Overproduction of ABA in rootstocks alleviates salinity stress in tomato shoots

Cristina Martínez-Andújar1 | Ascensi/S Martínez-Pérez1 | Alfonso Albacete1 | Purificación A. Martínez-Melgarejo1 | Ian C. Dodd2 | Andrew J. Thompson3 | Fady Mohareb3 | Lucia Estelles-Lopez3 | Zoltan Kevei3 | Almudena Ferrández-Ayela4 | José Manuel Pérez-Pérez4 | Miriam L. Gifford5 | Francisco Pérez-Alfocea1

1Department of Plant Nutrition, CEBAS-CSIC, Murcia, Spain
2The Lancaster Environment Centre, Lancaster University, Lancaster, UK
3Cranfield Soil and AgriFood Institute, Cranfield University, Bedfordshire, UK
4Instituto de Bioingeniería, Universidad Miguel Hernández, Elche, Spain
5School of Life Sciences and Warwick Integrative Synthetic Biology Centre, University of Warwick, Coventry, UK

Correspondence
Francisco Pérez-Alfocea, Department of Plant Nutrition, CEBAS-CSIC, Murcia, Spain.
Email: alfocea@cebas.csic.es

Funding information
European Union’s Seventh Framework Programme, Grant/Award Number: 289365; MINECO-FEDER, Grant/Award Number: RTI2018-099113-B-I00

Abstract
To determine whether root-supplied ABA alleviates saline stress, tomato (Solanum lycopersicum L. cv. Sugar Drop) was grafted onto two independent lines (NCED OE) over-expressing the SlNCED1 gene (9-cis-epoxycarotenoid dioxygenase) and wild type rootstocks. After 200 days of saline irrigation (EC = 3.5 dS m⁻¹), plants with NCED OE rootstocks had 30% higher fruit yield, but decreased root biomass and lateral root development. Although NCED OE rootstocks upregulated ABA-signalling (AREB, ATHB12), ethylene-related (ACCs, ERFs), aquaporin (PIPs) and stress-related (TAS14, KIN, LEA) genes, downregulation of PYL ABA receptors and signalling components (WRKYs), ethylene synthesis (ACOs) and auxin-responsive factors occurred. Elevated SlNCED1 expression enhanced ABA levels in reproductive tissue while ABA catabolites accumulated in leaf and xylem sap suggesting homeostatic mechanisms. NCED OE also reduced xylem cytokinin transport to the shoot and stimulated foliar 2-isopentenyl adenine (iP) accumulation and phloem transport. Moreover, increased xylem GA3 levels in growing fruit trusses were associated with enhanced reproductive growth. Improved photosynthesis without changes in stomatal conductance was consistent with reduced stress sensitivity and hormone-mediated alteration of leaf growth and mesophyll structure. Combined with increases in leaf nutrients and flavonoids, systemic changes in hormone balance could explain enhanced vigour, reproductive growth and yield under saline stress.

KEYWORDS
9-cis-epoxycarotenoid dioxygenase, abscisic acid, plant hormones, root gene expression, rootstocks, salt stress, tomato (Solanum lycopersicum)

1 | INTRODUCTION

Limited water availability is a shared component of drought and salinity stresses that constrains crop growth and yield. In addition, salinity stress limits plant growth and agricultural productivity through nutritional imbalance and ion toxicity. Roots sense their environment, triggering transcriptomic and biochemical responses that allow the plant to adapt to such conditions through local and systemic responses.
with hormones playing a key role in such adaptive responses (Achard et al., 2006). Root-targeted alteration of hormone metabolism and signalling has been proposed as a biotechnological strategy to overcome the effects of saline soils, and to enable this we must understand the specific adaptive roles of plant hormones (Albacete, Martínez-Andújar, & Pérez-Alfocea, 2014; Ghanem et al., 2011).

Crops dynamically regulate their root system architecture (RSA) in response to environmental stresses to fulfil their mineral and water requirements. In dry and saline soils, plants reduce lateral root initiation and elongation while promoting root hair density and the growth of the primary root to reach deeper water and nutrient sources (Brown et al., 2012; Koevoets, Venema, Elzenga, & Testerink, 2016; Li et al., 2021; Xu et al., 2013) Depending on the level of salt tolerance of the plant species or genotype, low-moderate salinity (2–8 dS m⁻¹) can promote root growth while high salt levels (8–16 dS m⁻¹) restrict root development (Julkowska & Testerink, 2015).

Among the different plant hormones, tissue-specific Abscisic acid (ABA) levels (and responses) change dynamically according to developmental and environmental stimuli. Although ABA is generally considered to inhibit growth of well-watered plants, low ABA concentrations (<1 μM) can stimulate root growth of Arabidopsis (Ephritikhine, Fellen, Vannini, Lapous, & Barbier-Brygoo, 1999; Fujii, Verslues, & Zhu, 2007). Phenotypic comparisons between wild-type (WT) and ABA-deficient mutants demonstrates that WT Abscisic acid (ABA) levels are necessary to sustain primary root growth in maize seedlings grown under low water potential (Sharp & LeNoble, 2002), and for leaf expansion and shoot development in tomato (Sharp, LeNoble, Else, Thorne, & Gherardi, 2000) and Arabidopsis (LeNoble, Spollen, & Sharp, 2004) under well-watered conditions. ABA may stimulate growth by restricting the biosynthesis of ethylene, a growth inhibitor (reviewed in Sharp et al., 2004). Within the roots, ABA alters gene expression that induces changes in RSA (Sharp et al., 2004), increases root hydraulic conductivity (Thompson et al., 2007), modifies nutrient and ionic transport and changes primary metabolism leading to osmotic adjustment (Martínez-Andújar et al., 2020; Sharp & LeNoble, 2002).

Plants growing in dry or saline soil can show stomatal closure before shoot water status (the trigger for leaf ABA accumulation) begins to decline (Dodd, 2005; Gowing, Jones, & Davies, 1993), coincident with root ABA accumulation and export to the shoot as a root-to-shoot signal (Wilkinson & Davies, 2002; Zhang & Davies, 1989). However, experiments with reciprocal grafts of ABA-deficient and WT plants showed that stomatal closure of WT scions in response to dry (Holbrook, 2002) or saline (Li, de Ollas, & Dodd, 2018) soil was rootstock independent. Instead, roots in drying soil alkalize xylem sap causing a redistribution of existing pools of ABA within the leaf that affects stomatal closure (Wilkinson, Corlett, Oger, & Davies, 1998), and other non-ABA chemical signals such as sulphate (Malcheska et al., 2017) or jasmonic acid (De Ollas, Arbona, Gómez-Cadenas, & Dodd, 2018) may also be involved. ABA detected in the root system may either be synthesized locally or translocated from the shoot via the phloem (McAdam, Brodribb, & Ross, 2016), and ABA can recirculate between roots and shoots, with roots either acting as a sink for ABA or as a net exporter of ABA to the shoot, depending on plant nutrient and water status (Peuke, 2016).

Genetically increasing endogenous ABA levels is a promising strategy to improve resistance to abiotic stresses such as drought and salinity. The enzyme 9-cis-epoxycarotenoid dioxygenase (NCED) is rate-limiting for ABA biosynthesis, and over-expression of NCED genes increased ABA content of tissues, as first shown in tobacco and tomato by overexpressing the tomato gene SINCED (Thompson et al. 2000, 2007b). This work provided transgenic tomato lines with different levels of expression of SINCED1 and ABA contents (SP12 and SP5) and offers the opportunity to study the effects of high ABA on root-to-shoot communication. In previous reciprocal grafting experiments between WT, SP12 and SP5, ABA in xylem sap collected from de-topped roots was mainly determined by the root genotype, as might be expected in the absence of the shoot. In addition, root cultures (again independent of the shoot) of SP12 and SP5 had higher ABA content that WT, thus overexpression of SINCED1 was sufficient to increase ABA biosynthesis in the root alone (Thompson, Mulholland, et al., 2007), despite the much lower level of NCED substrate available in roots compared to leaves (Taylor, Sonneveld, Bugg, & Thompson, 2005). In contrast, stomatal conductance in well-watered reciprocal grafting experiments was significantly affected only by the shoot genotype (Thompson, Mulholland, et al., 2007). Overexpression of NCED has now been explored in many systems, and its limiting effect on stomatal conductance confers improved water use efficiency (WUE; Thompson, Andrews, et al., 2007) and resistance to terminal drought (withdrawal of irrigation in pot experiments). Lower transpiration rate and slower soil moisture depletion of these NCED-overexpressing lines maintains turgor of tobacco (Qin & Zeevaart, 2002), grapevine (He et al., 2018) and petunia (Estrada-Melo, Ma, Reid, & Jiang, 2015) in drying soil. NCED overexpression also increased growth relative to WT under osmotic stress (NaCl, mannitol) in tobacco (Zhang, Yang, Lu, Cai, & Guo, 2008) and improved transpiration and reduced chloride accumulation in Arabidopsis grown in ‘a 150 mM chloride dominant solution’ (Zhang, You, Fan, & Ran, 2015). However, the effect of rootstocks overexpressing NCED on plant growth and yield responses to saline soil has not been investigated.

ABA interacts with other hormones to mediate local and systemic stress responses (Sah, Reddy, & Li, 2016): it antagonizes the growth inhibitory effects of ethylene production in tomato shoots (Sharp et al., 2000), Arabidopsis shoots (LeNoble et al., 2004) and maize roots (Spollen, Lenoble, Samuels, Bernstein, & Sharp, 2000), and also during grain-filling in wheat (Yang, Zhang, Liu, Wang, & Liu, 2006). Moreover, root-supplied ABA from WT rootstocks was sufficient to revert xylem 1-aminoacyclopropane-1-carboxylic acid (ACC) concentrations and foliar ethylene production of ABA-deficient scions, while enhancing their leaf area (Dodd, Theobald, Richer, & Davies, 2009). However, nighttime maize leaf expansion of water-stressed plants did not appear to be regulated by either ABA or ethylene (Voisin et al., 2006), but probably by more complex hormone interactions.

Many hormones (ABA, ethylene, JA and brassinosteroids) modify the development of RSA in saline stress conditions (Duan et al., 2013;
Gibberellins might mediate the integration of auxin and cytokinin antagonistic mechanisms, because auxin induces degradation of DELLAs proteins and enhances cell cycle activity, whereas gibberellins limit cytokinin-mediated growth inhibition (reviewed in Petricka, Winter, & Benfey, 2012). Although salinity causes root, xylem and leaf ABA accumulation in tomato (Albacete, Martínez-Andújar, Pascual, Acosta, & Pérez-Alfocea, 2008b; Li et al., 2018), it is not clear whether it directly controls plant responses, since other hormonal factors (such as the ethylene precursor ACC and the ratio ACC/ABA) co-varied with the productivity (biomass), photosynthetic parameters and WUE (Cantero-Navarro et al., 2016). These two root-derived hormones were positively (ABA) or negatively (ACC) correlated with productivity in a salinized population of plants in which a common scion was grafted onto rootstocks representing a recombinant inbred line population from the cross S. lycopersicum × S. cheesmaniae (Albacete et al., 2009).

Grafting is commonly applied to many woody and herbaceous horticultural species in commercial practice (Albacete et al., 2014). Tomato is one of the most important economic crops in the world and is commonly propagated by grafting high productivity scions onto vigorous rootstocks to alleviate soilborne diseases and abiotic stress effects (Bletsos & Olympios, 2008; Martínez-Andújar, Albacete, & Pérez-Alfocea, 2020). Cultivated tomato is moderately tolerant to salinity with a threshold of tolerance of 2.5 dS m⁻¹ but there is a subsequent yield loss of 10% for each unit of salinity increase (François & Maas, 1994), which means that 30–40% yield losses due to salinity are quite common in many horticultural areas such as the tomato-producing region of Southeast Spain. Root-specific traits such as RSA, sensing of edaphic stress and root-to-shoot communication can be exploited to improve resource (water and nutrients) capture and plant development under resource-limited conditions. Root system engineering and rootstock breeding provides new opportunities to maintain sustainable crop production under changing environmental conditions. We hypothesize that grafting a commercial tomato cultivar scion onto ABA over-producing tomato rootstocks would enhance growth and yield under saline conditions, potentially through multiple local and systemic mechanisms.

2 | MATERIAL AND METHODS

2.1 | Plant culture

Two independent tomato transgenic lines, SP5 and SP12, in the genetic background of the WT cultivar Ailsa Craig (AC; Thompson, Mulholland, et al., 2007) were used in this study as rootstocks of the commercial cherry variety Sugar Drop (SD, Unigenia Semillas, Murcia, Spain). SP5 and SP12 transgenic rootstocks constitutively overexpress the SlNCED1 gene (Thompson et al., 2000), under the control of the Gelvin superpromoter (SP) and contain elevated ABA levels compared to WT, with SP5 accumulating more ABA than SP12 (Thompson et al. 2007 b). Since germination rates differed between genotypes, different sowing dates were used to synchronize development of the three genotypes: SP12 and SP5 seeds were sown one and two weeks before the WT, respectively, as described previously (Martínez-Andújar, Martínez-Pérez, et al., 2020). Seeds of the scion SD were sown 5 days earlier than AC seeds (7 days earlier than SP12 and 14 days earlier than SP5) to ensure equal stem diameters at grafting.

For all genotypes, seeds were sown in commercial vermiculite, watered with deionized water and kept at 26–28°C and 80–90% relative humidity in the dark until germination. Grafting was performed using the splicing method at the two to three true leaf stages (3–4 weeks after sowing) where the scion was attached at the first node of the rootstock (Savvas et al., 2011). Grafting with the two transformants and the WT AC resulted in three graft combinations: SD/SP5, SD/SP12 and SD/AC (Figure S1).

One month later, when the grafted plants were well established, they were cultivated under commercial-like conventional plastic greenhouse conditions using a sand substrate during an autumn-winter season, in Almería area (Spain). Fertilizers and water were supplied by a drip fertigation. From 10 days after transplanting, a low salinity treatment with an electrical conductivity (EC) of 3.5 dS m⁻¹ was applied for a period of 200 days (Figure S1). Six plants per graft combination were randomly cultivated and distributed in blocks.

2.2 | Plant phenotyping

Throughout the experiment (after 130, 163 and 180 days of salt treatment, DST), photosynthesis (Aₕ), stomatal conductance (gₛ) and substomatal CO₂ (Cₐ) were measured in the youngest fully expanded leaves (one leaf per plant) using a CIRAS-2 (PP Systems, Massachusetts, USA) between 09.00 and 12.00 hr (lights were turned on at 08.00 hr). CO₂ was set at ambient levels (400 ppm) and radiation matched the chamber conditions (1,500 μmol m⁻² s⁻¹ PPFD). Intrinsic water-use efficiency (WUE) was calculated as the ratio between the values of Aₕ and gₛ.

After 130 DST, the second fully expanded mature leaf over the fourth truss (with actively growing fruits) of six plants per graft combination was assayed for various physiological parameters (described above), then detached to weigh and determine leaf area using an LI-3100 AC area meter (LI-Cor, Lincoln, NE, USA). Plant stem diameter was also measured at the second node level using an electronic liquid-crystal display (LCD) digital vernier caliper (0–150 mm). At the end of the experiment (200 DST), the shoot and root were detached and weighed to determine biomass.

Young fully expanded leaves and young roots were immediately frozen in liquid nitrogen and stored at −80°C for hormonal and gene expression analysis. Leaf, root and truss xylem sap were obtained by applying a pneumatic pressure (between 0.6 and 0.7 MPa) to excised organs. Sap was collected with a pipette, immediately frozen in liquid nitrogen and stored at −80°C for hormonal analysis. Phloem exudate was collected using the method described by Pérez-Alfocea, Balibrea, Alarcón, and Bolarín (2000). The distal stem with the shoot apex and...
the two youngest expanded leaves were excised and the basal 2–3 cm immediately immersed in a 150 ml glass containing 30 ml of 20 mM Ethylenediamine tetraacetic acid (EDTA) (pH 6, adjusted with LiOH to avoid interactions with cation measurements). Each container with the plant material was placed in a plastic bag and hermetically sealed. The exudate was obtained by incubating the plant material for 20 hr in the dark at room temperature.

Total yield was calculated using all the fruits collected from each plant during the harvest period. Fully ripe fruits were harvested weekly for 2 months. The truss length and fruit weight were also recorded in the third truss. Fruit at green and mature stages were harvested with the plant material was placed in a plastic bag and hermetically sealed. The exudate was obtained by incubating the plant material for 20 hr in the dark at room temperature.

2.3 | Nutritional, hormonal and flavonoid analysis

For ionome composition, leaves were dried for 48 hr at 80°C, milled to a powder and 200 mg dry tissue was digested with an HNO₃:HClO (2:1, vol/vol) solution. Samples were analysed by using inductively coupled plasma spectrometry (ICP-OES, Thermo ICAP 6000 Series). Total C and N contents were determined in 200 mg of dry leaf material by the combustion method using an elemental analyser (LECO TRUSPEC, The Netherlands).

The main classes of plant hormones, cytokinins [trans–zeatin (t-Z), zeatin riboside (ZR) and isopentenyladenine (IP)], gibberellic acid (GA₃), indole acetic acid (IAA), abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA) and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), as well as the ABA catabolites (dihydrophaseic acid (DPA) and phasic acid (PA)) and flavonoids (luteolin, taxifolin, genistein, quercetin and cyanidin) were identified and analysed as described previously in Albacete et al. (2008a) with some modifications. Fresh plant material (0.1 g FW of leaf or root) was homogenized in liquid nitrogen and incubated in 1 ml of cold (−20°C) extraction mixture of methanol/water (80/20, vol/vol) for 30 min at 4°C. Solids were separated by centrifugation (20,000g, 15 min at 4°C) and re-extracted for another 30 min at 4°C with 1 ml of extraction solution. Pooled supernatants were passed through Sep-Pak Plus C18 cartridges (previously conditioned with 3 ml of extraction buffer) to remove interfering lipids and some plant pigments. The supernatant was collected and evaporated under vacuum at 40°C. The residue was dissolved in 1 ml methanol/water (20/80, vol/vol) solution using an ultrasonic bath. The dissolved samples were filtered through 13 mm diameter Millex filters with 0.22 μm pore size nylon membrane (Millipore, Bedford, MA) and placed into opaque microcentrifuge tubes.

Ten microlitre of filtered extract (xylem, leaf or root) were injected in a Ultra high performance liquid chromatography (UHPLC) coupled with mass spectrometry (MS) system consisting of an Acquity Series U-HPLC (ThermoFisher Scientific, Waltham, MA) coupled to an Exactive mass spectrometer (ThermoFisher Scientific, Waltham, MA) using a heated electrospray ionization (HESI) interface. Mass spectra were obtained using the Xcalibur software version 2.2 (ThermoFisher Scientific, Waltham, MA). To quantify the plant hormones, calibration curves were constructed for each analysed component (0, 1, 10, 50 and 100 μg L⁻¹). ABA catabolites [dihydrophaseic acid (DPA) and phasic acid (PA)] and flavonoids (luteolin, taxifolin, genistein, quercetin and cyanidin) were identified by extracting the exact mass of the target catabolite from the full scan chromatogram obtained in the negative mode, adjusting a mass tolerance of ±1 ppm. The concentrations were semi-quantitatively determined from the extracted peaks using the calibration curve of ABA (catabolites) or the total area (flavonoids).

2.4 | RNA isolation for real-time quantitative PCR and microarray hybridization

Total RNA from frozen tomato roots (150 mg) was extracted using TRI-Reagent (Sigma-Aldrich, St Louis, MO). Contaminating genomic DNA was removed by 20 min incubation at 37°C with four units of DNase I (Thermo Fisher Scientific, Waltham, MA). After DNase I inactivation at 70°C for 15 min, RNA was ethanol-precipitated and resuspended in 30 ml of diethylpyrocarbonate (DEPC)-treated water.

2.5 | First-strand cDNA synthesis and real-time quantitative PCR

The expression of a set of ABA, stress, hormone and root-development related genes previously selected (Ferrández-Ayela et al., 2016; Martínez-Andújar, Martínez-Pérez, et al., 2020) was analysed in roots by real-time quantitative PCR (RT-qPCR). First-strand cDNA was synthesized with one μg of purified RNA using the iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad, Hercules, CA). The resulting cDNA was diluted by adding 40 μl of sterile distilled water.

Primers were designed to amplify 79–143 bp of the cDNA sequences as described previously (Ferrández-Ayela et al., 2016). To avoid amplifying genomic DNA, forward and reverse primers were designed to hybridize across consecutive exons, except in the case of SINCED1 gene. RT-qPCR reactions were prepared with 5 μl of the SsoAdvanced SYBR Green Supermix (Bio-Rad), 1 μM of specific primer pairs, 0.8 μl of cDNA and DNase-free water (up to 10 μl of total volume reaction). PCR amplifications were carried out in 96-well optical reaction plates on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). Three biological and two technical replicates were performed per genotype and treatment. The thermal cycling programme started with a step of 30 s at 95°C, followed by 40 cycles (5 s at 95°C, 10 s at 55°C and 20 s at 72°C) and a melt curve (from 65 to 95°C, with increments of 1°C every 5 s). Dissociation kinetic analyses and agarose gel loading and sequencing of the PCR product were used to confirm its specificity.

Primer pair validation and relative quantification of gene expression levels were performed using the comparative Ct method (Schmittgen & Livak, 2008). Data were represented as the relative gene expression normalized to the Ct value for the tomato housekeeping gene SlACTIN2 (Solyc04g011500) as previously described.
(Ferrández-Ayela et al., 2016). In each gene, mean fold-change values relative to the expression levels of WT were used for graphic representation. ΔCt values were analysed using SPSS 21.0.0 (SPSS, Inc.) by applying the Mann–Whitney U test for determining statistical differences between samples (p-value ≤ 0.05).

2.6 | Microarray hybridization and data analysis

Four biological replicates per genotype were used for RNA extraction using the method described above. RNA (200 ng) was used for cDNA synthesis and Cy3-labelling using the Low Input Quick Amp Labelling Kit for One-Colour Microarray-Based Gene Expression Agilent analysis (Agilent, Santa Clara, CA). Linearly amplified and labelled cDNA (1.65 μg) was hybridized for 17 hr at 65°C on 4 X 180 k format 60-mer oligonucleotide probes designed against the S. lycopersicum cv. Heinz 1706 build SL2.40 (annotation 2.3) genome [Agilent design ID = 069672; see Gene Expression Omnibus (GEO) record GPL21602]. Each array contained ~5 probes for 34,619 transcripts. Arrays were imaged using an MS200 microarray scanner using only the 480 nm laser using the autogain feature of the NimbleScan software (Roche NimbleGen, Madison, WI, USA). Image (tiff) files were imported into the Agilent Feature Extraction software for quality control assessment, grid alignment and expression value extraction at the probe and transcript level with the RMA algorithm (Irizarry et al., 2003) used to carry out background subtraction, quantile normalization and summarization via median polish and output log2 normalized gene expression levels (GEO record GSE79307; Ferrández-Ayela et al., 2016). Linear Models for Microarray Data (package LIMMA in R) was then used to fit linear models to pairs of samples, identifying genes that contrasted the most between the experimental pairs (Smyth, 2004). Transcripts were deemed to be differentially expressed if they showed a Benjamini-Hochberg adjusted p ≤ 0.05 when comparing rootstocks genotypes.

The molecular pathways where differentially expressed genes were involved in the biosynthesis of plant hormones (Figure S2) and hormone signal transduction (Figure S3) were marked in the relevant Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Kanehisa & Goto, 2000).

2.7 | Leaf anatomy and scanning electron microscopy

For mesophyll structure imaging, the third fully expanded mature leaf samples were prefixed in 3% glutaraldehyde solution in 0.1M cacodylate buffer (during 3 hr at 4°C), rinsed in 0.1M cacodylate buffer and 0.1M sucrose, then kept overnight. The next day, samples were fixed in 1% tetroxide (during 2 hr) and rinsed again in 0.1M cacodylate buffer and 0.1M sucrose and kept overnight. The fixed material was dehydrated with an acetone series (30, 50, 70, 90 and 100%) for 10 min at each concentration. Samples were dried in the critical point dryer (LEICA CPD 030) and coated with gold, before being examined under scanning electron microscopy (SEM; JEOL-6100 model). Stomatal density and epidermal cell size were determined in the adaxial and abaxial surface of mature fully expanded leaves using SEM micrographs at ×330 magnification.

2.8 | Assay of root xylem ABA under salinity stress in grafted plants

In grafted plants with either WT (AC) or SP12 rootstocks, the effect of salinity on ABA accumulation was investigated: 60-day old self-grafted WT plants (AC/AC) and WT scion grafted onto the rootstock of NCED OE line SP12 (AC/SP12) were cultivated for 21 days in 0.5 L pots using vermiculite as substrate and irrigated with ½ strength Hoagland nutrient solution alone (control) and supplied with 35, 70 and 100 mM NaCl (salinity). At the end of the experiment, root xylem sap ABA concentration was analysed as described previously.

2.9 | ABA sensitivity

Surface-sterilized (washed in 5% NaOCl) WT and SP12 seeds were germinated in Petri dishes containing 1/5 Hoagland nutrient solution supplemented with 10 g L−1 agar and 1% sucrose. Seedlings were transferred to culture medium supplied with 0, 1.5, 3 and 5 μM (+)-cis, trans-ABA (Sigma-Aldrich) when the cotyledons were developed (6 days for WT and 9 days for SP12). After 30 days of ABA treatment, main total root length was measured using WinRHIZO software (Pro 2016, Regent, Canada).

2.10 | Statistical analysis

Data were subjected to analysis of variance (ANOVA) to test the main effects of genotype. Genotypic means were compared using Tukey’s test at 0.05 of confidence level. All analyses were performed using SPSS for Windows (Version 22.0, SPSS, Inc., Chicago, IL).

3 | RESULTS

3.1 | Plant growth, gas exchange, leaf nutrients and yield

To determine whether rootstock ABA overproduction can alleviate salt stress, two independent tomato transgenic lines, SP5 and SP12, in the genetic background of the WT cultivar AC, as previously reported (Thompson et al., 2000), were used as rootstocks of the commercial cherry variety SD. At the end of the growing cycle (up to 200 days of irrigation with saline water), plants grafted onto NCED OE rootstocks had almost twice the leaf area, leaf and shoot biomass (shoot fresh weight; SFW) and stem diameter of plants grafted onto WT rootstocks (Figure 1a,b). However, the root biomass of SP12 and SP5...
rootstocks was 30% and 60% smaller than WT rootstocks, respectively (Figure 1b). Visually, these NCED OE grafts had less a complex RSA (the spatial configuration of a root system in the soil), than the WT (Figure 1a). Moreover, plants grafted onto NCED OE rootstocks had up to 20–30% increases in length and weight of the third fruiting truss, fruit number, fruit weight and total fruit yield (Figure 1b). Thus, NCED OE rootstocks promoted shoot (and fruit) growth but reduced the root system growth.

Plants grafted onto NCED OE rootstocks had higher photosynthesis rate ($\text{AN}$) on certain measurement occasions (Figure 2a), with similar $g_s$ (Figure 2b) and transpiration (data not shown) to plants grafted on WT rootstocks. Accordingly, NCED OE rootstocks increased WUEi (Figure 2b). Electron microscopy revealed that leaves of scions grafted on SP12 rootstocks had altered leaf and mesophyll structure, with a more disorganized palisade and spongy cell layers (Figure 2c) and smoother and more elongated epidermis and trichome cells in the adaxial surface (Figure 2e; Table 1) than those grafted on WT rootstocks. Those differences could explain the lower substomatal CO$_2$ concentration ($\text{Ci}$) in the leaves grafted onto the NCED OE lines (Figure 2d). The SP12 rootstock also seems to lead to fewer epicuticular wax crystals on both adaxial and abaxial leaf surfaces, without affecting stomatal density and aperture (Figure 2e; Table 1), supporting the lack of effect on $g_s$ (Figure 2b) and transpiration. Foliar C, N, P, K, Na, B and Zn concentrations did not differ between graft combinations, but plants grafted onto NCED OE rootstocks had increased S, Mg, Ca and Mn concentrations, but decreased Fe concentrations (Table 2). Thus, NCED OE rootstocks affected leaf structure, nutritional status and function.

3.2 Hormone accumulation

Since hormones mediate many physiological changes (Albacete et al., 2008a; Ghanem et al., 2008), we measured hormone levels of several root and shoot tissues and xylem and phloem exudates of grafted plants (Figures 3 and 4; Table S1).

Generally, NCED OE grafts produced few significant effects on ABA concentrations in tissues and transport pathways compared to the WT rootstock (Figures 3a and 4). Interestingly, the NCED OE rootstocks significantly increased ABA concentrations in the xylem.
sap of a flowering truss 180 days after transplanting, but those differences decreased during green fruit stage and disappeared at maturity stage. Moreover, mature fruit (juice) ABA concentration of plants grafted onto SP12 rootstocks was more than twofold higher than in plants grafted on WT rootstocks. Leaf phloem exudate ABA concentrations decreased in plants grafted on NCED OE rootstocks (Figure 3a). SP12 rootstocks had higher root and root xylem sap concentrations of the ABA catabolites PA and DPA, respectively, with leaves of plants grafted on SP12 having higher DPA concentrations (Figure 3b). Thus, rootstock NCED OE had significant effects on ABA and metabolites concentrations only in few shoot tissues.
Plants grafted onto NCED OE rootstocks had lower total CKs (t-Z and iP type) in the xylem sap of roots and flowering truss, as well as in leaf tissue and green fruits mainly due to lower t-Z levels (Figure 4; Table S1). The different graft combinations had similar t-Z and iP concentrations in leaf xylem sap and root tissues. However, iP type CK concentrations on leaf tissue (130 DST) and leaf phloem exudate were 5-14-fold higher in plants grafted on NCED OE rootstocks than on WT rootstocks, with iP the only hormone increasing in leaf phloem exudate (Figure 4; Table S1). Thus, rootstock NCED OE significantly affected CK concentrations in root xylem sap and shoot tissues.

Rootstock genotype also significantly affected auxin (IAA) and ethylene precursor (ACC) measurements. Leaf phloem exudate and root tissue ACC concentrations were 3–25 times lower in plants grafted on NCED OE rootstocks, while they had a higher ACC concentration in xylem sap of a mature fruit truss (Figure 4; Table S1). Leaf phloem exudate and xylem of mature fruit truss had up to sixfold lower IAA concentrations when grafted on the SP5 rootstock (Figure 4; Table S1), otherwise there were no significant rootstock impacts on IAA levels. Similar to ABA, leaf xylem sap of trusses and transport fluid concentrations of other acidic hormones.

When comparing SP rootstocks to WT, differentially expressed genes were enriched in several classes, including serine-type endopeptidases, defence response genes, oxygen binding, snoRNA binding, chlorophyll binding and glucuronosyltransferase activity (Figure 5c).

To interpret the gene expression data in a physiological context, we analysed DEGs related to hormone metabolism (Figure S2) and signalling (Figure S3) pathways, initially focusing on ABA-related genes because of the known role of NCED. Both PCR and transcriptomic data showed that SINCED1 gene expression was higher in SP5 than SP12 (Figure 6a; Table S2–S5), confirming previous results (Martínez-Andújar, Martínez-Pérez, et al., 2020; Thompson, Andrews, et al., 2007). Other ABA-metabolic genes were mostly not affected, corroborating their lack of differential regulation in roots of whole plants under control conditions (Martínez-Andújar, Martínez-Pérez, et al., 2020). AREB1 (Solyc04g078840) and ATHB12 (Solyc01g096320) were induced in SP12 and SP5 rootstocks, respectively,
FIGURE 3  Abscisic acid (ABA) concentrations in mature fruit juice (180 DST), mature, green and flower truss xylem sap (180 DST), leaf (130 DST), leaf phloem (180 DST), leaf xylem sap (130 DST), root xylem sap (200 DST) and root (200 DST) of tomato cv Sugar Drop grafted onto the WT AC (SD/AC) and the NCED OE lines SP12 (SD/SP12) and SP5 (SD/SP5) grown under 3.5 dS m$^{-1}$ (equivalent to 35 mM NaCl) in greenhouse conditions. Different letters indicate significant differences between genotypes ($n = 3$, $p \leq .05$) (a). Dihydrophaseic acid (DPA) and phaseic acid (PA) concentrations in leaf (130 DST), root xylem sap (200 DST) and root (200 DST) of tomato cv Sugar Drop grafted onto the WT AC (SD/AC) and the NCED OE line SP12 (SD/SP12). * indicates statistically significant difference between graft combinations ($n = 3$, $p \leq .05$) (b)
while other ABA signalling-related genes WRKYs (e.g., WRKY80/WRKY6, Solyc03g095770) and ABA-receptor PYLs (e.g., PYL6, solyc05g052420) were down-regulated in the NCED OE grafts, indicating a reduced response/or sensitivity to ABA compared to the WT (Figure 6a). Additional experiments determined the sensitivity of root responses to salinity and ABA. Root xylem sap ABA accumulation of SP12 rootstocks grafted to WT scions increased under control conditions compared to the WT rootstocks (22.8 vs. 5.8 ng ml$^{-1}$, $p < .01$, respectively), but it was stable as salt concentrations increased from 35 to 100 mM NaCl (Figure 6b). However, stress-induced root xylem ABA accumulation in the rootstocks of WT self-grafted plants diminished as salt concentrations increased. Whereas root length of WT plants almost halved as exogenous ABA concentrations increased from 1.5 to 5 $\mu$M, SP12 root length increased with exogenous ABA concentration (Figure 6c). Thus, increased SlNCED1 gene expression altered some ABA perception and signalling components, and reduced sensitivity to stress.

Regarding stress-related genes (Figure 7a; Table S2 and S3), the TAS14 (Solyc02g084850), KIN2 (Solyc03g095510), LEA (Solyc03g116390), MYB49 (Solyc10g008700) and MYB62 (Solyc03g119370) were upregulated in SP12 rootstocks, while most of those and other MYB genes were not affected or down-regulated in SP5 rootstocks (Figure 7a). Most aquaporin PIP genes analysed were down-regulated in NCED OE rootstocks (Figure 7b), while SP12 rootstocks upregulated PIP1.7 (Solyc03g096290) in SP5 and NIP6.1 (Solyc03g117050; Figure 7b). Both NCED OE rootstocks upregulated two genes involved in flavonoid synthesis, a flavanone 3-hydroxylase-like protein (Solyc03g080190) and a flavonoid oxidoreductase (cytochrome P450, Solyc03g111290; Table 3, Figure 7c). To investigate whether other upregulated genes in the root affect leaf metabolites, flavonoids were analysed in root xylem sap and leaves of grafted plants (Figure 7d). Luteolin and cyanidin concentrations increased in xylem sap and leaves of plants grafted on the SP12 rootstock, with no significant differences in taxol in, genistein and quer cetin concentrations. Thus, increased SlNCED1 gene expression either directly or indirectly generally decreased genes associated with response to stress and water transport, but increased flavonoid biosynthetic genes.

Rootstock NCED overexpression seems to interact with other hormone-related genes in the roots. These rootstocks downregulated IPT7 (Solyc01g080150), and a beta-glucosidase gene (Solyc03g119080) involved in biosynthesis of bioactive CKs (Figure 8a). While SP12 upregulated a GA biosynthesis gene (GA20ox-2, Solyc01g108870), SP5 upregulated four GA2 oxidases that are involved in GA deactivation (Figure 8b; Tables S2 and S3). Furthermore, both NCED OE rootstocks downregulated a gene involved in GA-deactivation (GA2ox3, Solyc01g079200 – qRT-PCR data). Transcriptomic data revealed that many JA-related genes in SP lines (LOX, JA1, MEJA, JAZ) were downregulated, particularly in SP5 (Figure 8c; Tables S2 and S3). RT-qPCR analysis revealed that JA2 was also downregulated in SP5, but up-regulated in SP12, confirming the data obtained in the roots of whole NCED OE plants (Martínez-Andújar, Martínez-Pérez, et al., 2020).

Both NCED rootstocks upregulated the ACC synthase genes (ACC2, Solyc01g095080; ACS1a, Solyc08g081540) and most ethylene response factors (ERFs; Figure 9a; Table 3). SP12 and SP5 rootstocks upregulated 2 and 1 ACC oxidase genes, respectively, but
downregulated 6 and 13 ACC oxidase genes, respectively (Figure 9a; Tables S2 and S3). SP12 rootstocks increased expression of genes involved in IAA conjugation (IAAsGH3, Solyc02g064830) but decreased expression of genes involved in IAA flux (PIN9, Solyc10g078370), along with the downregulation of most auxin-responsive proteins (Figure 9b; Tables S2 and S3).

Overall, these results are consistent with NCED OE rootstocks having enhanced ACC synthesis and ethylene signalling pathways, but with less conversion to ethylene as the majority of ACC oxidase genes were down-regulated. Moreover, SINCED1 gene overexpression decreased root auxin activity, while SP5 rootstocks showed greater changes in GA-related gene expression than SP12 rootstocks. The NCED OE rootstocks should have diminished CK biosynthesis.

4 | DISCUSSION

Roots sense a complex soil environment and change their architecture and function to optimize resources and restore plant functional equilibrium. Rootstock-specific SINCED1 overexpression altered root ABA biosynthesis, shoot phenotypes and enhanced stress-tolerance, likely via multiple mechanisms, including altered root-to-shoot signalling (Dodd, 2005; Pérez-Alfocea et al., 2000). NCED OE rootstocks increased vegetative and reproductive growth, with enhanced xylem ABA concentrations in flower trusses and ABA catabolites (PA and DPA) in roots, root xylem sap and leaves (Figure 3) and diminished root system development (Figures 1 and 6c). However, changes in root xylem ABA were more evident in young vegetative plants and diminished with salt stress, compared to the WT (Figure 6b; Martínez-Andújar, Martínez-Pérez, et al., 2020). Although root ABA biosynthesis and catabolism are enhanced and ABA is exported to the shoots, it did not accumulate in most tissues analysed. Alternatively, multiple changes in other hormone groups in many different tissues (Figure 4; Table S1) suggest that SINCED1 plays a complex role in regulating growth. Thus, it is necessary to understand how NCED OE in the roots alters shoot phenotype through both local and systemic responses affecting root gene expression and root-shoot communication.
| ID | LogFC | AveExpr | Adj.P.Val | Description |
|----|-------|---------|-----------|-------------|
|    |       |         |           | Upregulated genes |
| Solyc07g056570.1.1 | 6.74  | 11.71   | 2.15E⁻⁴  | 9-cis-epoxycarotenoid dioxygenase |
| Solyc01g095080.2.1 | 1.74  | 11.11   | 1.70E⁻³  | 1-aminocyclopropane-1-carboxylate synthase |
| Solyc04g072800.2.1 | 1.70  | 9.90    | 2.50E⁻⁴  | 2 3-bisphosphoglycerate-dependent phosphoglycerate mutase |
| Solyc09g015660.2.1 | 1.51  | 11.61   | 8.26E⁻⁷  | Bromodomain containing 2 |
| Solyc03g080190.2.1 | 1.50  | 12.54   | 7.13E⁻⁵  | Flavanone 3-hydroxylase-like protein |
| Solyc02g093040.2.1 | 1.49  | 8.77    | 1.15E⁻³  | Cathepsin B-like cysteine proteinase |
| Solyc03g111290.1.1 | 1.46  | 10.23   | 1.58E⁻⁴  | Cytochrome P450 |
| Solyc01g011450.1.1 | 1.45  | 7.47    | 4.01E⁻⁶  | Unknown protein |
| Solyc05g007950.2.1 | 1.44  | 10.74   | 8.07E⁻⁴  | Ribonuclease T2 |
| Solyc12g017460.1.1 | 1.41  | 10.84   | 1.93E⁻⁵  | GDSL esterase/lipase At1g28590 |
| Solyc06g052020.2.1 | 1.40  | 11.47   | 1.65E⁻⁶  | Unknown protein |
| Solyc04g012050.2.1 | 1.35  | 11.64   | 8.85E⁻⁵  | Ethylene responsive transcription factor 2a |
| Solyc09g098160.2.1 | 1.27  | 12.00   | 4.01E⁻⁴  | Pirin-like protein |
| Solyc07g056320.2.1 | 1.26  | 9.55    | 7.11E⁻⁴  | ER glycerol-phosphate acyltransferase |
| Solyc03g111720.2.1 | 1.25  | 12.79   | 1.26E⁻³  | Peptide methionine sulfoxide reductase msrA |
| Solyc08g013760.1.1 | 1.24  | 7.47    | 1.10E⁻³  | F-box family protein (AHRD V1 ***- B9GFH4_POPTR) |
| Solyc10g055200.1.1 | 1.22  | 9.92    | 6.40E⁻⁴  | Disease resistance response |
| Solyc06g065870.2.1 | 1.17  | 9.17    | 1.33E⁻³  | Unknown protein |
| Solyc07g054470.1.1 | 1.14  | 9.09    | 2.58E⁻⁴  | Unknown protein |
| Solyc03g096670.2.1 | 1.14  | 10.93   | 4.87E⁻⁴  | Integrin-linked kinase-associated serine/threonine phosphatase 2C |
| Solyc08g044490.1.1 | 1.14  | 6.27    | 6.90E⁻⁵  | Kinesin heavy chain-like protein |
| Solyc01g006170.2.1 | 1.13  | 9.45    | 1.60E⁻⁴  | rRNA processing protein ebna1-binding protein-related |
| Solyc01g110960.2.1 | 1.13  | 8.81    | 2.23E⁻⁶  | Glutamic acid-rich protein |
| Solyc02g111300.1.1 | 1.10  | 10.15   | 4.68E⁻⁴  | Cytochrome P450 |
| Solyc03g007170.2.1 | 1.10  | 10.82   | 7.59E⁻⁵  | FK506-binding protein 4, Peptidyl-prolyl cis-trans isomerase |
| Solyc03g096700.2.1 | 1.14  | 10.93   | 4.87E⁻⁴  | Integrin-linked kinase-associated serine/threonine phosphatase 2C |
| Solyc08g0063350.2.1 | 1.93  | 8.89    | 2.90E⁻⁴  | 3-methyl-2-oxobutanoate dehydrogenase (2-methylpropanoyl-transferring) |
| Solyc03g059870.1.1 | 1.96  | 9.63    | 4.00E⁻⁴  | Unknown protein |
| Solyc09g020080.2.1 | 1.97  | 11.93   | 1.59E⁻⁴  | Proteinase inhibitor II |
| Solyc02g068170.1.1 | 2.04  | 8.85    | 5.34E⁻⁶  | Unknown protein |
| Solyc10g036660.1.1 | 2.06  | 8.85    | 5.34E⁻⁶  | Unknown protein |
| Solyc09g072700.2.1 | 2.07  | 6.93    | 1.65E⁻⁶  | Peroxidase 57 |
| Solyc07g044900.1.1 | 2.08  | 5.63    | 1.37E⁻⁶  | Unknown protein |
| Solyc01g006300.2.1 | 2.17  | 8.36    | 5.77E⁻⁶  | Peroxidase |
| Solyc08g079930.1.1 | 2.31  | 8.63    | 5.61E⁸   | Subtilisin-like protease |
| Solyc12g087940.1.1 | 2.32  | 9.27    | 7.13⁵    | Aspartic proteinase nepenthesin-1 |
| Solyc08g079850.1.1 | 2.35  | 7.64    | 5.62E⁻⁷  | Subtilisin-like protease |
| Solyc04g076190.1.1 | 2.37  | 8.62    | 1.08E⁻⁵  | Aspartic proteinase nepenthesin-1 |
| Solyc08g079890.1.1 | 2.37  | 8.90    | 5.13E⁻⁷  | Subtilisin-like protease |
| Solyc08g079920.1.1 | 2.39  | 7.65    | 5.61E⁻⁸  | Subtilisin-like protease |
| Solyc09g097770.2.1 | 2.59  | 11.39   | 6.29E⁻⁴  | Cell wall protein |
| Solyc05g005560.2.1 | 2.69  | 8.18    | 5.34E⁻⁶  | BURP domain-containing protein |
| Solyc01g008620.2.1 | 2.77  | 9.65    | 4.54E⁻⁴  | beta-1-3-glucanase |
| Solyc08g079860.1.1 | 2.78  | 8.80    | 3.08E⁻⁷  | Subtilisin-like protease |
### Table 3 (Continued)

| ID                  | LogFC | AveExpr | Adj.P.Val | Description                     |
|---------------------|-------|---------|-----------|---------------------------------|
| Solyc08g079840.1.1 | −2.85 | 8.62    | 5.61E-8   | Subtilisin-like protease         |
| Solyc08g079900.1.1 | −2.92 | 9.74    | 5.61E-8   | Subtilisin-like protease         |
| Solyc08g079870.1.1 | −2.95 | 8.76    | 5.61E-8   | Subtilisin-like protease         |
| Solyc09g007020.1.1 | −3.03 | 11.09   | 5.70E-8   | Pathogenesis-related protein     |
| Solyc08g079910.1.1 | −3.06 | 8.11    | 5.61E-8   | Subtilisin-like protease         |
| Solyc08g079880.1.1 | −3.15 | 9.16    | 5.61E-8   | Subtilisin-like protease         |
| Solyc09g007010.1.1 | −3.37 | 11.95   | 1.68E-3   | Pathogenesis-related protein PR-1|

Note: The 25 most upregulated genes (largest logFC values) and the 25 most downregulated genes (smallest, most negative logFC values) are given with their mean relative expression (AveExpr) level and the adjusted p-value (Adj.P.val).

**Figure 6**  ABA related genes differentially expressed in root tissues comparing plants of SD/SP12 and SD/SP5 against SD/AC in response to 3.5 dS m⁻¹ (equivalent to 35 mM NaCl) for 200 days in greenhouse conditions. Real time PCR quantification (RT-qPCR) of some ABA-related selected genes is also given (a). Root xylem sap ABA concentration (as a percentage with respect to control conditions – no salt, data in the embedded table- for each genotype) as a function of salt concentration in the medium (35, 70 and 100 mM NaCl) of tomato cv Ailsa Craig self-grafted (AC/AC, open circles) and grafted onto the NCED OE line SP12 (AC/SP12, closed circles) during 27 days. Each point represents the mean value of four replicates. Different letters indicate significant differences between treatments within each graft combination (p ≤ .05). * and ** indicate significant difference between graft combinations at p ≤ .05 and p ≤ .01, respectively (b). The relationship between main root total length (RL) and ABA concentration in the culture medium (0, 1.5, 3 and 5 μM ABA) in tomato cv AC (open circles) and the transgenic line SP12 (SP12, closed circles) grown in vitro during 30 days. Each point represents the mean value of four replicates along with its standard error. Different letters indicate significant differences between treatments within each graft combination (p ≤ .05). * and ** indicate significant difference between graft combinations at p ≤ .05 and p ≤ .01, respectively (c).
4.1 NCED OE rootstocks have reduced gene expression for ABA receptors and signalling components

Rootstock SINCED1 overexpression (Figure 6a) was consistent with transgene expression level in own-rooted plants (Martínez-Andújar, Martínez-Pérez, et al., 2020; Thompson, Mulholland, et al., 2007), implying that shoot-to-root signalling has little effect on constitutive (root-specific in grafted plants) SINCED expression. Although bulk root ABA status did not increase in fruiting plants (Figure 3a), previously ABA in root exudates from approximately 7-week old de-topped plants (Thompson, Andrews, et al., 2007), in root cultures (Thompson, Mulholland, et al., 2007) and in bulk root tissue and xylem sap of younger ungrafted plants (Martínez-Andújar, Martínez-Pérez, et al., 2020) was elevated. Moreover, bulk root ABA concentration of grafted plants was determined by the root genotype and increased in SP5 and SP12 (Thompson, Mulholland, et al., 2007), as in the root xylem sap prior to stress (Figure 6b). Therefore, the lack of bulk root ABA accumulation in this study is consistent with increased root export (Figures 3a and 6b) and catabolism of ABA (Figure 3b).

NCED OE rootstocks showed differential gene expression compared to the WT grafts (Figure 5). NCED OE roots downregulated 7 PYL ABA receptors and 3 WRKY factors, consistent with decreased sensitivity to ABA (Figure 6c), as in own-rooted plants grown in optimal conditions (Martínez-Andújar, Martínez-Pérez, et al., 2020). Several ABA PYR/PYL receptors are highly expressed in tomato roots compared to other tissues (González-Guzmán et al., 2014), allowing root system adaptation to low water potential including via modulation of osmoregulation and architectural changes (Des Marais et al., 2012; Duan et al., 2013; Fernandez et al., 2020; Sharp et al., 2004). Loss or gain-of-function of several pyr/pyl loci reduced (González-Guzmán et al., 2014; Park et al., 2009) or enhanced (Fernandez et al., 2020; García-Maquilon et al., 2021) root ABA sensitivity and signalling, respectively, altering the root phenotype. Moreover, NCED OE rootstocks downregulated most auxin-responsive and auxin-induced genes (ARFs, MYBs, SAURs) and the auxin transporter PIN9, while upregulating the auxin deactivation gene IAA9GH3 in SP12 (Figure 9b), without changing root IAA concentration (Figure 4). Therefore, antagonistic ABA-auxin interactions can account for decreasing lateral and main root development (Duan...
et al., 2013; Hong, Seah, & Xu, 2013; Ma et al., 2018; Shkolnik-Inbar & Bar-Zvi, 2010; Song & Liu, 2015) as in the whole plants under control conditions (Martínez-Andújar, Martínez-Pérez, et al., 2020). Furthermore, genes involved in ABA biosynthesis (FLC/AAO), signalling (AREB, ATHB12) and stress responses (MYBs, PIPs) were slightly induced, not affected or attenuated in SP rootstocks (Figures 6a and 7a,b). Thus, downregulation of PYLs in NCED OE rootstocks may account for their reduced sensitivity to ABA and saline stress and limited root system development, favouring resource allocation to the vegetative and reproductive structures of the scion.

### 4.2 Enhanced photosynthesis of grafted plants with NCED OE rootstocks

Interestingly, NCED OE rootstocks enhanced leaf nutritional (S, Mg, Ca, Mn) status without affecting leaf Na concentration (Table 2), thus uncoupling root function from (diminished) root growth. Moreover, scions grafted on SP12 rootstocks maintained photosynthesis under low salinity (Figure 2a,b) without changing gs, thereby increasing intrinsic WUE (Figure 2b). Similarly, reciprocal grafting experiments under non-stressed conditions indicated that only NCED OE scions decreased gs with only modest effects on A, while NCED OE rootstocks had no effect on gs (Thompson, Mulholland, et al., 2007).

Irrespective of environmental stresses, elevated ABA tissue concentrations can promote developmental changes in stomata and leaf anatomy that mimic the effects of water deficit (Franks & Farquhar, 2001; Galmés et al., 2011; Quarrie & Jones, 1977). Enhanced cuticular wax deposition and changes in its composition can protect photosynthesis (Ziv, Zhao, Gao, & Xia, 2018). In this study, grafting scions onto NCED OE rootstocks increased elongation of leaf epidermal cells and reduced the number of cuticular wax crystals on leaf adaxial and abaxial surfaces (Figure 2e; Table 1). Similarly, scions grafted onto autotetraploid Rangpur lime rootstocks with high ABA levels had higher expression of the wax synthesis WAX2 gene than scions grafted onto the diploid equivalent with lower ABA levels (Allario et al., 2013). In contrast, there was a positive relationship between ABA level and wax deposition in ABA-deficient tomato mutants and following exogenous ABA application (Martin, Romero, Fich, Domozych, & Rose, 2017). NCED OE rootstocks may diminish wax deposition by directly downregulating wax synthesis pathways, or indirectly by alleviating salinity stress, thereby allowing greater leaf expansion and consequently diluting wax deposition or attenuating stress-induced wax synthesis. Furthermore, rootstocks can improve photosynthesis by affecting leaf structure to enhance mesophyll conductance to CO₂ ($g_m$; Fullana-Pericàs, Conesa, Pérez-Alfocea, & Galmés, 2020), with $g_m$ negatively correlated to sub-stomatal and/or ambient CO₂ concentration under long-term stress (Flexas et al., 2012, 2013). Here, grafting onto NCED OE rootstocks disorganized laminar mesophyll structure (Figure 2c), possibly explaining decreased Ci (Figure 2d) by enhancing CO₂ diffusion to the cells (Flexas et al., 2012, 2013).

Other rootstock-derived metabolites may also protect root and leaf function. Two genes involved in flavonoid synthesis, a flavanone 3-hydroxylase-like protein and a flavonoid oxidoreductase, were among the most upregulated genes in NCED OE rootstocks (Table 3; Figure 7c). Flavonoid accumulation leads to chilling and salt stress tolerance in tomato and Arabidopsis by reducing reactive oxygen species (ROS) accumulation and sensitivity to ABA (Li, Liu, & Yao, 2017; Mahajan & Yadav, 2014; Meng, Zhang, Deng, Wang, & Kong, 2015), which is supported by the down-regulation of several peroxidase genes in the NCED OE rootstocks (Table 3). Furthermore, rootstock-derived flavonoids were xylem-transported to the leaves (Albacete et al., 2015).
Overall, NCED OE rootstocks enhanced tomato productivity under low salinity via at least three mechanisms that improved assimilate supply for scion growth: (a) altered ABA metabolism and signalling restricted root growth, making more assimilate available for other sinks; (b) enhanced leaf nutrition and protection; (c) increased AN and decreased sub-stomatal CO₂ associated with changes in leaf mesophyll structure.

4.3 NCED OE rootstocks alter scion cytokinin status and affect root-shoot signalling

Plants grown on NCED OE rootstocks had lower xylem sap concentrations of bioactive CKs in leaves and fruit trusses (Figure 4; Table S1) and downregulated root expression of CK-metabolic genes (Figure 8a), supporting an antagonistic interaction with ABA (Gawronska, Deji, Sakakibara, & Sugiyama, 2003; Ghanem et al., 2011; Peleg & Blumwald, 2011). Despite attenuated root-to-shoot CK signalling, activation of shoot-to-root CK signalling (enhanced phloem iP concentrations) might act as a putative signal to restore root CK status (Hirose et al., 2008; Matsumoto-Kitano et al., 2008). Moreover, leaf area and AN were positively correlated with foliar iP accumulation (\( r = .85 \) and 0.73; \( p \leq .01 \)) across the different graft combinations, possibly explaining altered leaf mesophyll structure, since this hormone preferentially accumulates in the leaf mesophyll and vascular bundles (Veselov et al., 2018). By facilitating CO₂ diffusion to carboxylation sites (Flexas et al., 2012, 2013), iP/ABA-mediated mesophyll alteration favoured CO₂ assimilation. Indeed, both ABA and iP have been proposed as signalling components of the reticulate leaf phenotype in Arabidopsis, which has altered mesophyll structure and reduced CO₂ fixation capacity (Lundquist, Rosar, Bräutigam, & Weber, 2014). Interestingly, a phosphoglycerate mutase gene (Solyc04g072800), whose function is reduced in reticulate mutants (Lundquist et al., 2014), was 2 and 1.4-fold upregulated in SP12 and SP5 rootstocks, compared to the WT (Table 3). This enzyme is key in ATP production and reducing power from glycolysis (Zhao &
Assmann, 2011) and could contribute to active transport and root assimilatory processes such as nutrient uptake and Na$^+$ exclusion (Malagoli, Britto, Schulze, & Kronzucker, 2008; Munns, Passioura, Colmer, & Byrt, 2020) and nitrate or sulphate reduction (Wang et al., 2004), thereby enhancing leaf nutrient status. Moreover, iP-type CKs were related with greater xylem development and plant growth, vigour and yield in tomato (Qi et al., 2020). Since root-to-shoot CK-mediated plant vigour under salinity (Albacete et al., 2009, 2014; Albacete, Ghanem, et al., 2008a; Ghanem, Albacete, et al., 2011) was associated with decreased ABA levels, ABA-CK interactions in rootstock-mediated improvement of the scion physiology require further investigation.

### 4.4 Ethylene and gibberellin related responses in NCED OE grafted plants

ABA signalling maintains shoot and root growth in both well-watered and droughted tomato (Dodd et al., 2009; Sharp et al., 2000, 2004) and Arabidopsis (LeNoble et al., 2004) plants by suppressing ethylene production (LeNoble et al., 2004; Sharp et al., 2000; Spollen et al., 2000). Surprisingly, NCED OE rootstocks upregulated genes for biosynthesis of the ethylene precursor ACC (ACC2, Solyc01g095080; ACS1a, Solyc08g081540) and ethylene signalling (several ERFs), while most genes responsible for the final step in ethylene biosynthetic genes (e.g., ACCO, Solyc07g049550; ACCO-like protein, Solyc12g

![Fig 10](https://wileyonlinelibrary.com)
006380) were down-regulated (Figure 9a). Root and leaf phloem ACC concentrations were significantly reduced, as in own-rooted NCED OE plants (Martínez-Andújar, Martínez-Pérez, et al., 2020). Since diminished (lateral) root development in the NCED OE rootstocks is consistent with the phenotype of the ethylene overproducing mutant epinastic under control (Negi, Sukumar, Liu, Cohen, & Muday, 2010) and saline (Ortiz, 2017) conditions, higher up-regulation of ERFs may be involved (Figure 9a). ERFs induce GA2 oxidases to inactivate GAs and root growth by stabilizing DELLA proteins (Hetherington, Kakkar, Topping, & Lindsey, 2021; Julkowska & Testerink, 2015). Whether these local changes in ethylene and GA responses are involved in systemic signalling is less clear, as reproductive tissues of scions grafted on NCED OE rootstocks had increased ACC and GA3 levels (Figure 4; Table S1). These enhanced GA3 levels are consistent with the elongated truss phenotype (Figure 1). Overall, ABA-ethylene-GA interactions seem involved in regulating root growth, while long-distance ACC and GA signalling cannot be ruled out.

NCED OE rootstocks also upregulated other stress-adaptive processes (Table 3) involved in membrane protection (Glycero-3-phosphate acyltransferase, Solyc07g056320) through lipid metabolism (Zhao et al., 2020; Ziv et al., 2018) and epigenetic regulation (Bromodomain containing 2, Solyc09g015660) through RNA binding and chromatin remodelling (Chaturvedi & Rao, 2016; Liu et al., 2017). Finally, regulation of pathogenesis-related proteins and subtilin-like proteases genes seems highly sensitive to elevated natural (Zhang, Cao, Li, Chen, & Xu, 2019) or transgenic (this study) constitutive ABA production, which deserves further investigation.

5 | CONCLUSION

Grafting WT scions onto constitutively NCED OE producing rootstocks produced local (root) and systemic (scion) responses mediated by root-shoot communication. Evidence that rootstock SINCED1 overexpression changed root-to-shoot ABA signalling included increased ABA concentrations in scion reproductive tissues and increased ABA catabolites in leaves, but lower leaf phloem ABA concentrations. ABA overproduction altered stress-mediated responses associated with: decreasing root expression of PYL ABA receptors; reduced auxin signalling (lower auxin concentration in leaf phloem and decreased root expression of auxin-responsive factors); enhanced root expression of most ethylene signalling gene (ERFs); and decreased lateral root development. Moreover, rootstock NCED overexpression downregulated root expression of CK biosynthesis genes and reduced t-Z in root xylem sap and leaf, suggesting reduced CK transport from root to shoot. However, iP increased in the leaf and leaf phloem, potentially as part of feedback loop to restore CK homeostasis. Increased root glycolytic activity may mediate increased nutrient uptake and flavonoid synthesis and transport for stress protection in the scion. Rootstock NCED overexpression modified leaf growth and anatomy and enhanced photosynthesis, possibly due to iP, JA and ABA accumulation in the leaf and leaf phloem. Enhanced GA3 in truss xylem sap was consistent with increased truss length, weight and overall yield. Considering whole plant source-sink relationships, the stimulation of leaf photosynthesis and reduction in root assimilate requirements for biomass could explain the more productive scion phenotypes (vegetative vigour, truss length, fruit number and yield) when grafted on NCED OE rootstocks. Overall, NCED OE rootstocks may be of great value in generating plants with higher yields under abiotic stresses (Figure 10).

ACKNOWLEDGMENTS

The authors are very grateful to María del Puerto Sánchez-Iglesias for her technical assistance on hormonal analysis. Research was also supported by the Spanish MINECO-FEDER (project RTI2018-099113-B-I00) and by the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement # 289365 (project ROOTPOWER). AJT and ZK were partly supported by Biotechnology and Biological Sciences Research Council (grant BB/L01954X/1) and MLG was partly supported by Biotechnology and Biological Sciences Research Council (grant BB/H109502/1).

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Francisco Pérez-Alfocea: Planned and designed the research. Ascensión Martínez-Pérez and Cristina Martínez-Andújar: Performed all the stress experiments. Almudena Ferrández-Ayela and José Manuel Pérez-Pérez: Executed the qPCR analysis. Andrew J. Thompson, Fady Mohareb, Lucia Estelles-Lopez, Zoltan Kevei and Miriam L. Gifford: Carried out the transcriptomic analysis. Cristina Martínez-Andújar, Ascensión Martínez-Pérez, Purificación A. Martínez-Melgarejo and Alfonso Albacete: Performed the physiological analysis. Alfonso Albacete: Carried out the hormone profiling experiments. Cristina Martínez-Andújar: Performed the data analysis. Cristina Martínez-Andújar and Francisco Pérez-Alfocea: Wrote the original draft preparation. Cristina-Martínez-Andújar, Ian C. Dodd, Andrew J. Thompson and Francisco Pérez-Alfocea: Reviewed and edited the final manuscript.

DATA AVAILABILITY STATEMENT

All raw and processed microarray data are openly available in the Gene Expression Omnibus (GSE79307) at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE79307

ORCID

Cristina Martínez-Andújar https://orcid.org/0000-0002-3684-9765
Zoltan Kevei https://orcid.org/0000-0002-3065-3198
José Manuel Pérez-Pérez https://orcid.org/0000-0003-2848-4919

REFERENCES

Achard, P., Cheng, H., De Grauwe, L., Decat, J., Schoutteten, H., Moritz, T., ... Harberd, N. P. (2006). Integration of plant responses to environmentally activated phytohormonal signals. Science, 311, 91–94.
Albacete, A., Ghanem, M. E., Martínez-Andújar, C., Acosta, M., Sanchez-Bravo, J., Martínez, V., ... Perez-Alfocea, F. (2008a). Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (Solanum lycopersicum L.) plants. Journal of Experimental Botany, 59, 4119–4131.

Albacete, A., Martínez-Andújar, C., Ghanem, M. E., Acosta, M., Sánchez-Bravo, J., Asins, M. J., ... Pérez-Alfocea, F. (2009). Rootstock-mediated changes in xylem ionic and hormonal status are correlated with delayed leaf senescence, and increased leaf area and crop productivity in salinized tomato. Plant, Cell and Environment, 32, 928–938.

Albacete, A., Martínez-Andújar, C., Martínez-Perez, A., Thompson, A. J., Dodd, I. C., & Pérez-Alfocea, F. (2015). Unravelling rootstock xccin interactions to improve food security. Journal of Experimental Botany, 66, 2211–2226.

Albacete, A. M., Martínez-Andújar, C., Pascual, J. A., Acosta, M., & Pérez-Alfocea, F. (2008b). Increasing vegetative growth, yield and seed quantity in tomato by inducing plant vigour at the earliest seedling stage. Acta Horticulturae, 782, 265–272.

Albacete, A. M., Martínez-Andújar, C., & Pérez-Alfocea, F. (2014). Hormonal and metabolic regulation of source-sink relations under salinity and drought: From plant survival to crop yield stability. Biotechnology Advances, 32, 12–30.

Allario, T., Brumos, J., Colmenero-Flores, J. M., Iglesias, D. J., Pina, J. A., Navarro, L., ... Morrill, R. (2013). Tetraploid Rangpur lime rootstock increases drought tolerance via enhanced constitutive root abscisic acid production. Plant, Cell & Environment, 36, 856–868.

Bletsos, F., & Olympios, C. (2008). Rootstocks and grafting of tomatoes, peppers and eggplants for soil-borne disease resistance, improved yield and quality. European Journal of Plant Science and Biotechnology, 2, 62–73.

Brown, L. K., George, T. S., Thompson, J. A., Wright, G., Lyon, J., Dupuy, L., ... White, P. J. (2012). What are the implications of variation in root hair length on tolerance to phosphorus deficiency in combination with water stress in barley (Hordeum vulgare)? Annals of Botany, 110, 319–328.

Cantero-Navarro, E., Romero-Aranda, R., Fernández-Muñoz, R., Martínez-Andújar, C., Pérez-Alfocea, F., & Albacete, A. (2016). Improving agronomic water use efficiency in tomato by rootstock-mediated hormonal regulation of leaf biomass. Plant Science, 251, 90–100.

Chaturvedi, S., & Rao, A. L. N. (2016). A shift in plant proteome profile for the bromodomain containing RNA binding protein (BRP1) in plants infected with cucumber mosaic virus and its satellite RNA. Journal of Proteomics, 131, 1–7.

De Ollas, C., Arbona, V., Gwaldowska, H., Deji, A., Sakakibara, H., & Sugiyama, T. (2003). Hormone-mediated nitrogen signaling in plants: Implication of participation of a bromodomain containing RNA binding protein (BRP1) in plants infected with cucumber mosaic virus and its satellite RNA. Journal of Proteomics, 131, 1–7.

Duan, L., Dietrich, D., Ng, C. H., Yeen Chan, P. M., Bhalerao, R., Bennett, M. J., & Dinneny, J. R. (2013). Endodermal ABA signaling promotes lateral root quiescence during salt stress in Arabidopsis seedlings. Plant Cell, 25, 324–341.

Ephritikhine, G., Fellner, M., Vannini, C., Lapous, D., & Barbier-Brygoo, H. (1999). The sox1 dwarf mutant of Arabidopsis thaliana shows altered sensitivity of growth responses to abscisic acid, auxin, gibberellins and ethylene and is partially rescued by exogenous brassinosteroid. The Plant Journal, 18, 303–314.

Estrada-Melo, A. C., Ma, C., Reid, M. S., & Jiang, C. Z. (2015). Over-expression of an ABA biosynthesis gene using a stress-inducible promoter enhances drought resistance in petunia. Horticulture Research, 2, 1–9.

Ferrández-Ayela, A., Sánchez-García, A. B., Martínez-Andújar, C., Kevei, Z., Gifford, M. L., Thompson, A. J., ... Pérez-Pérez, J. M. (2016). Identification of novel stress-responsive biomarkers from gene expression datasets in tomato roots. Functional Plant Biology, 43, 783.

Flexas, J., Barbour, M. M., Brendel, O., Cabrera, H. M., Carriquí, M., Díaz-Peréz, A., ... Warren, C. R. (2012). Mesophyll diffusion conductance to CO2: An unappreciated central player in photosynthesis. Plant Science, 193–194, 70–84.

Flexas, J., Niinemets, Ü., Gallé, A., Barbour, M. M., Centritto, M., Díaz-Peréz, A., ... Medrano, H. (2013). Diffusional conductances to CO2 as a target for increasing photosynthesis and photosynthetic water-use efficiency. Photosynthesis Research, 117, 45–59.

Fujii, H., Verslues, P. E., & Zhu, J. K. (2007). Identification of two protein kinases required for abscisic acid regulation of seed germination, root growth, and gene expression in Arabidopsis. Plant Cell, 19, 485–494.

Fullana-Pericàs, M., Conesa, M., Pérez-Alfocea, F., & Galmés, J. (2020). The influence of grafting on crops photosynthetic performance. Plant Science, 295, 110250.

Galmés, J., Conesa, M. A., Ochogavia, J. M., Perdomo, J. A., Francis, D. M., Ribas-Carbó, M., ... Cifre, J. (2011). Physiological and morphological adaptations in relation to water use efficiency in Mediterranean accesses of Solanum lycopersicum. Plant, Cell and Environment, 34, 265–269.

Gawronska, H., Deji, A., Sakakibara, H., & Sugiyama, T. (2003). Hormone-mediated nitrogen signaling in plants: Implication of participation of abscisic acid in leaf senescence. Journal of Experimental Botany, 51, 928–938.

García-Maquilon, I., Coego, A., Lozano-Juste, J., Messenger, M., de Ollas, C., Julian, J., ... Ruíz-Partida, R. (2021). PYL8 ABA receptors of Phoenix dactylifera play a crucial role in response to abiotic stress and are stabilized by ABA. Journal of Experimental Botany, 72, 757–774.

Geng, Y., Wu, R., Wei, C. W., Xie, F., Wei, X., Chan, P. M. Y., ... Dinneny, J. R. (2013). A spatio-temporal understanding of growth regulation during the salt stress response in Arabidopsis. Plant Cell, 25, 2132–2154.

Ghanem, M. E., Albacete, A., Martínez-Andújar, C., Acosta, M., Romero-Aranda, R., Dodd, I. C., ... Pérez-Alfocea, F. (2008). Hormonal changes during salinity-induced leaf senescence in tomato (Solanum lycopersicum L.). Journal of Experimental Botany, 59, 3039–3050.

Ghanem, M. E., Albacete, A., Smigocki, A. C., Frébort, I., Pospíšilová, H., Martínez-Andújar, C., ... Pérez-Alfocea, F. (2011). Root-synthesized cytokinins improve shoot growth and fruit yield in salinized tomato (Solanum lycopersicum L.) plants. Journal of Experimental Botany, 62, 125–140.

Ghanem, M. E., Hichri, I., Smigocki, A. C., Albacete, A., Fauchonner, M. L., Diatloff, E., ... Pérez-Alfocea, F. (2011). Root-targeted biotechnology to mediate hormonal signalling and improve crop stress tolerance. Plant Cell Reports, 30, 807–823.
González-Guzmán, M., Rodríguez, L., Lorenzo-Orts, L., Pons, C., Sarrión-Perdigués, A., Fernández, M. A., ... Rodríguez, P. L. (2014). Tomato PYR/PYL/RCAR abscisic acid receptors show high expression in root, differential sensitivity to the abscisic acid agonist quinabactin, and the capability to enhance plant drought resistance. *Journal of Experimental Botany, 65*, 4451–4464.

Gowing, D. J. G., Jones, H. G., & Davies, W. J. (1993). Xylem-transported abscisic acid: The relative importance of its mass and its concentration in the control of stomatal aperture. *Plant, Cell and Environment, 16*, 453–459.

He, R., Zhuang, Y., Cai, Y., Agüero, C. B., Liu, S., Wu, J., ... Zhang, Y. (2018). Overexpression of 9-cis-epoxycarotenoid dioxygenase csgene in grapevine increases drought tolerance and results in pleiotropic effects. *Frontiers in Plant Science, 9*, 970.

Hetherington, F. M., Kakkar, M., Topping, J. F., & Lindsey, K. (2021). Giberellin signaling mediates lateral root inhibition in response to K deprivation. *Plant Physiology, 185*, 1198–1215.

Hirose, N., Takei, K., Kuroha, T., Kamada-Nobusada, T., Hayashi, H., & Sakakibara, H. (2008). Regulation of cytokinin biosynthesis, compartmentalization and translocation. *Journal of Experimental Botany, 59*, 75–83.

Holbrook, N. M. (2002). Stomatal control in tomato with ABA-deficient roots: Response of grafted plants to soil drying. *Journal of Experimental Botany, 53*, 1503–1514.

Hong, J. H., Seah, S. W., & Xu, J. (2013). The role of the ABA action in environmental stress response. *Plant Cell Reports, 32*, 971–983.

Irizarry, R. A., Hobbs, B., Collin, F., Beazer-Barclay, Y. D., Antonellis, K. J., Scherf, U., & Speed, T. P. (2003). Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics, 4*, 249–264.

Julkowski, M. M., & Testerink, C. (2015). Tuning plant signaling and growth to survive salt. *Trends in Plant Science, 20*, 586–594.

Kaneshita, M., & Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research, 28*, 27–30.

Koevoets, I. T., Venema, J. H., Elzenga, J. T. M., & Testerink, C. (2016). Roots withstanding their environment: Exploiting root system architecture responses to abiotic stress to improve crop tolerance. *Frontiers in Plant Science, 7*, 1335.

Le Noble, M. E., Spollen, W. G., & Sharp, R. E. (2004). Maintenance of shoot growth by endogenous ABA: Genetic assessment of the involvement of ethylene suppression. *Journal of Experimental Botany, 55*, 227–245.

Li, C., Liu, S., & Yao, X. (2017). PfNfEH, a flavonane 3-hydroxylase from the Antarctic moss *Pohlia nutans*, confers tolerance to salt stress and ABA treatment in transgenic Arabidopsis. *Plant Growth Regulation, 83*, 1–12.

Li, P., Yang, X., Wang, H., Pan, T., Wang, Y., Xu, Y., ... Yang, Z. (2021). Genetic control of root plasticity in response to salt stress in maize. *Theoretical and Applied Genetics, 134*, 1475–1492.

Li, W., de Ollas, C., & Dodd, I. C. (2018). Long-distance ABA transport can mediate distal tissue responses by affecting local ABA concentrations. *Journal of Integrative Plant Biology, 60*, 16–33.

Liu, Z., Wang, P., Chen, H., Wold, E. A., Tian, B., Brasier, A. R., & Zhou, J. (2017). Drug discovery targeting bromodomain-containing protein 4. *Journal of Medicinal Chemistry, 60*, 4533–4558.

Lundquist, P. K., Rosar, C., Bräutigam, A., & Weber, A. P. M. (2014). Plastid 3-hydroxylase gene confers tolerance to salt stress and Alternaria solani in transgenic tobacco. *Plant Molecular Biology, 85*, 551–573.

Malagoli, P., Britto, D. T., Schulze, L. M., & Kronzucker, H. J. (2008). Fuitive Na+ cycling at the root plasma membrane in rice (*Oryza sativa* L.): Kinetics, energetics, and relationship to salinity tolerance. *Journal of Experimental Botany, 59*, 4109–4117.
Savvas, D., Savva, A., Ntatsi, G., Ropokis, A., Karapanos, I., Krumbein, A., & Olympios, C. (2011). Effects of three commercial rootstocks on mineral nutrition, fruit yield, and quality of salinized tomato. Journal of Plant Nutrition and Soil Science, 174, 154–162.

Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. Nature Protocols, 3, 1101–1108.

Sharp, R. E., & LeNoble, M. E. (2002). ABA, ethylene and the control of shoot and root growth under water stress. Journal of Experimental Botany, 53, 33–37.

Sharp, R. E., LeNoble, M. E., Else, M. A., Thorne, E. T., & Gherardi, F. (2000). Endogenous ABA maintains shoot growth in tomato independently of effects on plant water balance: Evidence for an interaction with ethylene. Journal of Experimental Botany, 51, 1575–1584.

Sharp, R. E., Poroyko, V., Hejlek, L. G., Spollen, W. G., Springer, G. K., Bohnert, H. J., & Nguyen, H. T. (2004). Root growth maintenance during water deficits: Physiology to functional genomics. Journal of Experimental Botany, 55, 2343–2351.

Shkolnik-Inbar, D., & Bar-Zvi, D. (2010). ABI4 mediates abscisic acid and cytokinin inhibition of lateral root formation by reducing polar auxin transport in arabidopsis. Plant Cell, 22, 3560–3573.

Smyth, G. K. (2004). Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Statistical Applications in Genetics and Molecular Biology, 3, 1–25.

Song, L., & Liu, D. (2015). Ethylene and plant responses to phosphate deficiency. Frontiers in Plant Science, 6, 1–14.

Spollen, W. G., Lenoble, M. E., Samuels, T. D., Bernstein, N., & Sharp, R. E. (2000). Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. Plant Physiology, 122, 967–976.

Taylor, I. B., Sonneveld, T., Bugg, T. D. H., & Thompson, A. J. (2005). Regulation and manipulation of the biosynthesis of abscisic acid, including the supply of xanthophyll precursors. Journal of Plant Growth Regulation, 24, 253–273.

Thompson, A. J., Andrews, J., Mulholland, B. J., McKee, J. M. T., Hilton, H. W., Horridge, J. S., ... Taylor, I. B. (2007). Overproduction of abscisic acid in tomato increases transpiration efficiency and root hydraulic conductivity and influences leaf expansion. Plant Physiology, 143, 1905–1917.

Thompson, A. J., Jackson, A. C., Symonds, R. C., Mulholland, B. J., Dadswell, A. R., Blake, P. S., ... Taylor, I. B. (2000). Ectopic expression of a tomato 9-cis-epoxycarotenoid dioxygenase gene causes overproduction of abscisic acid. Plant Journal, 23, 362–374.

Thompson, A. J., Mulholland, B. J., Jackson, A. C., Mckee, J. M. T., Hilton, H. W., Symonds, R. C., ... Taylor, I. B. (2007). Regulation and manipulation of ABA biosynthesis in roots. Plant, Cell and Environment, 30, 67–78.

Veselov, S. Y., Timergalin, L. N., Akhiyarova, G. R., Kudoyarova, G. R., Korobova, A. V., Ivanov, I., ... Prinsen, E. (2018). Study of cytokinin transport from shoots to roots of wheat plants is informed by a novel method of differential localization of free cytokinin bases or their ribosylated forms by means of their specific fixation. Protoplasma, 255, 1581–1594.

Vissenberg, K., Claeijs, N., Balcerowicz, D., & Schoenaers, S. (2020). Hormonal regulation of root hair growth and responses to the environment in Arabidopsis. Journal of Experimental Botany, 71, 4124–4227.

Voisin, A. S., Reidy, B., Parent, B., Rolland, G., Redondo, E., Gerentes, D., ... Muller, B. (2006). Are ABA, ethylene or their interaction involved in the response of leaf growth to soil water deficit? An analysis using naturally occurring variation or genetic transformation of ABA production in maize. Plant, Cell and Environment, 29, 1829–1840.

Waidmann, S., Sarkel, E., & Kleine-Vehn, J. (2020). Same same, but different: Growth responses of primary and lateral roots. Journal of Experimental Botany, 71, 2397–2411.

Wang, R., Tischner, R., Gutiérrez, R. A., Hoffman, M., Xing, X., Chen, M., ... Crawford, N. M. (2004). Genomic analysis of the nitrate response using a nitrate reductase-null mutant of arabidopsis. Plant Physiology, 136, 2512–2522.

Wilkinson, S., Corlett, J. E., Oger, L., & Davies, W. J. (1998). Effects of xylem pH on transpiration from wild-type and flaca tomato leaves: A vital role for abscisic acid in preventing excessive water loss even from well-watered plants. Plant Physiology, 117, 703–709.

Wilkinson, S., & Davies, W. J. (2002). ABA-based chemical signalling: The co-ordination of responses to stress in plants. Plant, Cell and Environment, 25, 195–210.

Xu, W., Ji, L., Shi, W., Liang, J., Zhou, F., Li, Q., & Zhang, J. (2013). Abscisic acid accumulation modulates auxin transport in the root tip to enhance proton secretion for maintaining root growth under moderate water stress. New Phytologist, 197, 139–150.

Yang, J., Zhang, J., Liu, K., Wang, Z., & Liu, L. (2006). Abscisic acid and ethylene interact in wheat grains in response to soil drying during grain filling. New Phytologist, 171, 293–303.

Zhao, Z., & Assmann, S. M. (2011). The glycolytic enzyme, phosphoglycerate mutase, has critical roles in stomatal movement, vegetative growth, and pollen production in Arabidopsis thaliana. Journal of Experimental Botany, 62, 5179–5189.

Zhang, Z., Cao, B., Li, N., Chen, Z., & Xu, K. (2019). Comparative transcriptome analysis of the regulation of ABA signaling genes in different rootstock grafted tomato seedlings under drought stress. Environmental and Experimental Botany, 166, 103814.

Zhang, J., & Davies, W. J. (1989). Sequential response of whole plant water relations to prolonged soil drying and the involvement of xylem sap ABA in the regulation of stomatal behaviour of sunflower plants. New Phytologist, 113, 167–174.

Zhao, J., Long, T., Wang, Y., Tong, X., Tang, J., Li, J., ... Zhang, J. (2020). Rm52 encoding a GDSL lipase mediates lipid homeostasis in anthers to determine rice male fertility. Plant Physiology, 182, 2047–2064.

Zhang, Y., Yang, J., Lu, S., Cai, J., & Guo, Z. (2008). Overexpressing SnNCED1 in tobacco increases ABA level, antioxidant enzyme activities, and stress tolerance. Journal of Plant Growth Regulation, 27, 151–158.

Zhang, W. W., Yang, H. Q., You, S. Z., Fan, S. L., & Ran, K. (2015). MmNCED3, a gene encoding 9-cis-epoxycarotenoid dioxygenase in Malus hupehensis Rehd., enhances plant tolerance to Cl- stress by reducing Cl- accumulation. Plant Physiology and Biochemistry, 89, 85–91.

Ziv, C., Zhao, Z., Gao, Y. G., & Xia, Y. (2018). Multifunctional roles of plant cuticle during plant-pathogen interactions. Frontiers in Plant Science, 9, 1088.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.