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A study on ABA involvement in the response of tomato to suboptimal root temperature using reciprocal grafts with *notabilis*, a null mutant in the ABA-biosynthesis gene *LeNCED1*

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ABSTRACT

To elucidate the role of abscisic acid (ABA) in tomato (*Solanum lycopersicum* L.) responses to suboptimal root temperature (T), a near-isogenic line carrying the *notabilis* null mutation in the ABA biosynthesis gene *LeNCED1* was reciprocally grafted with its parental cultivar Ailsa Craig. Exposure of tomato to suboptimal root T (15 °C) decreased leaf area expansion, shoot elongation and plant biomass in comparison with optimal root T (25 °C). Both suboptimal root T and null mutation of the *LeNCED1* gene in root and shoot reduced leaf area and total plant biomass, but these two factors did not interact. Transpiration rates and stomatal conductances decreased, while net CO_2 assimilation was not influenced by root exposure to suboptimal T. However, *notabilis* scions exhibited higher net assimilation rates, stomatal conductances and transpiration rates than Ailsa Craig scions. Moreover, *notabilis* plants invested much more biomass into the root than Ailsa Craig self-grafts, thereby improving their water uptake capacity. Lipid peroxidation as well as polyamines and guaiacol-peroxidase (G-POD), which are considered to possess antioxidant properties, increased in the leaves of all grafting combinations of tomato when exposed to suboptimal root T. All grafting combinations showed an increase in shoot ABA levels when exposed to suboptimal root T. However, the levels of ABA in the shoot of *notabilis* did not differ significantly from those found in Ailsa Craig, indicating that inactivation of *LeNCED1* did not impair ABA accumulation in the leaves at low root-zone T. The genes *LeNCED2* and *LeNCED6* were not up-regulated at low root-zone T and therefore not responsible for ABA biosynthesis in *notabilis*. Increased ABA levels in tomato exposed to suboptimal root T were associated with elevated levels of the polyamine putrescine which contributes to ABA biosynthesis. In conclusion, the introgression in Ailsa Craig that contains the mutation resulting from inactivation of the *LeNCED1* gene includes further genetic differences that influence responses to suboptimal root-zone T, such as plant growth restriction and ROS scavenging modification.

1. Introduction

Tomato (*Solanum lycopersicum* L. Mill.) is an economically important crop (Mauromicale et al., 2011) that is adapted to warm climates. Therefore, growth and development of agriculturally used cultivars are inhibited at temperatures below 12 °C (Criddle et al., 1997) and tomatoes are produced during the summer period in the open field and throughout the year indoors (Venema et al., 2008). Consequently, energy costs for greenhouse heating represent a large percentage of the total production costs of tomato (Venema et al., 2008). According to Elings et al. (2005), a 16% saving in energy costs could be achieved through a reduction of 2 °C in the mean air temperature (T) in the greenhouses, while the same reduction restricts the CO_2 emission by about 200 t/ha. This could be achieved through an increase in tomato tolerance to suboptimal T, i.e. in a range between minimum (8–12 °C) and optimum T (25–27 °C) (Schwarz et al., 2010). However, this approach failed due to the very low genetic diversity of the commercially used tomato cultivars that did not allow the identification of genes conferring tolerance to suboptimal T (Schwarz et al., 2010).

A promising tool to enhance the tolerance of tomato against environmental stresses is grafting onto rootstocks that are known

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to affect canopy development, disease resistance or cold hardiness (Schwarz et al., 2010). Increased tolerance to cooling/suboptimal temperature of the root zone could be achieved through the use of *S. habrochaites* LA 1777 as rootstock (Venema et al., 2008). This wild tomato species originates from an altitude of 3200 m (Rick et al., 1994) where an adaptation to low temperatures can be expected (Patterson et al., 1978). According to Jensen et al. (2003), acropetal and basipetal metabolite transport is modified by hormonal messengers under low T conditions. Hence grafting, which results in two different genotypes in root and shoot, is an excellent tool to investigate interactions of metabolite transport with suboptimal T. Indeed, Zhou et al. (2007) demonstrated that some signals (i.e. ABA/CK) originating from chilling-resistant cucumber rootstocks contributed to protection of leaf photosynthesis in a chilling-sensitive scion. Nevertheless, the underlying signaling pathways are poorly understood.

Plants have evolved adaptive mechanisms to alleviate cell damage and retain their ability to reproduce under suboptimal T conditions. Chilling-susceptible tomato plants did not close their stomata at 5 °C until a water loss of 20% of the fresh weight was reached (Bloom et al., 2004), while transpiration of cold-adapted plants was more responsive to reduced water status at that T (Guye and Wilson, 1987). This was associated with a higher ABA content in cold-acclimated plants (Daie and Campbell, 1981). ABA is a plant hormone that can be produced in both root and shoot, and transported acropetally through the xylem and basipetally through the phloem, respectively (Taiz and Zeiger, 2007), making signal transduction via ABA possible in both directions. ABA may function as a growth promoter under stress conditions such as soil compaction (Mulholland et al., 1996, 1999) and drought (Sharp and Le Noble, 2002), but may also reduce shoot growth in plants exposed to water stress (Creelman et al., 1990; Saab et al., 1990; Zhang and Davies, 1990). Moreover, as reported by Bloom et al. (2004), several other signals such as cytokinins and nitric oxide appear to be associated with ABA in promoting stomatal closure. However, little is known about the role of ABA in tomato responses to suboptimal T.

Plant metabolism is modulated due to interaction of stress- with sugar-signaling pathways under abiotic stress conditions (Gupta and Knaur, 2005). Indeed, during cold stress, sucrose accumulation in leaves leads to a feed-back inhibition of photosynthesis (Chiou and Bush, 1998; Ruelland et al., 2009). As reported by Zhou et al. (2007), some signals originating from the roots of chilling-resistant cucumber rootstock (i.e. ABA and cytokinins) protected leaf photosynthesis in shoots of a chilling-sensitive scion. Under stress conditions such as low temperature, the reactions of the Calvin cycle are down-regulated, and this may lead to over-reduction of the photosynthetic light reactions and generation of reactive oxygen species (ROS) (Ensminger et al., 2006). Zhou et al. (2009) demonstrated that increased ABA concentration in xylem sap could enhance the activity of antioxidants and induce stomata closure which could lead to decreased CO_2 fixation and increased ROS generation. The accumulated sugars may act as osmolytes or substrates for cellular respiration (Gupta and Knaur, 2005) or as antioxidative agents (Bogdanovic et al., 2008) that scavenge ROS (Noctor and Foyer, 1998). ROS-scavenging capacity is also enhanced by hormones (e.g. cytokinins) as reported by Zhou et al. (2007).

The polyamines putrescine (Put), spermidine (Spd) and spermine (Spm) can also protect cells from stress damage by acting as ROS scavengers (Alcazar et al., 2010; Rhee et al., 2007; Zhao and Yang, 2008) or by stimulating non-photochemical quenching (Ioannidis and Kotzabasis, 2007). Put levels increase upon cold stress in many plant species (Kushad and Yelenosky, 1987; Lee et al., 2012; Shen et al., 2000), which may affect ABA biosynthesis through the activation of the Arabidopsis *AtNCED3* gene (Cuevas et al., 2008). Regulation of ABA homeostasis is an important process during plant adaptation to abiotic stress conditions and involves changes in ABA biosynthesis, catabolism, transport and conjugation in a tissue-specific manner (Baron et al., 2012; Nambara and Marion-Poll, 2005). ABA biosynthesis is controlled by the genes encoding 9-*cis*-epoxycarotenoid dioxygenases (*NCED*) that use substrates of the xanthophyll-cycle to produce xanthoxin, a precursor of ABA (Seo and Koshiba, 2002). Since at least two *NCED*s (*NCED1* and *NCED2*) have an important role in ABA biosynthesis, the use of mutants with impaired function of *NCED*s provide a useful tool to elucidate the involvement of ABA homeostasis in stress tolerance (Taylor et al., 2005). Focusing on tolerance to suboptimal T in the root zone, the comparison of rootstock/scion combinations with different tolerance to suboptimal T should provide a better understanding of the root to shoot interactions with respect to hormonal transport and signaling.

The research presented in this paper was designed to contribute to a better understanding of the implication of ABA biosynthesis and homeostasis in the responses of tomato to suboptimal T stress in the root zone. To distinguish between the functions of root- and shoot-synthesized ABA, self- and reciprocal grafting of the tomato mutant *notabilis*, which carries a null mutation in the ABA biosynthesis gene *LeNCED1* (Burbidge et al., 1999), and its near-isogenic wild-type counterpart Ailsa Craig, was performed. Links between ABA, sugar, and polyamine metabolism were studied under both control/optimal and suboptimal T in the root zone.

2. Materials and methods

2.1. Plant material and growth conditions

The *notabilis* mutant is a near-isogenic line derived from repeated backcrossing to the cultivar Ailsa Craig which carries to a function the wild-type *LeNCED1* gene (Maxon-Smith and Ritchie, 1983). Self- and reciprocal grafting of the mutant and Ailsa Craig was conducted in a heated glasshouse at the Leibniz-Institute of Vegetable and Ornamental Crops, Großbeeren, Germany (latitude 52°20′ N, longitude 13°18′ E, altitude 40 m). Seeds were germinated in vermiculite for about 20 days. Splice grafting (Savvas et al., 2011) was performed when seedlings had developed 3–4 true leaves. The rootstock/scion combinations were, using conventional tomato genetics notation, +/+, +/*not*, *not*/+ and *not*/*not*, where *not* represents the *notabilis* genotype and “+” the wild-type functional allele. In October 2010, grafted tomato plants were transferred into gullies ($8\,m \times 0.2\,m \times 0.07\,m$) in which a standard nutrient solution for tomato (De Kreij et al., 1997) was re-circulating. Prior to transplanting, the roots of the young seedlings were carefully washed in tap water to remove aggregates of the growing medium. The gullies were continuously supplied with nutrient solution which was pumped from a 150 L supply tank at a flow rate of $2\,L\,min^{-1}$. Twelve plants were accommodated in each gully with a plant density of approximately 2 plants m^{-2}. During the growth period of 30 days (from planting until harvest), the nutrient solution was replenished on a daily basis. The pH in the re-circulating nutrient solutions was adjusted daily to 5.6–5.7 by adding proper amounts of 1N HNO_3 stock solution. The experimental installation consisted of two groups of channels differing in the temperature (T) of the re-circulating nutrient solution, which was either optimal (day and night $25 \pm 0.6\,°C$) or suboptimal (day and night $15 \pm 0.4\,°C$). The target solution temperatures were accurately maintained by cooling and heating pipe systems which were connected to the respective solution tanks. The mean daily air T in both treatments was $25 \pm 0.8\,°C$ with a maximum and minimum at 22.4 and 25.2 °C, respectively. The mean relative humidity was 70%, the CO_2 concentration 400 $\mu mol\,mol^{-1}$ and the mean daily photosynthetically active radiation 15 $mol\,m^{-2}\,d^{-1}$.