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Constitutive gibberellin response in grafted tomato modulates root-to-shoot signaling under drought stress

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ABSTRACT

Plants are sessile organisms that must perceive and respond to various environmental constraints throughout their life cycle. Among these constraints, drought stress has become the main limiting factor to crop production around the world. Water deprivation is perceived primarily by the roots, which efficiently signal the shoot to trigger drought responses in order to maximize a plant's ability to survive. In this study, the tomato (*Solanum lycopersicum* L.) mutant *procera* (*pro*), with a constitutive response to gibberellin (GA), and its near isogenic line cv. Micro-Tom (MT), were used in reciprocal grafting under well-watered and water stress conditions to evaluate the role of GA signaling in root-to-shoot communication during drought stress. Growth, oxidative stress, gene expression, water relations and hormonal content were measured in order to provide insights into GA-mediated adjustments to water stress. All graft combinations with *pro* (i.e. *pro/pro*, MT/*pro* and *pro*/MT) prevented the reduction of growth under stress conditions without a reduction in oxidative stress. The increase of oxidative stress was followed by upregulation of *SlDREB2*, a drought-tolerance related gene, in all drought-stressed plants. Scions harboring the *pro* mutation tended to increase the abscisic acid (ABA) content, independent of the rootstock. Moreover, the GA sensitivity of the rootstock modulated stomatal conductance and water use efficiency under drought stress, indicating GA and ABA crosstalk in the adjustment of growth and water economy.

1. Introduction

Water stress is one of the main constraints for crop production around the world. In addition, there are predictions that this will worsen in the next years due to global warming and climate changes (Dai, 2011; Bornman et al., 2015; Trnka et al., 2015). Climate changes can strongly impact rainfall regime, which is one of the greatest limitations to crop expansion in agricultural systems (Skirycz and Inzé, 2010; Dai, 2011; Sardans and Peñuelas, 2013; Wheeler and Von Braun, 2013). Under this climatic changing context, plants would be more vulnerable to severe drought conditions (Dai, 2012). Water stress adversely affects many aspects of the physiology of plants by reducing stomatal conductance to maintain leaf water status and, consequently, result in lower leaf internal CO_2 concentrations that negatively impact photosynthesis and plant growth under stress conditions (Granier and Tardieu, 1999; Skirycz and Inzé, 2010; Tramontini et al., 2013; Ollas et al., 2015). These modifications are coordinated by an intricate network of molecular and biochemical signals (González et al., 2013; Meyer et al., 2014; Qazi et al., 2014; Sellin et al., 2014). The expression of various genes with functions in the water stress responses has been identified in many species (Guo and Wang, 2011; González et al., 2013; Blum, 2014; Ober et al., 2014). In addition, the involvement of general physiological processes associated with drought-responsive gene expression include oxidative stress molecules production (Ashraf and Foolad, 2007; Ahmed et al., 2014; Tesfaye et al., 2014) and plant hormone biosynthesis and signaling (Pospíšilová, 2003; Colebrook et al., 2014; Cui et al., 2015; Ollas and Dodd, 2016).

The plants take up water from the soil by the roots. Therefore, the

Abbreviations: *A*, CO_2 assimilation; ABA, abscisic acid; DAS, days after sowing; DW, dry weight; *E*, water transpiration; FW, fresh weight; GAs, gibberellins; GID1, GIBBERELLIN-INSENSITIVE DWARF 1; *gs*, stomatal conductance; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; POD, peroxidase; ROS, reactive oxygen species; RWC, relative water content; TW, turgid weight; WUE_i, intrinsic water use efficiency

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reduction in water availability in the soil is readily sensed by plant roots to respond to local moisture (Holbrook et al., 2002; Tramontini et al., 2013; Martorell et al., 2015). Thus, in order to limit water loss during soil drying, plants can control the stomata aperture to reduce water transpiration even before the water status declines in the root and shoot (Gollan et al., 1986; Stoll et al., 2000; Augé and Moore, 2002; Holbrook et al., 2002; Osakabe et al., 2014) which implicates root-to-shoot communication to modulate shoot response to drought. This suggests the existence of a biochemical signal from the roots that triggers adaptive mechanisms in the shoot. There is compelling evidence that the abscisic acid (ABA) plays an important role for long-distance signaling considering that the levels of this water-stress associated hormone increases in both xylem sap and leaves controlling stomata closure under stress (Jacobsen et al., 2009; Carvalho et al., 2011; Vijayalakshmi et al., 2014; Ollas and Dodd, 2016). However, several experiments have demonstrated that, under drought conditions, the stomata close can occur independently of the ABA biosynthesis by the roots (Stoll et al., 2000; Augé and Moore, 2002; Holbrook et al., 2002). For instance, grafting experiments with tomato (*Solanum lycopersicum* L.) mutants with reduced ABA biosynthesis (*flacca* and *sitiens*) revealed that stomatal closure occurred independently of ABA production by the roots, but rather ABA biosynthesis in the leaves represents a key signal for stomatal behavior (Holbrook et al., 2002). Thus, the nature of the root-derived systemic signal induced by water stress has remained elusive. Recent investigation indicates a crosstalk mechanism between ABA and gibberellins (GAs) during water-limited conditions, in which ABA biosynthesis and the control of stomatal conductance were regulated by the soluble receptor for GA, GIBBERELLIN-INSENSITIVE DWARF 1 (GID1) under water stress (Du et al., 2015). The *gid1* rice (*Oryza sativa* L.) mutant, which impairs GA signaling, showed reduced levels of ABA and increased stomatal conductance in comparison to wild-type plants under drought stress (Du et al., 2015).

The phytohormone GA is involved in the adaptive response to various abiotic stresses such as cold, salinity, heat, flooding and drought (Achard et al., 2008; Colebrook et al., 2014; Khan et al., 2015). However, the role of GAs during drought stress adaptation is still unclear. Reduction of GAs levels in maize (*Zea mays* L.), wheat (*Triticum aestivum* L.) and ramie [*Boehmeria nivea* (L.) Gaud] have been described during drought conditions (Wang et al., 2008; Coelho Filho et al., 2013; Liu et al., 2013a, 2013b). Moreover, a GA application could recover plant growth under stress conditions, providing greater growth and maintenance of photosynthesis, as well as oxidative stress reduction (Kaya et al., 2006; Akter et al., 2014). On the other hand, there is a range of studies demonstrating that reduced sensitivity to GAs may induce a greater tolerance to water stress. For instance, wheat *Rht8*, *Rht-1b* and *Rht-D1b* mutants, with reduced GA sensitivity, were more tolerant to drought stress compared to the wild-type (Landjeva et al., 2008; Alghabari et al., 2014; Alghabari et al., 2016). Likewise, plants with reduced levels of active GAs, such as the mutants of *Arabidopsis thaliana* (*ga20ox1/2* and *ga3ox1/2*) and the transgenic tomato overexpressing *AtGAMT1*, a gene from Arabidopsis that encodes an enzyme that induces GA deactivation, induce greater tolerance to water-limiting conditions (Colebrook et al., 2014; Nir et al., 2014). However, the involvement of GAs signaling in root-to-shoot communication to coordinate growth and development at the whole-plant level in response to drought stress is largely unexplored.

Furthermore, the recent discovery of GA_{12} transported by vascular bundles (Regnault et al., 2015) allows us to raise the following questions: i) Do GAs act in the perception of water deprivation by the roots? ii) Are GAs the biochemical signal transported to long-distance from the roots to the shoot controlling drought stress responses? iii) If so, is the role of GAs during drought stress negative or positive? To provide insights into these questions, we used the tomato mutant *procera* (*pro*), which has a constitutive response to GA (Carrera et al., 2012), and its near isogenic line cv. Micro-Tom (MT) in reciprocal grafting under well-watered and water stress conditions.

2. Material and Methods

2.1. Plant material and grafting

Seeds of tomato (*Solanum lycopersicum* L.) mutant *procera* (*pro*), which exhibits constitutive GA response due to a point mutation in the gene encoding DELLA protein (Bassel et al., 2008), and a near isogenic line cv. Micro-Tom (MT) were germinated in boxes containing a mixture of 1:1 (v/v) commercial pot mix (BioPlant, Brazil) and vermiculite. Fifteen days after sowing (DAS), the plants were transferred to pots filled with the same sowing mixture, and grafting was performed by splice method combining MT and *pro* in reciprocal grafting (MT/MT, *pro/pro*, MT/*pro*, *pro*/MT; the first genotype indicates the scion, and the second genotype indicates the rootstock). Immediately, the grafted plants were placed in a floating moist chamber and were kept until complete healing of the grafting region (*c.* 15 days), and then were transferred to a glasshouse.

2.2. Water stress conditions

All plants were watered daily until the beginning of water stress. To establish the stress treatment, irrigation was suspended for a seven-day period in the grafted plants (37 DAS). As a control, plants were daily watered by maintaining water availability close to the capacity of the potting mix. After seven days under the respective growth conditions (well-watered and drought stress), plants (45 DAS) were taken for analysis as described below.

2.3. Growth analysis

Plant height was obtained using a graduated ruler. The leaf area was measured using an Image Analysis System (Delta-T Devices, Cambridge, UK), whereas the root area was measured using a Hewlett Packard 125C scanner; the image of each plant was analyzed by Delta-T Scan software. Subsequently, the weights of both the roots and shoot fresh mass were recorded. Afterwards, they were oven-dried at 60 °C for 72 h, and the dry weight was determined using an analytical balance (Denver Instrument Company AA-200).

2.4. Chlorophylls and carotenoids contents

The pigments were extracted from the third fully expanded leaf as described by Alves et al. (2017) and were determined spectrophotometrically at 661.6 nm (Chlorophyll *a*), 644.8 nm (Chlorophyll *b*) and 470 nm (Carotenoids), and the concentration of each pigment was estimated by the equations of Lichtenthaler (1987).

2.5. Lipid peroxidation and H_2O_2 content

Lipid peroxidation was estimated by the content of thiobarbituric acid reactive substances (TBARS). Malondialdehyde (MDA) was estimated by measurements at 535 and 600 nm, and the concentration was calculated using an extinction coefficient of 1.55×10^{-5} mol^{-1} cm^{-1} (Gratão et al., 2012). MDA content was expressed in nmol g^{-1} fresh weight.

The content of hydrogen peroxide (H_2O_2) was determined by a reaction with potassium iodide, as described by Alexieva et al. (2001). The absorbance was read at 560 nm, and the H_2O_2 content for all samples was determined using a known H_2O_2 concentration curve as a standard. H_2O_2 content was expressed in mol g^{-1} fresh weight (Alves et al., 2017).

2.6. Peroxidase activity (POD EC 1.11.1.7)

Approximately 500 mg of plant tissue were macerated in the presence of liquid nitrogen and homogenized with 50 mM potassium