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Phytochromes are key regulators of abiotic stress responses in tomato

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ABSTRACT

Phytochromes are the best characterized and most frequently studied plant photoreceptors. A plethora of studies have revealed important roles for phytochromes in plant development, and more recently, evidence indicates that these photoreceptors also modulate responses to a multitude of abiotic and biotic stresses. Thus, the present work aimed to investigate whether tomato phytochromes phyA, phyB1 and phyB2 are involved with responses to low water potential (polyethylene glycol 6000 at Ψ_w of −0.3 MPa), high salinity (100 mM NaCl), cadmium contamination (65 mM $CdCl_2$), high temperature (42 °C for six hours during three days) and ultraviolet B radiation (UV-B – 280–320 nm for eight hours during three days) stresses. For this purpose, seedlings of tomato mutants impacted by phytochrome A (*fri*), phytochrome B1 (*tri*) and phytochrome B2 (*phyB2*) were subjected to abiotic stresses and evaluated for their growth, pigment and osmoprotectant accumulation and lipid peroxidation. Under the conditions of this study, the results did not shown large variations of phyA mutant when compared to the wild genotype. However, the tomato phytochromes B1 and B2 mainly act as negative regulators of growth, pigment maintenance and osmoprotectant accumulation during responses to the different abiotic stresses.

1. Introduction

Light is one of the most important environmental signals regulating plant development. Fluctuations in light quality and quantity can deeply modify how, when and where a plant will grow; therefore, light is a crucial signal for a species to thrive in its environment. Thus, it is not surprising that plants have evolved different receptors to perceive light signals. Among these types of photoreceptors, phytochromes are, so far, the best characterized and more frequently studied (Carvalho et al., 2011a). Phytochromes are dimeric proteins (~130 kDa) covalently linked to the phytochromobilin, a linear tetrapyrrole that acts as a chromophore and recognizes specific light signals to interconvert the phytochrome from its inactive state, which perceives red light wavelengths (Pr), to the active state, which perceives far-red light wavelengths (Pfr) (Chen et al., 2005). Tomato (*Solanum lycopersicum* L.) is an important crop species in which the characterization of the molecular functions and modes of action of phytochromes have been constantly studied. The tomato plant harbors the following five phytochrome types: phyA, phyB1, phyB2, phyE and phyF (Pratt et al., 1997).

Since its discovery, a plethora of studies have revealed important roles of phytochromes in plant development, from seed germination to flowering (Carvalho et al., 2010; Toledo-Ortiz et al., 2010). Initial evidence for the involvement of plant photoreceptors as mediators of stress responses date back from the 1970s (Williams et al., 1972), but the topic has only recently been gaining more interest. The knowledge of how phytochromes work at the molecular level, the identification of transcription factor families whose action is regulated by phytochromes (Castilon, 2007; Zheng et al., 2014) and the increased availability of phytochrome mutants in different plant species provide ideal tools for studying the participation of these photoreceptors in biotic and abiotic stress responses.

With respect to the multitude of abiotic and biotic stresses modulated by phytochromes, they have been shown to be important components of plant signaling pathways involved in responses to insect herbivory (Ballaré, 2009; Howe and Jander, 2008), temperature fluctuations (Auge et al., 2012; Donohue et al., 2008; Foreman et al., 2011), harmful light radiation (e.g., ultraviolet B (UV-B)) (Boccalandro et al., 2001; Kreslavski et al., 2013, 2015; Mani and Guruprasad, 2015), salt stress (Balestrasse et al., 2008a; Datta et al., 2008), water stress (D'amico-Damião et al., 2015; Kidokoro et al., 2009) and even heavy

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metal intoxication (Cui et al., 2011). Because those environmental conditions can greatly affect plant productivity, the studies on phytochrome regulation of stress responses have become a hot spot of research.

Photomorphogenetic mutants have constantly been employed to evaluate the role of phytochromes in stress responses (Carvalho et al., 2011b; D'amico-Damião et al., 2015). For example, Indorf et al. (2007) found that phyA and phyB *Arabidopsis thaliana* mutants showed a reduced expression of *STO,* a gene involved in salt stress responses, when treated with red light, suggesting that the phytochrome family contributes to salt stress responses. In rice modified with maize phytochrome interacting factor 3, it was shown to improve drought and salt stress tolerance (Gao et al., 2015). In fact, phyB seems to be a fundamental part of abiotic stress responses. Studies with rice *phyB* mutants showed that the presence of the *phyB* genotype reduced water loss per unit of leaf area, conferring better tolerance to water stress (Liu et al., 2012). Arabidopsis phytochrome B mutants indicate that this photoreceptor is involved in responses to high temperature (Njimona et al., 2014) and prolonged UV-B radiation (Boccalandro et al., 2001; Huang et al., 2012; Li et al., 2013; Rusaczonek et al., 2015).

However, although the control of stress response by phytochromes is an old topic (Williams et al., 1972; Thomsen et al., 1992; Cockburn et al., 1996), recently, evidence has emerged revealing new roles of these molecules. Transgenic plants overexpressing the chromophore biosynthesis enzymes have been associated with increased tolerance to mercury toxicity (Shen et al., 2011). Transgenic plants expressing the hyperactive mutant S599A-PhyA showed improved tolerance to zinc (Gurunani et al., 2016), while phyA mutants in tomato showed altered response to nutritional stress (Carvalho et al., 2016). These studies suggest a complex, multifaceted control of the stress response by phytochromes, raising questions about how and which phytochromes modulate different abiotic stresses. Thus, the present work aimed to investigate if tomato phytochromes phyA, phyB1 and phyB2 are involved in responses to low water potential, high salinity, cadmium contamination, high temperature and UV-B radiation stresses. For this purpose, seedlings of tomato mutants impacted in phytochrome A (*fri*), phytochrome B1 (*tri*) and phytochrome B2 (*phyB2*) were subjected to abiotic stresses and their growth, pigment and osmoprotectant accumulation and lipid peroxidation were evaluated.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds from the tomato photomorphogenic mutants *far-red light insensitive* (*fri*), *temporarily red light insensitive* (*tri*) and *phyB2,* defective for the genes encoding the PHYA, PHYB1 and PHYB2 apoproteins, respectively, were obtained from the "Tomato Genetics Resource Center" (TGCR; Davis – California). All mutants were present in the Moneymaker cultivar (Van Tuinen et al., 1995a,b; Kerckhoffs et al., 1999), which was used as a wild type (WT) parent for all experiments. To avoid fungal contamination, seeds were pre-treated with a 5% sodium hypochlorite solution for 10 mins and then thoroughly washed with water before use. Germination was performed in plastic boxes containing two layers of filter paper embedded in water, under 25 °C in the dark. For all experiments described, plants were grown in a chamber under 12 h white light (60 μmol $m^{-2} s^{-2}$) at 25 °C.

2.2. Induction of low water potential, high salt concentration and cadmium contamination stresses

Three days after germination, 25 seedlings with 2 mm radicles were transferred to 1 L plastic pots containing two layers of filter paper embedded in 15 mL of different solutions prepared to induce the studied stressful condition. For low water potential, a solution of polyethylene glycol 6000 (PEG) was prepared to achieve a Ψ_w of −0.3 MPa, as described by Vilela et al. (1992). Solutions of 100 mM NaCl and 65 mM $CdCl_2$ were prepared to induce high salt concentration and cadmium contamination stresses, respectively. As a control condition, a set of pots was filled with 15 mL of distilled water. Each of the described conditions consisted of three pots that were maintained for seven days in a growth chamber adjusted to the same conditions aforementioned in Section 2.1.

2.3. Induction of high-temperature stress and prolonged UV-B light exposure

Four days after germination, 25 seedlings were transferred to 1 L plastic pots containing two layers of filter paper embedded in 15 mL of distilled water and placed inside a control chamber, set at 25 °C, with a 12 h photoperiod and 60 μmol $m^{-2} s^{-2}$ light intensity (Alexieva et al., 2001). A set of three pots was kept in this chamber as a control for the experiment. For high temperature stress, a set of three pots was moved to a chamber maintained at 42 °C for six hours and then returned to the control chamber. This procedure was repeated on the 6th and 7th days after germination, always returning the plates to the control chamber after treatment with high temperature. Plates were then kept in the control chamber for three extra days (10 days total) until further measurements. For prolonged UV-B light exposure, a set of three pots was moved to a chamber containing mercury lamps set to specifically emit UV-B light wavelength (8W-T5, BRAVO, peak at 305 nm – 310 nm) added to white lamp (20W-T10, NSK). Plates were kept under the UV-B light for eight hours and then returned to the control chamber. This procedure was also repeated on the 6th and 7th days after germination, always returning the plates to the control chamber after treatment with high temperature. Plates were then kept in the control chamber for three extra days (10 days total) until further measurements. Fig. 1 illustrates the strategy used for induction of high temperature (Fig. 1A) and UV-B light (Fig. 1B) stresses.

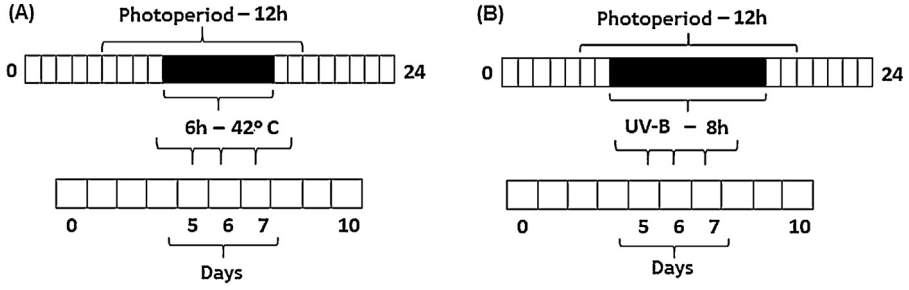

Fig. 1. Methodology used for high temperature (A) and prolonged UV-B light (B) stresses. Seedlings were kept in a control chamber (25 °C, 12 h photoperiod, see Methods) for an initial period of 4 days. For high temperature stress, seedlings were then transferred to a chamber set at 42 °C for six hours during the 5th, 6th and 7th days after germination, always returning the plates to the control chamber after treatment. For prolonged UV-B light exposure, seedlings were moved to a UV-B (305–310 nm) chamber for eight hours during the 5th, 6th and 7th days after germination, always returning the plates to the control chamber after treatment. For both treatments, plates were kept at the control chamber for three extra days (10 days total) until measurements were taken.