl, carotenoid and protein estimation

Chl and carotenoid contents were estimated from fully expanded leaves from the middle portion of the plants as described by Porra et al. (1989) and Welburn and Lichtenthaler (1984). Protein quantification was performed according to Bradford (1976).

2.3. Chlorophyll fluorescence and P700 measurement

Chl fluorescence was studied in detached leaves. The Chl fluorescence of PSII and the redox state of P700 were measured at room temperature using the Dual-PAM-100 fluorometer (Walz, Effeltrich, Germany) and Dual-PAM software. Actinic light from a 620 nm light-emitting diode (LED) and blue actinic light at $100\, \mu mol\, m^{-2}\, s^{-1}$ from 460 nm LED arrays was delivered to a guayule leaf for 5 min, along with saturating light pulses ($10{,}000\, \mu mol\, m^{-2}\, s^{-1}$) of 300 ms. The guayule leaves immediately after detachment were kept in the dark for 10 min before the measurement of minimum (F_0) and maximum (F_m) fluorescence (using saturated flash). Maximum quantum yield of PSII (F_v/F_m) was measured as (Schreiber, 2004). The F'_m (maximum fluorescence in light adapted leaf) was measured when the leaves were illuminated. The quantum yield Y(II) of PSII was measured as $(F'_m - F)/F'_m$ (Kramer et al., 2004). F is the steady state fluorescence in a light adapted leaf. The electron transport rate of PSII (ETRII) was estimated by Dual-PAM software (Walz, Effeltrich, Germany). NPQ was calculated as $(F_m - F'_m)/F'_m$ (Schreiber et al., 1986). Photochemical quenching (q_p) was calculated as $(F'_m - F)/(F'_m - F'_0)$ where F'_0 is the minimal fluorescence in light adapted leaf.

The P700 redox state was also measured by Dual-PAM-100 with a dual wavelength (830/875 nm) unit (Klughammer and Schreiber, 1994; Schreiber and Klughammer, 2008). The P700 signal (P) may vary from minimum (P700 fully reduced) to maximum (P700 fully oxidized). The maximum PSI signal (P_m), which is analogous to F_m, was determined with application of a saturation pulse after pre-illumination with far-red light. The minimum P700 signal (P_0), analogous to F_0, was calculated when complete reduction of PSI was induced after the saturation pulse and in the absence of far-red illumination. P'_m was determined similarly to Pm, but with background actinic light instead of far-red illumination. The quantum yield Y(I) of PSI was calculated as $(P'_m - P)/P'_m$ (Pfündel et al., 2008). Electron transport rates of PSI (ETRI) were analyzed using the Dual-PAM software.

2.4. Malondialdehyde assay

MDA, a measure of lipid peroxidation and an indicator of oxidative stress (Turan and Tripathy, 2013), was analyzed as described by Dhindsa and Matowe (1981). Leaf tissues were ground in 10 mL of 10% (w/v) TCA and homogenate was centrifuged at 10,000 rpm for 10 min. Afterward, 2 mL of 10% TCA containing 0.67% (w/v) thiobarbituric acid was added to 2 mL supernatant. The mixture was incubated at 100 °C for 15 min and centrifuged at $10{,}000 \times g$ at 4 °C for 10 min. The OD of the supernatant was measured at 532 nm (corrected for nonspecific turbidity by subtracting the A_{600}). MDA values were calculated using a molar extinction coefficient of $1.56910\ 5\ M^{-1}\, cm^{-1}$ and expressed relative to the leaf FW ($nmol\, g^{-1}$ FW).