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Research

Inhibition of gibberellin accumulation by water deficiency promotes fast and long-term 'drought avoidance' responses in tomato

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Summary

- Plants reduce transpiration to avoid dehydration during drought episodes by stomatal closure and inhibition of canopy growth. Previous studies have suggested that low gibberellin (GA) activity promotes these 'drought avoidance' responses.
- Using genome editing, molecular, physiological and hormone analyses, we examined if drought regulates GA metabolism in tomato (*Solanum lycopersicum*) guard cells and leaves, and studied how this affects water loss.
- Water deficiency inhibited the expression of the GA biosynthesis genes *GA20 oxidase1* (*GA20ox1*) and *GA20ox2* and induced the GA deactivating gene *GA2ox7* in guard cells and leaf tissue, resulting in reduced levels of bioactive GAs. These effects were mediated by abscisic acid-dependent and abscisic acid-independent pathways, and by the transcription factor *TINY1*. The loss of *GA2ox7* attenuated stomatal response to water deficiency and during soil dehydration, *ga2ox7* plants closed their stomata later, and wilted faster than wild-type (WT) M82 cv. Mutations in *GA20ox1* and *GA20ox2*, had no effect on stomatal closure, but reduced water loss due to the mutants' smaller canopy areas.
- The results suggested that drought-induced GA deactivation in guard cells, contributes to stomatal closure at the early stages of soil dehydration, whereas inhibition of GA synthesis in leaves suppresses canopy growth and restricts transpiration area.

Introduction

Drought is a common and devastating abiotic stress that reduces crop yield worldwide (Fahad *et al.*, 2017). Water deficiency inhibits plant growth, flowering and fruit development (Gupta *et al.*, 2020). It also suppresses directly and indirectly major biochemical pathways, including photosynthesis and primary carbon metabolism (Tardieu *et al.*, 2018). Plants use three major strategies to cope with and/or adapt to drought: drought escape, drought avoidance and drought tolerance (Skirycz & Inze, 2010; Kooyers, 2015). To escape from water-deficit stress, plants complete their life cycle before drought becomes severe. Tolerance to drought is acquired by osmotic adjustment, ROS scavenging and activation of stress-related genes. 'Drought avoidance' is a major plant adaptation strategy to survive transient water-deficit conditions. To avoid drought stress, plants reduce their transpiration and can use the available water in the soil more slowly and for a longer period before the arrival of the next rain. Two major mechanisms have been evolved to reduce water loss under drought: fast stomatal closure and long-term growth inhibition. Drought avoidance is regulated primarily by the stress hormone abscisic acid (ABA) (Cutler *et al.*, 2010). However, several studies have suggested that changes in the levels of the growth-promoting hormone gibberellin (GA) may also be involved (Colebrook *et al.*, 2014).

Gibberellin promotes major developmental processes throughout the plant life cycle, including seed germination, shoot elongation, leaf expansion, flowering and fruit development (Yamaguchi, 2008). The nuclear proteins DELLA suppress all GA responses by interacting with numerous transcription factors (Hauvermale *et al.*, 2012; Locascio *et al.*, 2013). When GA binds to its receptor GIBBERELLIN-INSENSITIVE DWARF1 (GID1) it induces DELLA degradation via the ubiquitin-proteasome pathway, leading to the activation of GA responses (Daviere & Achard, 2013). GA activity is controlled also at the level of hormone biosynthesis and deactivation. Both endogenous and environmental cues regulate the expression of three small gene families acting late in the GA biosynthetic pathway and

coding for 2-oxoglutarate-dependent-dioxygenases (2-ODDs). These include the GA 20-oxidases (GA20ox) that cleave C-20 to generate C-19 GAs, GA 3-oxidases (GA3ox) that form the bioactive GAs, GA_1 and GA_4 by 3β-hydroxylation and GA 2-oxidases (GA2oxs) that deactivate bioactive GAs or their C-19 and C-20 precursors (Hedden, 2020). Plants maintain GA homeostasis by a feedback response; reduced GA activity upregulates *GA20ox* and *GA3ox* and inhibits *GA2ox* expression, whereas increased GA activity has the opposite effect. These transcriptional feedback and feed-forward regulations are mediated by changes in DELLA levels (Middleton *et al.*, 2012; Fukazawa *et al.*, 2017).

Biotic and abiotic stresses affect GA levels by upregulating or downregulating *GA20ox*, *GA3ox* or *GA2ox* genes (Yamaguchi, 2008). Several studies have suggested that water-deficit conditions reduce GA levels (Colebrook *et al.*, 2014); drought induced the expression of *GA2ox* in *Populus* (Zawaski & Busov, 2014) and reduced the levels of bioactive GAs in maize leaves (Nelissen *et al.*, 2018). Moreover, overexpression of drought-related transcription factors from the *DEHYDRATION RESPONSIVE ELEMENT BINDING (DREB)* family in tomato and *Arabidopsis thaliana* (*Arabidopsis*) reduces GA levels and improves salt and drought tolerance (Magome *et al.*, 2008; Li *et al.*, 2012). Numerous studies have demonstrated that low GA activity increases plant tolerance to abiotic stresses, including salt and drought (Achard *et al.*, 2006; Magome *et al.*, 2008; Nir *et al.*, 2017; Illouz Eliaz *et al.*, 2020). Reduced GA levels have led to the activation of various stress-related genes (Tuna *et al.*, 2008), and accumulation of osmolytes (Omena Garcia *et al.*, 2019) and ROS scavenging enzymes (Achard *et al.*, 2008), all related to drought tolerance. Previously we have shown that suppression of GA accumulation in tomato reduced water loss under water-deficit conditions (Nir *et al.*, 2014). Moreover, transgenic tomato plants overexpressing the constitutively active stable tomato DELLA protein *procera*Δ*17* (*pro*Δ*17*), exhibited lower whole-plant transpiration due to a smaller canopy area and reduced stomatal aperture (Nir *et al.*, 2017). Expressing *pro*Δ*17* specifically in guard cells was sufficient to reduce stomatal aperture without affecting growth, suggesting that this effect of DELLA is cell autonomous. The effects of *pro*Δ*17* on stomatal closure and water loss were suppressed in the ABA-deficient *sitiens* (*sit*) mutant, indicating that these effects of DELLA are ABA dependent (Nir *et al.*, 2017). High levels of DELLA promoted the expression of the ABA transporter gene *ABA-IMPORTING TRANSPORTER1.1* (*AIT1.1*) in guard cells, and the *ait1.1* mutant suppressed the effect of DELLA on ABA-induced stomatal closure and transpiration (Shohat *et al.*, 2020). These changes suggest that *AIT1.1* mediates, at least partially, the effect of DELLA on stomatal closure. Taken together, these results suggested that low GA/high DELLA activity in tomato guard cells promotes ABA-induced stomatal closure. However, the effect of water availability on GA metabolism and content in the guard cells was not demonstrated.

To this aim, we have studied how water availability affects GA metabolism in tomato guard cells and leaf tissue and, in turn, how this affects transpiration. We show that water deficiency suppressed GA accumulation by downregulating the GA biosynthesis genes *GA20ox1* and *GA20ox2*, and by upregulating the GA deactivating gene *GA2ox7* in both leaf tissue and guard cells. The reduced GA levels in guard cells had a short-term impact on stomatal closure and the lower levels of GA in the leaf tissue had a prolonged impact by inhibiting leaf growth. Together, these changes reduced transpiration and promoted 'drought avoidance'.

Materials and Methods

Plant materials, growth conditions and hormone treatments

Tomato (*Solanum lycopersicum*) plants in M82 background (*sp*/*sp*) were used throughout this study. The *ga2ox7*, *sit*, transgenic line 35S:*pro*Δ*17* and the CRISPER-derived *ga20ox1*, *ga20ox2* and *tiny1* were backcrossed to or generated in M82 background. Plants were grown in a growth room set to a photoperiod of 12 h : 12 h, day : night, light intensity of 150 μmol m^{-2} s^{-1} and 25°C and irrigated to saturation. In other experiments, plants were grown in a glasshouse under natural day-length conditions, a light intensity of 700–1000 μmol m^{-2} s^{-1} and 18–30°C. The seeds were harvested from ripe fruits and treated with 1% sodium hypochlorite followed by 1% $Na_3PO_4{\cdot}12H_2O$, and incubated with 10% sucrose overnight at 37°C. Seeds were stored dry at room temperature. (±) ABA dissolved in dimethyl sulphoxide (DMSO; Sigma-Aldrich, St Louis, MO, USA), was applied to the plants by spraying.

Drought treatments

Plants were irrigated to saturation and then irrigation was stopped. Leaf relative water content (RWC, see Measurements of leaf RWC) was measured when the susceptible genotype (wild-type (WT) or mutant) lost turgor and wilted. In some experiments, soil volumetric water content (VWC; see Measurements of soil VWC) was measured to monitor soil dehydration and to determine drought severity.

CRISPR/Cas9 mutagenesis: cloning, plant transformation and selection of mutant alleles

Two single-guide RNAs (sgRNAs, Supporting Information Table S1) were designed to target *GA20ox1*, *GA20ox2* and *TINY1* genes, using the CRISPR-P tool (http://cbi.hzau.edu.cn/crispr). Vectors were assembled using the Golden Gate cloning system, as described by Weber *et al.* (2011). Final binary vectors, pAGM4723, were introduced into *Agrobacterium tumefaciens* strain GV3101 by electroporation. The constructs were transformed into M82 cotyledons using transformation and regeneration methods described by McCormick (1991). Kanamycin-resistant T0 plants were grown and independent transgenic lines were selected and self-pollinated to generate homozygous transgenic lines. The genomic DNA of each plant was extracted, and genotyped by PCR for the presence of the Cas9 construct. The CRISPR/Cas9-positive lines were further genotyped for mutations using a forward primer to the upstream sequence of the

sgRNA1 target and a reverse primer to the downstream of the sgRNA2 target sequence. The target genes in all mutant lines were sequenced. Several homozygous and heterozygous lines were identified and independent mutant lines for each gene were selected for further analysis. The Cas9 construct was segregated out by crosses to M82.

Isolation of guard cells

Guard cells from tomato leaves (leaves nos. 3 and 4 below the apex) were isolated according to Shohat *et al.* (2020). Briefly, four fully expanded leaves without the central veins were ground twice in a blender containing 100 ml cold distilled water, for 30 s each time. The blended mixture was poured onto a 100 μm nylon mesh (Sefar) and the remaining epidermal peels were rinsed thoroughly with 0.5 l of cold deionised water. The peels were then transferred into 2 ml Eppendorf tubes and frozen in liquid nitrogen. The samples were stained with 0.03% neutral red and cell vitality was examined under a light microscope.

RNA extraction and cDNA synthesis

Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen). For synthesis of cDNA, SuperScript II reverse transcriptase (18064014; Invitrogen, Waltham, MA, USA) and 3 mg of total RNA were used, according to the manufacturer's instructions.

RT-qPCR analysis

RT-qPCR analysis was performed using an Absolute Blue qPCR SYBR Green ROX Mix (AB-4162/B) kit (Thermo Fisher Scientific, Waltham, MA, USA). Reactions were performed using a Rotor-Gene 6000 cycler (Corbett Research, Sydney, NSW, Australia). A standard curve was obtained using dilutions of the cDNA sample. Expression was quantified using ROTOR-GENE software (Corbett Research). Three independent technical repeats were performed for each sample. Relative expression was calculated by dividing the expression level of the examined gene by that of *SlACTIN*. The gene to *ACTIN* ratio was then averaged. The values for control (mock and irrigation) and/or M82 WT treatments were set to 1. All primer sequences are presented in Table S2.

GA analysis in leaves

Sample preparation and analysis of GAs were performed according to the method described in Urbanova *et al.* (2013) with some modifications. Briefly, tissue samples of *c.* 10 mg dry weight (DW) were ground to a fine consistency using 3-mm zirconium oxide beads (Retsch GmbH & Co. KG, Haan, Germany) and a MM 301 vibration mill at a frequency of 30 Hz for 3 min (Retsch GmbH & Co. KG) with 1 ml of ice-cold 80% acetonitrile containing 5% formic acid as extraction solution. The samples were then extracted overnight at 4°C using a benchtop laboratory rotator Stuart SB3 (Bibby Scientific Ltd, Staffordshire, UK) after adding 17 internal gibberellins standards ($[^2H_2]GA_1$, $[^2H_2]GA_3$, $[^2H_2]GA_4$, $[^2H_2]GA_5$, $[^2H_2]GA_6$, $[^2H_2]GA_7$, $[^2H_2]GA_8$, $[^2H_2]GA_9$, $[^2H_2]GA_{15}$, $[^2H_2]GA_{19}$, $[^2H_2]GA_{20}$, $[^2H_2]GA_{24}$, $[^2H_2]GA_{29}$, $[^2H_2]GA_{34}$, $[^2H_2]GA_{44}$, $[^2H_2]GA_{51}$ and $[^2H_2]GA_{53}$); purchased from OlChemIm (Olomouc, Czech Republic). The homogenates were centrifuged at 36 670 ***g*** and 4°C for 10 min, corresponding supernatants were further purified using reverse-phase and mixed-mode SPE cartridges (Waters, Milford, MA, USA) and analysed by ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS; Micromass, Manchester, UK). GAs were detected using the multiple-reaction monitoring mode of the transition of the ion $[M-H]^-$ to the appropriate product ion. MassLynx 4.1 software (Waters) was used to analyse the data and the standard isotope dilution method (Rittenberg & Foster, 1940) was used to quantify the GAs levels.

GA analysis in guard cells

Hormone extraction was performed as described previously (Kojima *et al.*, 2009; Breitel *et al.*, 2016) with some modifications. Briefly, isolated guard cells samples were frozen in liquid nitrogen and grounded into powder using motor and pestle. Gibberellins were extracted from 200 mg of the ground sample in ice-cold methanol : water : formic acid (15 : 4 : 1, v/v/v) added with internal standards. Similar concentrations of isotope-labelled gibberellin internal standards were added into samples and calibration standards. The samples were then purified using Oasis MCX SPE cartridges (Waters) according to manufacturer's protocol and injected onto an Acquity UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm, Waters; mobile phases: gradients of 0.1% acetic acid in water or acetonitrile), connected to the Acquity UPLC H class system (Waters) coupled with a UPLC-ESI-MS/MS triple-quadrupole mass spectrometer for identification followed by quantification of hormones. The hormones were measured in negative mode, with two MRM transitions for each compound. External calibration curves were constructed using a series of diluted gibberellin standards and deuterium-labelled internal standards (Table S3) and used for absolute quantification (Balcke *et al.*, 2012). Hormone concentrations were derived by comparing ratios of MRM peak areas of analyte with its corresponding internal standard using TargetLynx (v.4.1; Waters) software.

Thermal imaging

Thermal images were obtained using an A655SC, FOV 15 (FLIR Systems, Wilsonville, OR, USA). The camera was mounted vertically above the plants. Mean temperature of leaflets from leaves nos. 3 and 4 below the apex were calculated using the customised region of interest (ROI) tool, according to the manufacturer's instructions.

Stomatal aperture measurements

Stomatal aperture was determined using the rapid imprinting technique described by Geisler *et al.* (2000). Light-bodied

vinylpolysiloxane dental resin (eliteHD+; Zhermack Clinical, Badia Polesine, Italy) was attached to the abaxial side of the leaflet and then removed as soon as it dried (minutes). The resin epidermal imprints were covered with transparent nail polish, which was removed once it dried and served as a mirror image of the resin imprint. The nail-polish imprints were put on glass cover-slips and photographed under a model ICC50 W bright-field inverted microscope (Leica Microsystem, Wetzlar, Germany). Stomatal images were later analysed to determine aperture size using the ImageJ software fit-ellipse tool (http://rsb.info.nih.gov/ij/). A microscopic ruler (Olympus, Waltham, MA, USA) was used for size calibration.

Transpiration rate and daily transpiration measurements

Whole-plant transpiration rate was determined using an array of lysimeters placed in the glasshouse (Plant array 3.0 system; Plant-Ditech) in the I CORE Center for Functional Phenotyping (http://departments.agri.huji.ac.il/plantscience/icore.phpon), as described in detail by Halperin *et al.* (2017). Briefly, plants were grown in 4-l pots under semicontrolled temperature conditions (20°C : 32°C, night : day), natural day-length and light intensity of *c.* 1000 μmol m^{-2} s^{-1}. Each pot was placed on a temperature-compensated load cell with digital output (Vishay Tedea-Huntleigh, Holon, Israel) and sealed to prevent evaporation from the surface of the growth medium. The weight output of the load cells was monitored every 3 min. The data were analysed using Spac analytics (Plant-Ditech, Yavne, Israel) software to obtain the following whole-plant physiological traits: daily transpiration (weight loss between predawn and sunset) and transpiration rate (weight loss between two 3-min time points) were calculated from the weight difference between the two data points.

Stomatal conductance (g_s) measurements

Stomatal conductance was determined using an SC-1 Leaf Porometer (Decagon Devices, Pullman, WA, USA) or an LI-6800 portable gas exchange system (LI-COR Biosciences, Lincoln, NE, USA). Measurements were performed at 09:00 h under a constant CO_2 concentration of 400 ppm and constant photosynthetic photon flux density of 1200 μmol m^{-2} s^{-1}.

Measurements of leaf RWC

Leaf RWC of irrigated and drought-treated plants were measured as follows: fresh weight (FW) was measured immediately after leaf detachment and then leaves were soaked for 24 h in 5 mM $CaCl_2$ and the turgid weight (TW) was recorded. Total DW was recorded after drying these leaves at 55°C for 48 h. Leaf RWC was calculated as (FW − DW)/(TW − DW) × 100.

Measurements of soil VWC

VWC was measured using the 5TM soil moisture and temperature sensor, combined with the 'ProCheck' interface reader (Decagon Devices, Pullman, WA, USA).

Measurements of leaf area

The plant's total leaf area was measured using a Li 3100 leaf area meter (Li-Cor area meter, model Li 3100, Lincoln, NE, USA).

Statistical analyses

All assays were conducted with three or more biological replicates and analysed using Jmp software (SAS Institute, Cary, NC, USA). Means comparison was conducted using analysis of variance (ANOVA) with post-hoc Tukey–Kramer honest significant difference (HSD) test (for multiple comparisons) and Student's *t*-test (for one comparison) ($P < 0.05$).

Gene annotation and accession numbers

GA2ox1 to *GA2ox5* were named here according to Pattison *et al.* (2015) and *GA2ox7* according to Schrager Lavelle *et al.* (2019) and all other *GA2oxs* were named by their accession numbers. *GA20ox1* to *GA20ox4* and *GA3ox1* and *GA3ox2* were named here according to Pattison *et al.* (2015), all other *GA20oxs* and *GA3oxs* were named by their accession numbers.

Sequence data from this article can be found in the Sol Genomics Network (https://solgenomics.net/) under the following accession numbers: *ACTIN*, Solyc11g005330; *GA2ox1*, Solyc05g053340; *GA2ox2*, Solyc07g056670; *GA2ox3*, Solyc01g079200; *GA2ox4*, Solyc07g061720; *GA2ox5*, Solyc07g061730; *GA2ox7*, Solyc02g080120; Other *GA2oxs*: Solyc01g058040; Solyc02g070430; Solyc08g016660; Solyc10g007570; Solyc01g058030; *GA20ox1*, Solyc03g006880; *GA20ox2*, Solyc06g035530; *GA20ox3*, Solyc11g072310; *GA20ox4*, Solyc01g093980; Other *GA20oxs:* Solyc06g050110; Solyc09g009110; Solyc10g046820; Solyc11g013360; *GA3ox1*, Solyc06g066820; *GA3ox2*, Solyc03g119910; Other *GA3oxs*: Solyc01g058250; Solyc05g052740; Solyc00g007180; Solyc01g067620; *TINY1*, Solyc06g066540; Other TINYs: Solyc08g066660; Solyc12g044390; Solyc01g090560; Solyc12g008350; Solyc03g120840.

Results

Water deficiency suppresses GA accumulation in guard cells

A rapid and efficient guard-cell isolation procedure (see methods in Shohat *et al.*, 2020) was used to examine if water deficiency affects GA accumulation in guard cells. This rapid procedure was taken to minimise the effect of the isolation process on transcription and hormone metabolism. Tomato M82 (WT) plants were grown under normal irrigation regime, or exposed to water deficiency (15% soil VWC) and then guard cells were isolated from leaves nos. 3 and 4 (top down). Microscopic analysis of the guard-cell enriched samples, stained with neutral red, confirmed the viability of the guard cells, but not of the remaining epidermal cells (Fig. S1). We then analysed GA content in the guard-cell enriched samples and found that water deficiency reduced significantly the levels of the bioactive GAs, GA_1 and GA_3 and

the C-20 intermediate, GA_{12} (Fig. 1a; Dataset S1). Although GA_3 is rather rare in plants (Hedden, 2020), previous studies have demonstrated its accumulation in tomato (Li *et al.*, 2020). The level of the bioactive GA_4 was much lower than that of GA_1 and GA_3, and was slightly, but not significantly, higher in the drought treatment.

Colebrook *et al.* (2014) suggested that abiotic stresses reduce GA accumulation by upregulating the GA deactivating gene *GA2ox*. We analysed the expression of the 11 tomato *GA2ox* genes (Pattison *et al.*, 2015; Chen *et al.*, 2016) in guard-cell enriched samples, following water-deficit treatment (15% soil VWC). Among the 11 *GA2ox* genes, only *GA2ox7* was strongly upregulated by drought treatment (Fig. 1b). Comparing transcript levels of all *GA2oxs* in isolated WT guard cells using available RNA-seq data (Shohat *et al.*, 2020), revealed that *GA2ox7* expression was much higher than all other *GA2oxs* (Fig. S2). The strong induction of *GA2ox7* by water deficiency can explain the low level of GA_{12} found under drought conditions, as this C-20 precursor is a substrate of GA2ox7 in tomato (Fig. 1c; Schrager Lavelle *et al.*, 2019). It is interesting to note that *GA2ox7* is the closest homologue of *Arabidopsis GA2ox8* (Chen *et al.*, 2016), which is expressed specifically in guard cells (Li *et al.*, 2019).

We next examined whether water deficiency also suppressed GA biosynthesis in guard cells via the inhibition of the GA biosynthesis genes, *GA20ox* or *GA3ox*. Tomato has eight *GA20ox* and six *GA3ox* genes (Pattison *et al.*, 2015). While none of the *GA3ox* genes was downregulated (Fig. S3), the expression levels of two *GA20ox* genes, *GA20ox1* and *GA20ox2* were suppressed (Fig. 1d). Surprisingly, the expression of *GA20ox3* was strongly upregulated under water-deficit conditions.

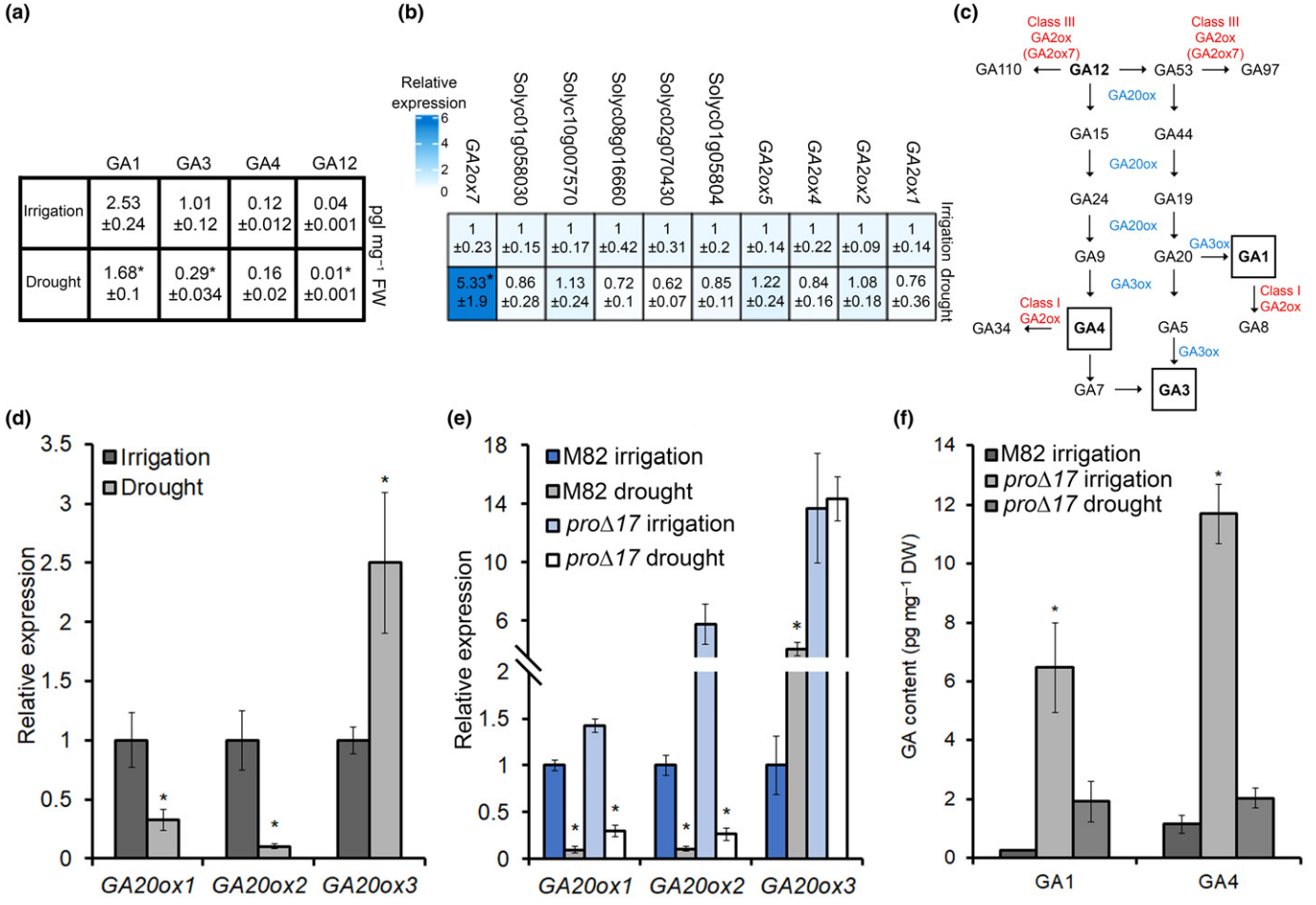

Fig. 1 Drought inhibits gibberellin (GA) biosynthesis in tomato guard cells and leaf tissue. (a) GA levels under irrigation and water-deficit conditions (soil volumetric water content (VWC) 15%) in guard cells isolated from leaves nos. 3 and 4 (top down). Values are means of four biological replicates ± SE. FW, fresh weight. (b) Heat map showing the relative expression of the tomato *GA 2-oxidase* (*GA2ox*) genes in isolated guard cells under irrigation or drought treatments (soil VWC 15%). Values are means of four biological replicates ± SE. (c) Scheme of GA metabolism showing the GA biosynthesis enzymes (blue), GA deactivation enzymes (red) and bioactive GAs (black squares). (d) *GA 20-oxidase1* (*GA20ox1*), *GA20ox2* and *GA20ox3* expression in isolated guard cells taken from M82 plants that grew with irrigation or exposed to water deficiency (soil VWC 15%). Values are means of four biological replicates ± SE. (e) *GA20ox1*, *GA20ox2* and *GA20ox3* expression in M82 and 35S:*proΔ17* leaves under irrigation or drought conditions (soil VWC 15%). (f) Levels of the bioactive GA_1 and GA_4 in leaves of irrigated M82 and 35S:*proΔ17* and drought-treated 35S:*proΔ17* (soil VWC 15%). Values are means of four biological replicates ± SE. DW, dry weight. Asterisks (a, b, d–f) represent significant differences between respective treatments by Student's *t*-test ($P < 0.05$).

To examine if these changes in GA metabolism are guard-cell specific, we analysed the expression of all the above-mentioned genes also in whole-leaf tissue following soil dehydration (15% soil VWC). Similar to guard cells, none of the *GA3ox* genes was downregulated, *GA2ox7* was upregulated, and *GA20ox1* and *GA20ox2* were downregulated (Fig. S4a–c). The expression of *GA20ox3* was, again, strongly upregulated by water-deficit conditions. GA analysis in drought-treated leaves (15% soil VWC) showed strong suppression of GA_1 but not GA_4 (Fig. S4d; Dataset S1).

Drought out-competes the DELLA-induced feedback response to maintain low GA levels under water-deficit conditions

We examined if the drought-induced *GA20ox3* upregulation is a result of a feedback response due to the reduced GA levels (Middleton *et al.*, 2012; Fukazawa *et al.*, 2017). As the upregulation of GA biosynthesis genes by reduced GA levels (feedback response) is mediated by the accumulation of DELLA (Middleton *et al.*, 2012), we analysed the expression of *GA20ox1*, *GA20ox2* and *GA20ox3* in leaves of WT and transgenic plants overexpressing stable DELLA protein (*35S:proΔ17*, Nir *et al.*, 2017), under irrigation and water deficiency. In well watered plants, all three genes showed higher expression levels in *proΔ17* leaves compared with WT (Fig. 1e), suggesting that all are upregulated by the DELLA-mediated feedback response. However, when these plants were exposed to water-deficit conditions, the expression of *GA20ox1* and *GA20ox2*, but not that of *GA20ox3*, was strongly suppressed in both WT and *proΔ17*. We also examined how these changes in gene expression in *proΔ17* affected GA content in leaves. The levels of the two bioactive GAs, GA_1 and GA_4, were much higher in *proΔ17* than in WT leaves and were strongly reduced under water-deficit conditions (Fig. 1f; Dataset S1). Moreover, we found a strong reduction in the level of the direct precursor of GA_1, GA_{20} and higher levels of GA_{19}, the precursor of GA_{20}, indicating an overall inhibition of GA20ox activity (Dataset S1; Fig. 1c; Hedden, 2020). Taken together, these results demonstrated that water deficiency out-competes the effects of DELLA on the transcriptional regulation of *GA20ox1* and *GA20ox2* and keeps them low, despite the activation of the feedback response by the accumulated DELLA. This overcomes the mechanism of homeostasis and maintains low GA levels.

ABA and *DREB-TINY1* regulate GA metabolism under drought conditions

ABA accumulates in tomato leaves under water-deficit conditions (Nir *et al.*, 2017). We examined if ABA mediates the effect of water deficiency on GA metabolism. ABA treatment suppressed the expression of *GA20ox2* and induced the expression of *GA2ox7*, but had no effect on *GA20ox1* (Fig. S5a). We further examined the effect of water deficiency on the expression of these three genes in the leaves of the ABA-deficient mutant *sit*. While *GA20ox1* expression was equally downregulated by drought in WT and *sit* (Fig. 2a), the expression of *GA20ox2* was strongly downregulated in WT, but was hardly affected in *sit* (Fig. 2b). The upregulation of *GA2ox7* by water deficiency was partially inhibited in *sit* (Fig. 2c). These results suggested that water-deficit conditions affected GA biosynthesis and deactivation via both ABA-dependent and ABA-independent pathways.

A previous study in tomato suggested a role for a DREB transcription factor from the subfamily TINY in GA metabolism (Li *et al.*, 2012). *In silico* analysis of *GA20ox1*, *GA20ox2* and *GA2ox7* promoters suggested the presence of several putative DREB-binding elements (Sakuma *et al.*, 2002) in *GA2ox7*, but not in *GA20ox1* and *GA20ox2*, promoters (Fig. S6). Tomato has six putative DREB-TINY genes. Only *TINY1* (Solyc06g066540) and Solyc01g090560 were upregulated by drought (Fig. 2d) and the effect on *TINY1* was much stronger. The upregulation of *TINY1* by water-deficit conditions was partially inhibited in *sit* (Fig. 2e) and the application of ABA increased its expression (Fig. S5b), suggesting that drought-induced *TINY1* expression is partially ABA dependent, similar to *GA2ox7*. To examine if *TINY1* affects the expression of *GA2ox7*, we generated CRISPR-Cas9-derived *tiny1* mutants (Fig. S7). The homozygous mutant lines (two alleles, *tiny1-1* and *tiny1-2*) showed a WT phenotype. The loss of *TINY1* had no effect on *GA20ox1* and *GA20ox2* downregulation by drought (Fig. S5c,d). However, drought-induced *GA2ox7* expression was strongly inhibited in *tiny1-1* and *tiny1-2* (Fig. 2f). Together, the results implied that drought-induced *GA2ox7* expression is regulated (directly or indirectly) by *TINY1*.

GA deactivation in guard cells promotes stomatal closure under water-deficit conditions

As water deficiency upregulated *GA2ox7* expression in guard cells, we examined the significance of GA2ox7 activity to stomatal closure. The *ga2ox7* mutant was recently characterised in tomato (Schrager Lavelle *et al.*, 2019). The mutant has an elongated epicotyl, but the leaf area and stomatal density are similar to WT (Figs S8, 3a). Under normal irrigation, the loss of *GA2ox7* had no effect on the basal stomatal aperture, transpiration rate and stomatal conductance (Fig. S9). However, when *ga2ox7* plants were exposed to water-deficit conditions, they wilted before the WT and exhibited a faster decrease in leaf RWC (Fig. 3a,b). We then examined if the rapid water loss was caused by a higher transpiration rate under water-deficit conditions. We analysed whole-plant transpiration in WT and *ga2ox7* mutant plants, grown in a glasshouse using an array of lysimeters (Illouz Eliaz *et al.*, 2020). Plants were grown with irrigation, and then water supply was terminated. Transpiration rate was not affected in the first 3 d into the drought treatment (Fig. 3c). However, on the fourth day, the transpiration rate in WT plants sharply decreased, but that of *ga2ox7* did not change. Only on the fifth day, *ga2ox7* plants reduced their transpiration. Whole-plant daily transpiration was also decreased in WT before *ga2ox7* (Fig. S10a). We further analysed the transpiration rate during gradual soil dehydration by thermal imaging. In well watered soil (60% soil VWC) leaf-surface temperature was similar in WT and *ga2ox7*, indicating a similar transpiration rate (Fig. 3d). However, when the soil was dehydrated to 35% VWC, the temperature of

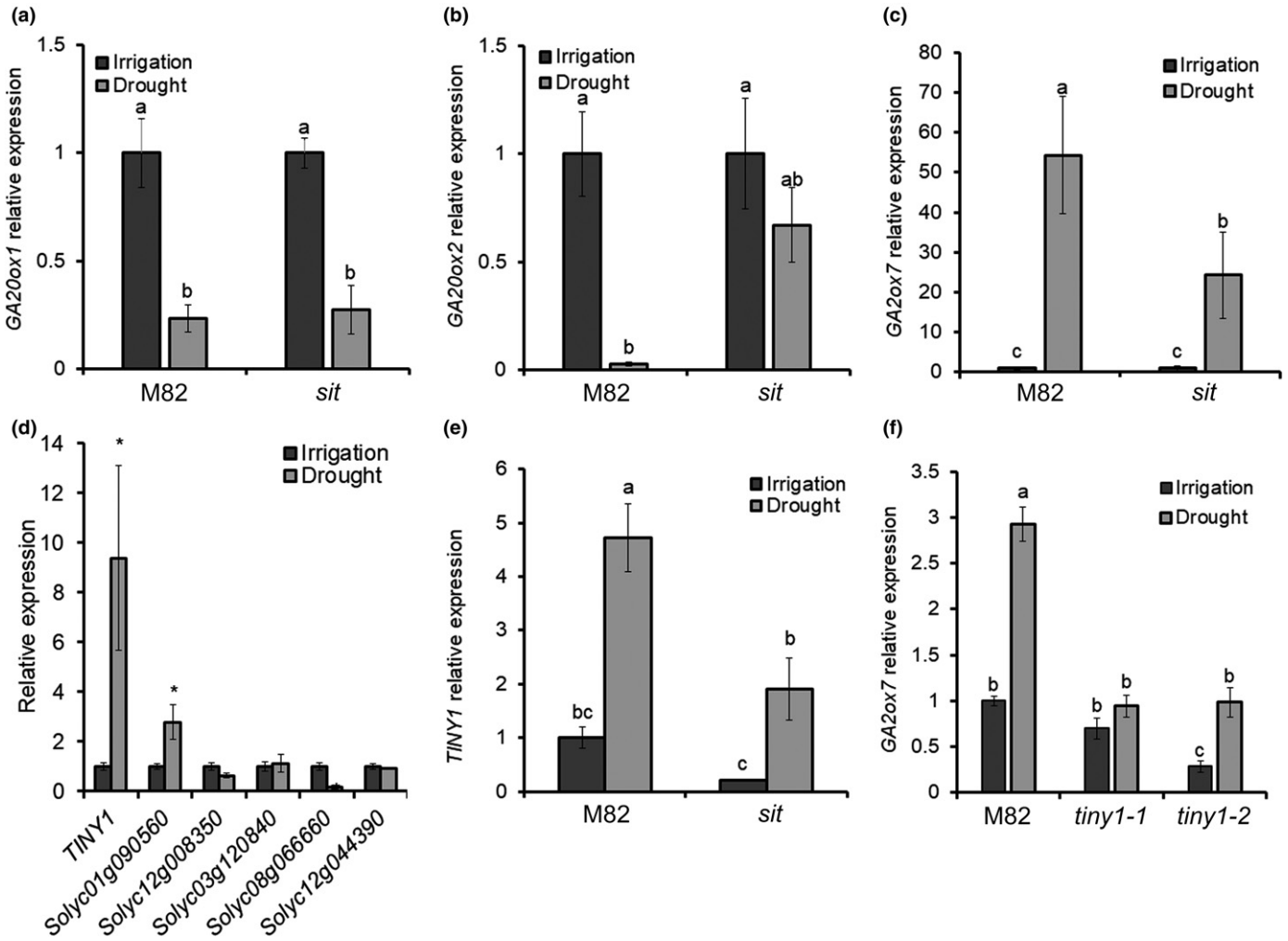

Fig. 2 Drought inhibition of gibberellin (GA) accumulation in tomato is partially mediated by the abscisic acid (ABA)-induced *TINY1*. (a–c) Relative expression of *GA 20-oxidase1* (*GA20ox1*) (a), *GA20ox2* (b) and *GA 2-oxidase7* (*GA2ox7*) (c) under irrigation and drought conditions (soil volumetric water content (VWC) 15%) in leaves of M82 and the ABA-deficient mutant *sitiens* (*sit*). Values are means of four biological replicates ± SE. (d) Relative expression of the six putative *TINY* genes in leaves of M82 plants grown with irrigation or exposed to drought condition (soil VWC 15%). (e) Relative expression of *TINY1* in leaves of M82 and the ABA-deficient mutant *sit* grown with irrigation or exposed to drought condition (soil VWC 15%). Values are means of four biological replicates ± SE. (f) Relative expression of *GA2ox7* under irrigation and drought conditions in leaves of M82 and *tiny1*. Values are the means of four biological replicates ± SE. Lowercase letters above the bars (a–c, e, f) or asterisks (d) represent significant differences between respective treatments by Tukey–Kramer HSD test ($P < 0.05$) or Student's *t*-test ($P < 0.05$), respectively.

WT leaves increased, but that of *ga2ox7* did not change, indicating that WT leaves, but not the mutant, closed their stomata. At 15% soil VWC, WT and *ga2ox7* leaves exhibited higher and similar temperatures, indicating that both closed their stomata. Microscopic analysis showed that, under mild water deficiency (35% soil VWC), 70% of WT stomata were closed while only 49% in *ga2ox7* (Fig. 3e). Finally, we analysed stomatal conductance in WT and *ga2ox7* plants at different soil VWC. In irrigated plants, stomatal conductance was similar in WT and the mutant (Figs 3f, S10b). However, when soil VWC was reduced to 40%, stomatal conductance in WT was significantly lower than in *ga2ox7*. Under severe drought (15% soil VWC), stomatal conductance was very low and similar in WT and *ga2ox7*. These results showed that *ga2ox7* stomata are hyposensitive to soil dehydration. Analysis of *GA2ox7* expression in guard cells during soil dehydration showed upregulation already at the early stages of soil dehydration (30% soil VWC; Fig. 3g). Taken together, the results suggested that GA deactivation at the early stages of soil dehydration contributed to stomatal closure.

Mutations in *GA20ox1* and *GA20ox2* promoted 'drought avoidance' by suppressing leaf expansion

To test if the reduced expression of *GA20ox1* and *GA20ox2* also affected stomatal closure and water status under water-deficit conditions, we generated CRISPR/Cas9-derived *ga20ox1* and *ga20ox2* loss-of-function mutants (Fig. S11). Both mutants exhibited reduced size with shorter stems, smaller leaves, but WT basal stomatal apertures in well watered plants (Figs 4a, S12). We first exposed WT, *ga20ox1* and *ga20ox2* plants to water-deficit

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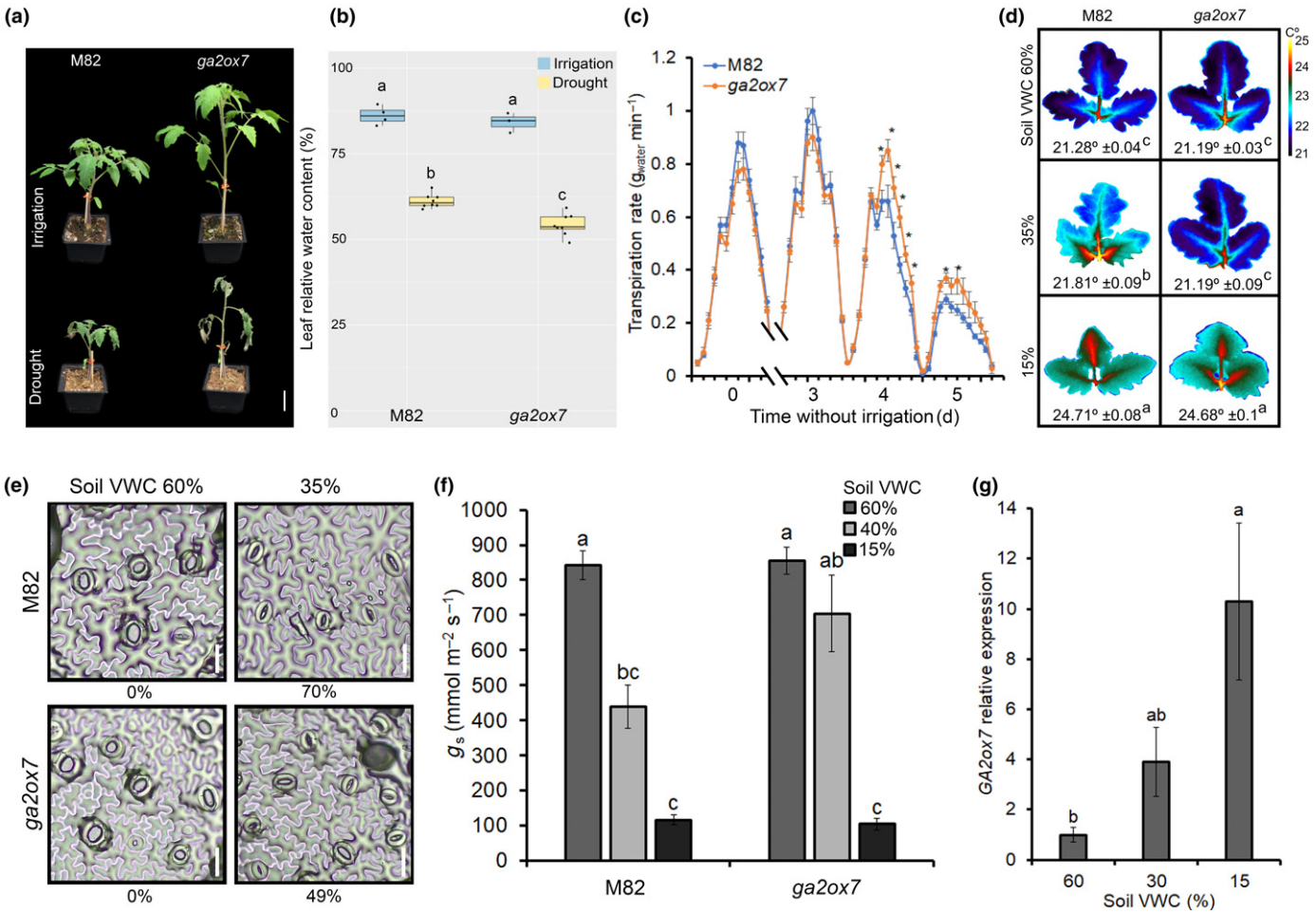

Fig. 3 Tomato *ga 2-oxidase7 (ga2ox7)* stomata are hyposensitive to soil dehydration. (a) Representative plants grown under irrigation or 7 d without irrigation. Bar, 5 cm. (b) Leaf relative water content in M82 and *ga2ox7* grown under irrigation or without irrigation for 7 d. Values are means of four (for irrigation) or eight (for drought) biological replicates ± SE. (c) M82 and *ga2ox7* whole-plant transpiration rate over the course of 24 h (06:00 h to 18:00 h) at days 0, 3, 4 and 5 after the termination of irrigation. Values are the means of six (for M82) or 10 (for *ga2ox7*) plants ± SE. M82 and *ga2ox7* plants were placed on lysimeters and pot (pot + soil + plant) weight was measured every 3 min. (d) Thermal imaging of representative leaves (leaf no. 4 below the apex) of M82 and *ga2ox7* plants exposed to different soil volumetric water content (VWC). Images were digitally extracted for comparison. Numbers below leaves are the average leaf-surface temperature, and the values are means of five biological replicates (plants), each measured six times ± SE. (e) Representative abaxial epidermal imprints taken from irrigated or drought (mild drought, soil VWC 35%) treated M82 and *ga2ox7*. Numbers below images represent the percentage of closed stomata in each treatment. Values are percentages of four biological replicates each with *c.* 100 measurements (stomata). Bars, 30 μm. (f) Stomatal conductance (g_s) of M82 and *ga2ox7* under different soil VWC. Values are the means of four biological replicates ± SE. (g) *GA2ox7* relative expression in M82 isolated guard cells under different soil VWC. Asterisk or lowercase letters represent significant differences between respective lines (c) by Student's *t*-test ($P < 0.05$) or lines and treatments (b, d, f, g) Tukey–Kramer HSD test ($P < 0.05$).

conditions and analysed the rate of water loss. Both mutants wilted later than WT, and maintained higher leaf RWC (Fig. 4a, b), indicating a reduced rate of water loss under drought. We then analysed the transpiration rate using thermal imaging. Both mutations had no effect on transpiration rate under irrigation or water-deficit conditions (Fig. 4c). In addition, stomatal conductance was similar between WT and the mutants during soil dehydration (Fig. 4d). Together, the results suggested that the reduced rate of water loss found in *ga20ox1* and *ga20ox2* plants under water-deficit conditions was caused by the smaller plant size (leaf area), and not by faster stomatal closure. We also generated the double mutant *ga20ox1/ga20ox2* by crosses. The double mutant exhibited an additive effect on growth; plants were dwarf and their leaves were very small (Fig. S13). Microscopic analysis of *ga20ox1/ga20ox2* abaxial leaf epidermis showed reduced stomatal apertures (Fig. 4e), similar to other strong GA mutants, such as overexpression of stable DELLA (Nir *et al.*, 2017). In line with this observation, stomatal conductance in the double mutant was lower than WT in well watered plants (Fig. 4f). These results suggested high redundancy between *GA20ox*1 and *GA20ox2* in the promotion of stomatal closure, but not in the regulation of leaf growth. *GA20ox1* and *GA20ox2* expression was much higher in whole-leaf tissue compared with guard cells (Fig. 4g). This may explain why they have a significant role in leaf growth but not in stomatal closure.

To confirm that inhibition of GA synthesis reduces water loss solely via growth suppression, whereas induction of GA deactivation through stomatal closure, we grew *ga20ox1* and *ga2ox7*

mutants, each in the same pot with WT and stopped irrigation when plants had four to five leaves. This eliminated the effect of plant size on the rate of soil dehydration, and both WT and the mutants were exposed to the same soil VWC at any time during the experiment. Six d into the drought treatment, both WT and *ga20ox1* lost their turgor and start wilting (Fig. 5a). At this time point, leaf RWC was similar in the two lines (Fig. 5b), supporting our suggestion that the loss of *GA20ox1* improved 'drought avoidance' solely via the reduced plant size and total transpiration area. Conversely, *ga2ox7* lost turgor and wilted 1 d before WT (Fig. 5c). At this time point (fifth day into the drought treatment), *ga2ox7* plants exhibited lower leaf RWC compared with WT (Fig. 5d), suggesting that the reduced 'drought avoidance' in *ga2ox7* plants was caused, as suggested above, by delayed stomatal closure.

We then compared the contribution of each response (early stomatal closure and growth suppression) to 'drought avoidance'. To this end, we generated a double mutant *ga20ox1/ga2ox7* by crosses. The homozygous double mutant plants exhibited elongated epicotyl, similar to *ga2ox7* and smaller leaves, similar to

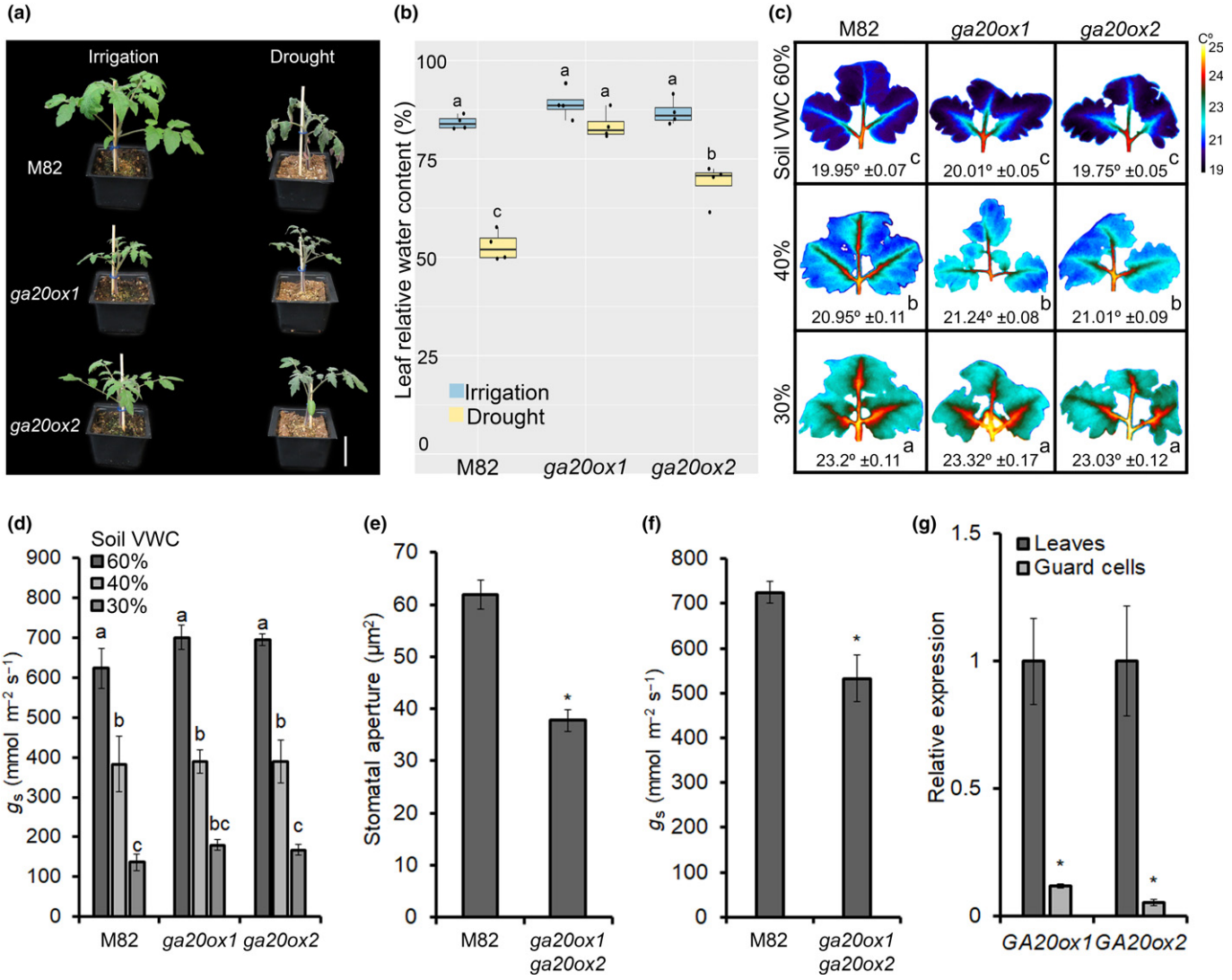

Fig. 4 Loss of the tomato *GA 20-oxidase1* (*GA20ox1*) and *GA20ox2* reduced canopy growth and inhibited water loss under water-deficit conditions. (a) Representative plants grown under irrigation or 7 d of drought. Bar, 5 cm. (b) Leaf relative water content in M82, *ga20ox1* and *ga20ox2* grown under irrigation or 7 d without irrigation. Values are the means of four biological replicates ± SE. (c) Thermal imaging of representative leaves of M82, *ga20ox1* and *ga20ox2* plants exposed to different soil volumetric water content (VWC). Images were digitally extracted for comparison. Numbers below plants are the average leaf-surface temperature, and the values are means of five biological replicates (plants), each measured six times ± SE. (d) Stomatal conductance (g_s) in M82, *ga20ox1* and *ga20ox2* under different soil VWC. Values are the means of four biological replicates measured four to eight times ± SE. (e) Stomatal aperture of M82 and the double mutant *ga20ox1/ga20ox2* measured on imprints of abaxial epidermis taken from leaf no. 3 below the apex. Values are the means of four biological replicates each with *c*. 100 measurements (stomata) ± SE. (f) Stomatal conductance (g_s) of M82 and *ga20ox1/ga20ox2*. Values are the means of four biological replicates ± SE. (g) Relative expression of *GA20ox1* and *GA20ox2* in whole-leaf tissue compared with isolated guard cells. Values are the means of four biological replicates ± SE. Lowercase letters (b–d) or asterisks (e–g) represent significant differences between respective treatments by Tukey–Kramer HSD test ($P < 0.05$) or Student's *t*-test ($P < 0.05$), respectively.

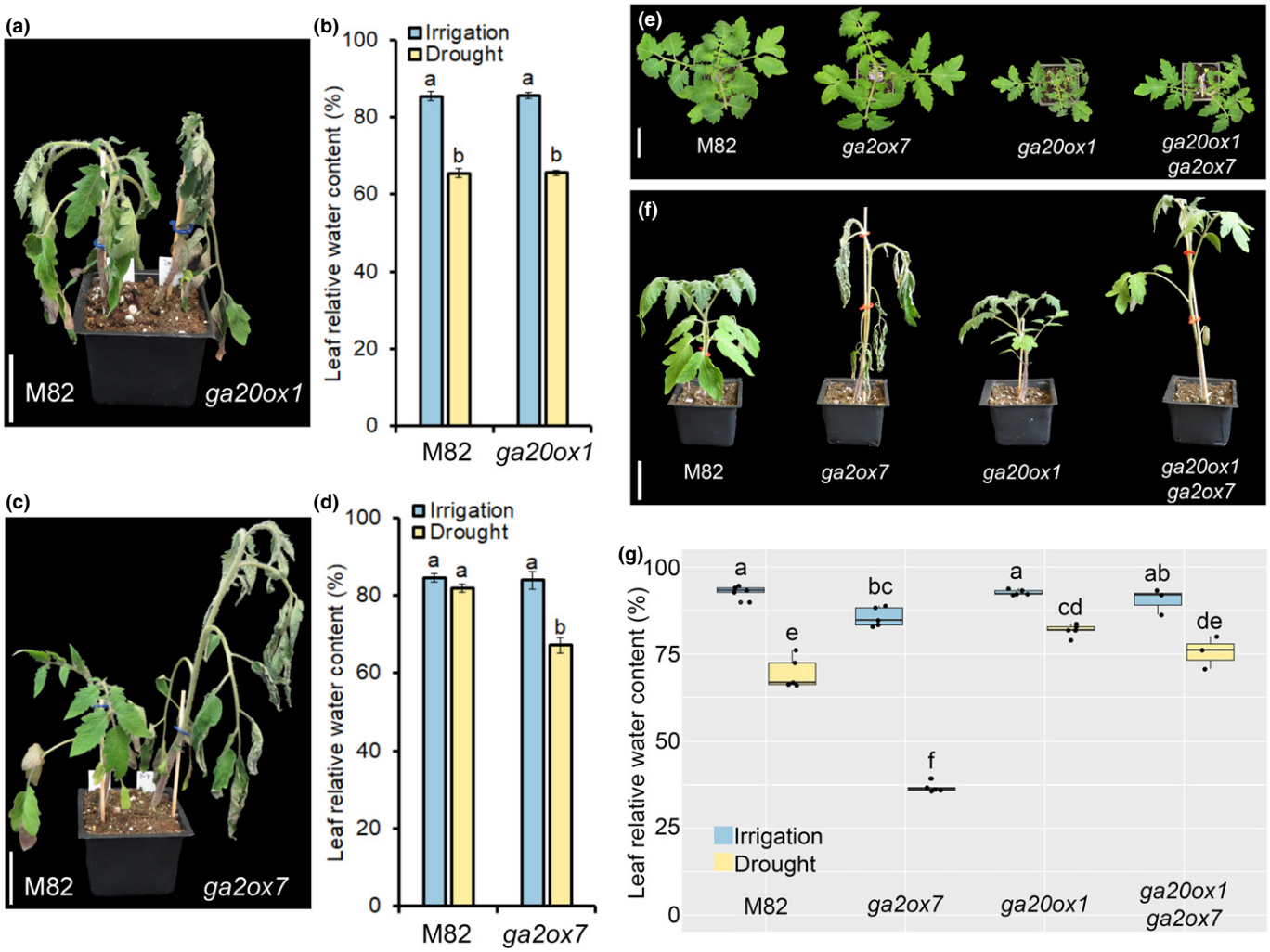

Fig. 5 Reduced gibberellin (GA) levels in tomato contribute to 'drought avoidance' by suppressing leaf growth and promoting stomatal closure. (a, b) Wild-type (WT) and *ga 20-oxidase1* (*ga20ox1*) were grown together in the same pot and then irrigation was stopped. Representative plants (a) and leaf relative water content (RWC) (b) 6 d into the drought treatment. (c, d) WT and *ga 2-oxidase7* (*ga2ox7*) plants (as in (a)) 5 d into the drought treatment. Values in (b) and (d) are the means of six biological replicates (terminal leaflets taken from leaf no. 3 below the apex) ± SE. (e, f) Representative plants of the double mutant *ga20ox1/ga2ox7* grown under irrigation (e) or 10 d without irrigation (f). Bars: (a, c, e, f) 5 cm. (g) Leaf RWC in M82, *ga2ox7*, *ga20ox1* and the double mutant *ga20ox1/ga2ox7* under irrigation or 10 d without irrigation. Values are the means of three to five biological replicates (four terminal leaflets taken from leaf no. 3 below the apex) ± SE. Lowercase letters (b, c, g) represent significant differences between respective treatments by Tukey–Kramer HSD test ($P < 0.05$).

ga20ox1 (Figs S14, 5e,f). While the *ga2ox7* single mutant exhibited rapid wilting and low RWC under water-deficit conditions, *ga20ox1* plants maintained higher leaf RWC and showed slower water loss (due to the smaller plant size). In the double mutant, the loss of *GA20ox1*, strongly suppressed the effect of *ga2ox7*; these plants maintained higher leaf RWC compared with WT, slightly lower than the single mutant *ga20ox1* (Fig. 5g). These results demonstrated the importance of GA regulation of leaf growth to 'drought avoidance'.

Discussion

The results of this study suggested that water deficiency reduced the levels of the bioactive GA_1 and GA_3 in tomato guard cells and this accelerated stomatal closure. The relatively high levels of GA_3 in stomata of well watered plants were unexpected as most studies found only trace amounts of this bioactive GA in plants (Hedden, 2020). However, a recent study showed the accumulation of GA_3 in tomato fruits (Li *et al.*, 2020). The reduced levels of bioactive GAs in guard cells under water deficiency was probably a result of the strong upregulation of the GA deactivating gene *GA2ox7*. *GA2ox7* belongs to class III *GA2oxs*. Enzymes of this class catalyse the deactivation of C-20 GA precursors, affecting the metabolic flow towards the production of C-19 bioactive GAs (Hedden, 2020). Water deficiency reduced in guard cells not only the level of the bioactive GAs but also the level of their C-20 precursor GA_{12}, the direct substrate of GA2ox7 (Schrager Lavelle *et al.*, 2019).

GA2ox7 was upregulated in guard cells at the early stages of soil dehydration and its loss-of-function inhibited stomatal

closure in response to mild soil dehydration. Under severe drought, however, stomata of *ga2ox7* were closed, similar to those of WT. Previously we have shown that reduced GA activity promoted ABA responses in guard cells (Nir *et al.*, 2017; Shohat *et al.*, 2020). Promoting ABA responses can have a significant effect on stomatal closure at the early stages of soil dehydration when ABA levels are still limited, but in dry soil, when ABA levels are saturated, the effect is probably neglected. Therefore, we propose that at the early stages of soil dehydration, *GA2ox7* is upregulated in guard cells, leading to a reduction in bioactive GA levels that, in turn, increases ABA activity to accelerate stomatal closure (Fig. 6).

It is not clear yet what is the contribution of *GA20ox1* and *GA20ox2* activity to these changes in guard-cell GA levels. The expression of these two genes in guard cells was suppressed under drought, but their loss-of-function had no effect on basal stomatal aperture or stomatal response to water deficiency. It had a strong effect, however, on plant size. Only in the double knockout mutant *ga20ox1/ga20ox2* were the basal stomatal aperture and conductance reduced, similar to overexpression of stable DELLA (Nir *et al.*, 2017). As these two genes exhibited low expression in guard cells (compared with whole-leaf tissue), their contribution to GA accumulation in these cells may be limited. It is possible that GA levels/activity in guard cells have to be below a certain threshold to affect stomatal closure.

Water-deficit conditions suppress growth to reduce transpiration area and relocate resources for adaptation (Eziz *et al.*, 2017). It has been shown previously that growth inhibition by abiotic stress is mediated by reduced GA accumulation (Achard *et al.*, 2006; Skirycz & Inze, 2010). Our results in tomato suggest that growth inhibition by drought is also mediated, at least partially, by a reduction in bioactive GA levels. *GA20ox1* and *GA20ox2* expression was suppressed by water deficiency also in leaf tissues. These two genes are highly expressed in leaves (http://bar.utoronto.ca/efp_tomato). Although the single *ga20ox* semidwarf mutants exhibited WT stomatal closure, both were able to maintain higher leaf RWC for longer time periods under water-deficit conditions. As this effect was eliminated when WT and *ga20ox1* were grown in the same pot and exposed to the same soil VWC, the effect of the mutation on 'drought avoidance' can be attributed solely to the reduced plant size. In the *ga2ox7/ga20ox1* double mutant, the effect of reduced leaf size, caused by the loss of *GA20ox1*, suppressed the fast water loss caused by *ga2ox7*, demonstrating the importance of growth suppression and plant size to 'drought avoidance'. It is interesting to note that the double mutant *ga2ox7/ga20ox1* exhibits smaller leaves, similar to *ga20ox1* and elongated epicotyl, similar to *ga2ox7*. This finding suggests that the high GA levels in the stem cannot compensate for the low GA levels in the leaves, and implies that translocation of GAs from stems to leaves is limited in tomato.

The expression of *GA20ox* genes is regulated by the GA feedback loop via the transcriptional complex DELLA-INDETERMINATE DOMAIN (IDD, Fukazawa *et al.*, 2017) to maintain GA homeostasis. A major question is how environmental conditions overcome this mechanism of homeostasis. In tomato leaves, water deficiency inhibited the expression of *GA20ox1* and *GA20ox2*, but induced the expression of *GA20ox3*. These three genes (*GA20ox1/2/3*) were upregulated in transgenic plants overexpressing stable DELLA protein (*proΔ17*), suggesting that all of them are regulated by the feedback response via DELLA to maintain GA homeostasis. Water deficiency, however, downregulated *GA20ox1* and *GA20ox2* (but not *GA20ox3*) in the presence of stable DELLA (*proΔ17*) and inhibited the accumulation of bioactive GAs. These findings suggest that water deficiency out-competes the effect of DELLA on the transcriptional upregulation of the two genes, and keeps the expression low despite DELLA accumulation and, therefore, suppresses the mechanism of homeostasis.

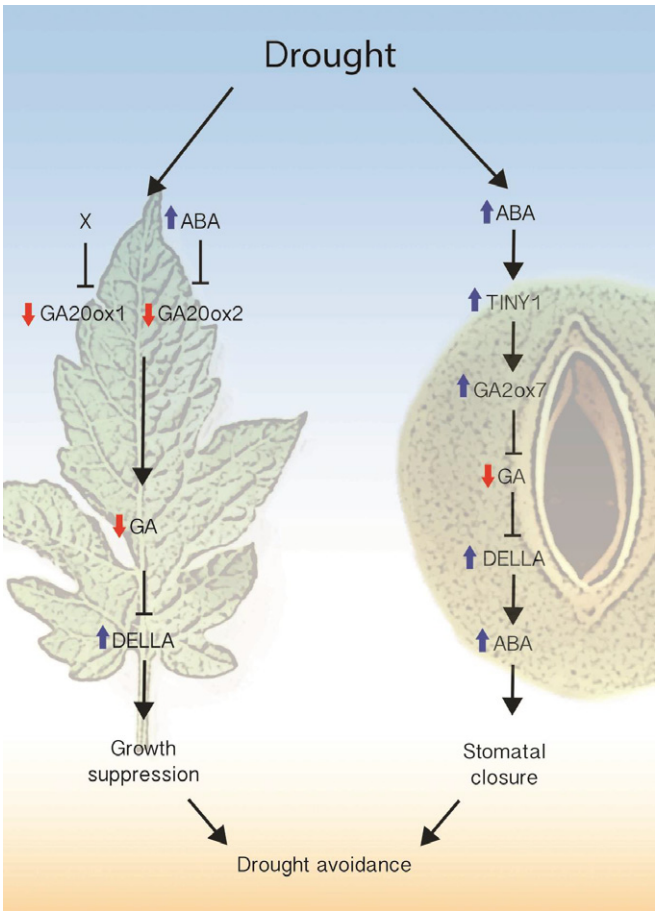

Fig. 6 Suggested model for the role of gibberellin (GA) in 'drought avoidance' in tomato plants. Water-deficit conditions, via abscisic acid (ABA)-dependent and ABA-independent pathways downregulates the expression of the GA biosynthesis genes *GA 20-oxidase1* (*GA20ox1*) and *GA20ox2* and upregulates the GA deactivating gene *GA 2-oxidase7* (*GA2ox7*) in leaf tissue and guard cells. The upregulation of *GA2ox7* by drought and ABA is mediated by the transcription factor *DEHYDRATION RESPONSIVE ELEMENT BINDING* (DREB)-TINY1. These molecular changes led to reduced levels of bioactive GAs and the accumulation of DELLA. In turn, DELLA inhibits leaf growth and promotes ABA-induced stomatal closure at the early stages of soil dehydration. In leaf tissue the levels of *GA20ox1* and *GA20ox2* expression has the dominant role in the regulation of growth, whereas in guard cells *GA2ox7* has a major role in stomatal closure. The inhibition of leaf growth and the earlier stomatal closure reduce transpiration and promote 'drought avoidance'.

Plant responses to drought and ABA are mediated by the ETHYLEN RESPONSE FACTOR (ERF)/AP2 transcription factors DREB (Sakuma *et al.*, 2002). DREBs regulate downstream responses, including stomatal closure, growth suppression and induction of stress-related genes (Lata & Prasad, 2011). DREB proteins are divided into six subfamilies (A1 to A6), and DREB-TINYs belong to subfamily A4, which contains 16 genes in Arabidopsis (Nakano *et al.*, 2006). An Arabidopsis TINY protein suppresses growth by inhibition of brassinosteroid activity and promotes ABA-induced stomatal closure (Xie *et al.*, 2019). In tomato, overexpression of DREB-*TINY1* suppresses *GA20ox1* and *GA20ox2* expression, reduces GA levels, inhibits growth, and promotes tolerance to drought (Li *et al.*, 2012). The tomato *TINY1* was also induced by high temperatures and its downregulation resulted in susceptibility to heat stress (Mao *et al.*, 2020). Our results showed that the loss of *TINY1* had no effect on the suppression of *GA20ox1* and *GA20ox2* by drought, suggesting that TINY1 is not the drought-induced regulator of these *GA20oxs*. Conversely, *GA2ox7* induction by drought was inhibited in *tiny1*, suggesting that *TINY1* mediates the effect of drought on *GA2ox7* expression. Whether *TINY1* is a direct regulator of *GA2ox7*, is not yet clear.

To conclude, we suggest that water-deficit conditions, via ABA-dependent and ABA-independent pathways, downregulate the expression of the GA biosynthesis genes *GA20ox1* and *GA20ox2* and upregulate the GA deactivating gene *GA2ox7* in leaf tissues and guard cells, leading to reduced levels of bioactive GAs. The lower GA levels in leaves suppress their growth and in guard cells promote ABA-induced stomatal closure at the early stages of soil dehydration (Fig. 6). The suppression of leaf growth is regulated mainly by the inhibition of GA biosynthesis (downregulation of *GA20ox*), whereas the accelerated stomatal closure, is regulated by GA deactivation (upregulation of *GA2ox*). These short-term and long-term responses reduce transpiration and promote 'drought avoidance' and adaptation to water-deficit conditions.

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Author contributions

HS and DW designed the research plan; HS, HC, HVK, NIE, SB and ZA performed the research; AA and DT contributed analytic tools; HS, HVK, AA and DT analysed data; HS, YE and DW wrote the paper.

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Data availability

All data can be found in the manuscript and in the Supporting Information.

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Supporting Information

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

Dataset S1 Gibberellin analyses in leaves and guard cells.

Fig. S1 Vitality of the isolated guard cells.

Fig. S2 *GA2ox7* exhibits the highest expression among all analysed *GA2ox* genes in guard cells.

Fig. S3 Drought regulation of *GA3oxs* and *GA20oxs* expression in guard cells.

Fig. S4 Drought regulation of *GA2ox*, *GA3ox* and *GA20ox* in leaves.

Fig. S5 Abscisic acid regulation of gibberellin metabolism genes.

Fig. S6 Putative DREB-responsive elements (DRE) in the *GA2ox7* promoter.

Fig. S7 Sequence analyses of *tiny1* CRISPR-Cas9 mutants.

Fig. S8 Loss of *GA2ox7* affects stem elongation but not leaf growth.

Fig. S9 Loss of *GA2ox7* did not affect transpiration rate and stomatal aperture in well watered plants.

Fig. S10 Loss of *GA2ox7* inhibited stomatal closure in response to mild soil dehydration.

Fig. S11 Sequence analyses of *ga20ox1 and ga20ox2* CRISPR-Cas9 mutants.

Fig. S12 Loss of *GA20ox1* and *GA20ox2* suppressed growth but had no effect on basal stomatal aperture.

Fig. S13 The double mutant *ga20ox1/ga20ox2*.

Fig. S14 The double mutant *ga20ox1/ga2ox7* has long epicotyl, similar to *ga2ox7* and small leaves, similar to *ga20ox1*.

Table S1 Single-guide RNAs used in this study.

Table S2 Primers used in this study.

Table S3 UPLC-MRM parameters used for measuring gibberellins by UPLC-QQQ-MS/MS.

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