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Plants are able to sense a few degrees rise in temperature, and appropriately adapt their metabolic and growth processes. To this end, plants produce various signaling molecules that act throughout the plant body. Here, we report that root-derived GA_{12}, a precursor of the bioactive gibberellins (GA), mediates thermo-responsive shoot growth in *Arabidopsis*. Our data suggest that root-to-shoot translocation of GA_{12} enables a flexible growth response to ambient temperature changes.

In plants, roots and shoots cooperate to ensure adaptive growth rates to fluctuating environmental cues. For example, the elevation of the temperature causes important changes to plant shape, promoting root and hypocotyl elongation, hyponastic growth and flowering[1-3]. Root-derived signals have been reported since long time to modulate shoot morphology, however, the contribution of these signals to temperature-induced shoot growth remains unclear.

Gibberellins (GA) are a class of phytohormones that play important role in diverse developmental processes, including seed germination, plant growth and flowering[4]. GA promote growth by activating

the destabilization of nuclear DELLA growth repressing proteins[4]. In recent years, GA pathway has been reported to integrate various environmental signals and in response, to optimize plant growth accordingly[5,6]. Notably, diverse studies have pointed out a role for GA signaling in temperature-sensing pathways. Whereas warmer temperatures enhance GA biosynthesis gene expression[7,8], lack of DELLA activity accelerates flowering at lower temperatures[3]. Thus, changes in GA synthesis and/or distribution have important effect on plant growth adaption to fluctuating temperatures. If it is admitted that GA are usually synthetized close to their site of action, they can also be transported throughout the plant[9,10]. This latest observation prompted us to investigate whether root-to-shoot translocation of GA contributes to accelerated shoot growth at elevated ambient temperatures.

To investigate this possibility, we measured the aerial growth of reciprocal grafts between *Arabidopsis* wild-type (Col-0) and GA-deficient mutant (*ga1-3*) at 20° and 28°C (Supplementary Figure 1a-c). Strikingly, whereas the rosettes of Col-0 self-grafts grown at 28°C were larger to those grown at 20°C, the rosette radius of wild-type scions grafted onto *ga1-3* rootstocks (*ga1-3*/Col-0 grafts) was smaller at 28°C (Figure 1a and Supplementary Figure 1b). Moreover, the diameter of three-week-old *ga1-3* rosettes grafted onto wild-type rootstocks (Col-0/*ga1-3* grafts) was significantly larger at 28°C than at 20°C (Figure 1b and Supplementary Figure 1c). Thus, root-derived GA contribute substantially to the shoot growth promotion triggered by high temperature. Remarkably, we found that *ga20ox1-2-3* triple mutant rootstocks, which produce GA_{12} but are unable to convert it into GA_{15} and following products[11] (Supplementary Figure 1a), were also able to increase the growth of *ga1-3* grafted scions at high temperature (Figure 1a,b and Supplementary Figure 1b,c); consistent with the premise that GA_{12} is a mobile growth signal in *Arabidopsis*[9].

To determine the nature of the root-derived GA signal leading to accelerated shoot growth at high temperature, we quantified the GA levels in scions of Col-0/*ga1-3* and *ga1-3*/Col-0 grafts, and wild-type and *ga1-3* self-graft controls. Consistent with the morphological parameters, bioactive GA_4 accumulated to higher levels in *ga1-3* mutant scions of Col-0/*ga1-3* grafts growing at 28°C compared with those at 20°C (Figure 1c). Interestingly, GA_{12} was also detected in higher amount in *ga1-3* mutant scions of Col-0/*ga1-3* grafts grown at 28°C. Thus, these data indicate that high temperatures increase the level of root-borne GA_{12} delivered to the shoots, which is then converted into GA_4 by the activities of GA 20-oxidases (GA20ox) and GA 3-oxidases (GA3ox)[11] (Supplementary Figure 1a). Consistent with this hypothesis, we found that GA_4 accumulated to higher level in wild-type scions of Col-0/Col-0 grafts compared with *ga1-3*/Col-0 grafts at 28°C (Figure 1c), and therefore could explain their difference in rosette diameter (Figure 1a). Unlike GA_4, GA_{12} was detected at a lower level in wild-type scions of Col-0/Col-0 and *ga1-3*/Col-0 grafts grown at 28°C compared with those grown at 20°C (Figure 1c). In wild-type shoots, the concentration of GA_{12} is determined by both the amount of root-derived and shoot-synthetized GA_{12}, and the rate of its metabolic conversion to GA_{15} by the

activity of GA20ox, which catalyze a rate-limiting step in GA metabolism[11]. As previously reported[7], we found that elevated ambient temperature enhanced *GA20ox1* expression, and thereby, the conversion of GA_{12} into GA_{15} (Figure S1d and Figure 1c). Thus, warm temperatures enhance both GA_{12} supply and metabolic conversion in wild-type shoots.

High ambient temperature could enhance the delivery of root-borne GA_{12} in the shoots via increased synthesis of GA_{12} in roots and/or enhanced transport of GA_{12} from the roots. To examine these possibilities, we first determined the concentration of endogenous GA in wild-type roots grown at 20° and at 28°C. The content in GA_{12} was not statistically different between the two conditions (Figure 1d), as well as the expression of GA metabolism genes catalyzing the first steps of the GA biosynthetic pathway (Supplementary Figure 1e). We also determined the concentration of GA_{12} in xylem exudates collected from shoots of five-week-old Col-0/*ga1-3* grafts grown at 20° and 28°C. Remarkably, both the concentration of GA_{12} and the GA_{12}/K^+ ratio (K^+ is used as a reference of the xylem sap flux[12], which fluctuates with the transpiration rate of the leaves) were increased at 28°C (Figure 1e). Altogether, these findings provide compelling evidence that high temperature increases the root-to-shoot translocation of GA_{12}, which in turn, after metabolic conversion into bioactive GA_4, promotes shoot elongation.

In *Arabidopsis*, thermo-sensing pathway is dependent on the transcription factor PIF4, which accelerates shoot growth through direct activation of auxin biosynthesis and signaling genes[1,3]. It has been reported that warm temperature enhances both *PIF4* expression and transcriptional activity, while GA-regulated DELLA proteins repress PIF4 activity by preventing its DNA binding capacity (Figure 2a)[1,3,13]. To investigate whether root-derived GA_{12} has an effect on PIF4 activity in shoots, we generated a transgenic line that produces GA_{12} solely in the root. To this end, we complemented the *kao1 kao2* double mutant (deprived of GA_{12} synthesis[14]) with a construct that expresses *KAO1* only in root, with the use of the *ROOT-SPECIFIC KINASE 1* (*ARSK1*) promoter (in *kao1 kao2 pARSK1:KAO1-RFP*). Indeed, as previously observed with *pARSK1:GUS* reporter line[15], the promoter of *ARSK1* is active in the entire root (except the root apex), mainly in epidermal and endodermal cells, but not in the hypocotyl and cotyledons (Supplementary Figure 2a-e). Moreover, to further validate the root-specific activity of *ARSK1* promoter, we also expressed *GA20ox1* under the regulation of the *ARSK1* promoter in the *ga20ox1-2-3* triple mutant (in *ga20ox1-2-3 pARSK1:GA20ox1-RFP*). Consistent with the assumption that GA_{12} is the main GA form translocated from root to shoot, *pARSK1:KAO1-RFP* entirely rescued the dwarf phenotype of *kao1 kao2* mutant seedlings (in *kao1 kao2 pARSK1:KAO1-RFP*) grown at 22°C in long day conditions, while *pARSK1:GA20ox1-RFP* only restored the growth of *ga20ox1-2-3* roots (in *ga20ox1-2-3 pARSK1:GA20ox1-RFP*); the hypocotyls being similar from that of *ga20ox1-2-3* triple mutant (Supplementary Figure 3a,b). Thus, the activity of the *ARSK1* promoter is restricted to the root at seedling stage. At adult stage, *ARSK1* promoter is

also active in rosette leaves (but not in inflorescence; Supplementary Figure 3e-i), and therefore, all further experiments were performed on 7-d-old seedlings. Noteworthy, complementary analysis revealed that ambient temperature does not modulate *ARSK1* promoter activity (Supplementary Figure 2f,g).

To further substantiate the previous results obtained with the grafts (Figure 1), we finally examined the hypocotyl length of *kao1 kao2 pARSK1:KAO1-RFP* and *ga20ox1-2-3 pARSK1:GA20ox1-RFP* seedlings at 20° and 28°C, compared to that of wild-type and *pif4-101* mutant. For this experiment, the seedlings were grown in short day conditions under low light intensity (30 µmol/m^2/s) to enhance hypocotyl elongation and therefore to monitor subtle changes in hypocotyl length. Whereas the seedlings exhibited similar growth patterns at 20°C (moderate temperature inhibits PIF action[1]), the hypocotyls of wild-type and *kao1 kao2 pARSK1:KAO1-RFP* were more elongated than those of *ga20ox1-2-3 pARSK1:GA20ox1-RFP* and *pif4-101* mutant at 28°C (Figure 2b,c). Consistent with this result, we found that hypocotyl growth of rootless wild-type seedlings (hence producing GA only in shoot) was significantly reduced at 28°C compared to that of intact wild-type seedlings (producing GA in both root and shoot), but less affected than *pif4* mutant, which is substantially resistant to temperature-induced shoot growth[3] (Supplementary Figure 4a). Furthermore, we found that elevated temperature decreased the abundance of the DELLA protein REPRESSOR OF GA1-3 (RGA) in hypocotyls of wild-type, *kao1 kao2 pARSK1:KAO1-RFP* and *pif4-101* mutant (compared to that at 20°C) but not in *ga20ox1-2-3 pARSK1:GA20ox1-RFP* hypocotyls (Figure 2d and Supplementary Figure 4b); hence confirming that GA signaling acts upstream of PIF4. Thus, increased root-to-shoot translocation of GA_{12} at 28°C enhances DELLA protein degradation, and as a consequence shoot growth, in accord with the growth parameters obtained with the grafts in Figure 1.

Subsequently, we analyzed the effects of root-to-shoot translocation of GA_{12} on the expression of PIF4 target genes in hypocotyls after a temperature shift from 20° to 28°C for 4h. Consistent with the above results, we found that *INDOLE-3-ACETIC ACID INDUCIBLE 19* (*IAA19*), *IAA29*, *YUCCA 8* (*YUC8*) and *PACLOBUTRAZOL RESISTANT 1* (*PRE1*) transcripts were increased by about twofold in wild-type and *kao1 kao2 pARSK1:KAO1-RFP* hypocotyls treated at 28°C, compared with those kept at 20°C (Figure 2e). By contrast, high temperature did not significantly induce the expression of the PIF4 target genes in *ga20ox1-2-3 pARSK1:GA20ox1-RFP* hypocotyls. Given that DELLA-PIF4 interaction blocks the DNA binding capacity of PIF4 to its target genes[13], these results suggest that root-derived GA_{12} induces PIF4-mediated temperature-responsive gene expression, by enhancing the destabilization of DELLA proteins.

Plant hormones play a critical role in long-distance communication, ensuring coordinated developmental processes in response to fluctuating environmental cues. For example, nitrate availability fine-tunes the amount of root-borne cytokinins delivered to the shoots to optimize their development[16]. Remarkably, whereas previous studies have revealed that GA can move over long

distances[9,10], the physiological relevance of this transport remained unclear. In this work, we demonstrated that root-derived GA_{12} contributes substantially to temperature-induced shoot growth, consistent with previous data showing that GA_{12} is the main mobile GA signal over long distances in *Arabidopsis*[9]. The amount of root-borne GA_{12} delivered to the shoots depends on its concentration in xylem sap and the leaf transpiration, which increases at elevated temperature. By analyzing the GA_{12}/K^+ ratio, our findings have revealed a specific effect of the temperature on the concentration of GA_{12} in xylem fluid (Figure 1e). Although further studies are needed to unravel the molecular mechanism, it is tempting to speculate that ambient temperature regulates the activity or the level of GA efflux transporters facilitating the translocation of GA_{12} into the xylem. Unfortunately, despite the recent identification of several GA influx transporters involved in the local movement of GA in *Arabidopsis*[10,17,18], GA efflux transporters remain to be discovered.

Climate change has begun to exert significant effects in plant morphology and behavior. Pioneering studies suggested that environmental signals such as flooding or soil temperature influence shoot growth, at least in part, via the modulation in the supply of GA in the xylem sap in tomato and in pine[19,20]. Although it remains unclear how the temperature signal is sensed in the root, our results show that root-derived GA_{12} permits a flexible growth response to ambient temperature changes (Supplementary Figure 5; Supplementary Discussion). Root-to-shoot translocation of GA_{12} might play an essential role in response to day and night air temperature oscillations for which, in contrast to soil temperature, the amplitude of the variations can rapidly rise in spring.

Methods

Plant material and growth conditions

Mutant lines are derived from Columbia-0 (Col-0) (*ga1-3*; *kao1-1 kao2-1*; *ga20ox1 ga20ox2 ga20ox3-1; pif4-101*) backgrounds. Plants were grown on soil or on plates containing 1x Murashige-Skoog (MS) medium (Duchefa Biochemical), 1% sucrose and 0.8% agar, at 20°C or at 28°C, as indicated. GA-deficient mutant seeds were pretreated at 4°C with 5 µM GA_3 (Sigma-Aldrich) for 3 days to synchronize germination, washed thoroughly 3 times, then surface sterilized before sowing.

Plasmid construction and plant transformation

To generate *pARSK1:GUS*, *pARSK1* promoter (2.7-kb fragment) was PCR amplified from Col-0 genomic DNA with appropriate primers (listed in Supplementary Table 3) and inserted into pDONR207 (Invitrogen) by Gateway cloning and recombined with pGWB633 [21]. To generate *pARSK1:KAO1-RFP* and *pARSK1:GA20ox1-RFP* constructs, *pARSK1* (2.7-kb fragment) was cloned into HindIII/SpeI sites of pB7RWG2 [22], replacing the *p35S* promoter. *KAO1* and *GA20ox1* cDNA amplified by RT-PCR were then inserted into pDONR207 and recombined with the new *pARSK1*-pB7RWG2 vectors. The plant binary vectors were introduced into *Agrobacterium tumefaciens* GV3101 strain by electroporation, and *Arabidopsis* Col-0, *kao1 kao2* and *ga20ox1-2-3* plants were

transformed by floral dip to respectively obtained *pARSK1:GUS*, *kao1 kao2 pARSK1:KAO1-RFP* and *ga20ox1-2-3 pARSK1:GA20ox1-RFP* transgenic lines.

Grafting

Micrografting between hypocotyls of rootstocks and scions was carried out without collars on 6-day-old seedlings, as previously described[23]. Successful grafts were transferred into soil and grown under continuous light at 20°C or at 28°C.

Xylem sap exudations

Stems of 5-week-old grafts (5 days after bolting) were decapitated 2 cm above the rosette with a razor blade and placed in closed box to maintain high humidity. Exudate was collected every 30 min for 3 h (the first drop was discarded), pooled and concentrated under vacuum centrifugation (Savant ThermoFisher).

GA determinations

GA contents in scions of grafted plants (Figure 1c) were determined by ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) using a Q-Exactive spectrometer (Orbitrap detector; ThermoFisher Scientific). Dry grounded material was suspended in 80% methanol-1% acetic acid including 17-$^2H_2$-labeled GA internal standards (Olchemim) and mixed by shaking during one hour at 4ºC. The extract was kept at -20ºC overnight, centrifuged and the supernatant dried in a vacuum evaporator. The dry residue was dissolved in 1% acetic acid and passed consecutively through a reverse phase column Oasis HLB column and a cationic exchange Oasis MCX eluted with MeOH. The dried eluate was dissolved in 5% acetonitrile-1% acetic acid, and the GAs were separated by UHPLC (Accucore C18 column 2.6 µm, 100 mm x 2.1 mm; ThermoFisher Scientific) with a 2 to 55% acetonitrile gradient containing 0.05% acetic acid, at 400 µL/min over 21 min. The concentrations of GAs in the extracts were analyzed by selected ion monitoring (SIM) using embedded calibration curves and the Xcalibur 4.0 and TraceFinder 4.1 SP1programs. The total dry masses of the samples are given in Supplementary Table 4.

Quantitative analysis of endogenous GA levels in roots (Figure 1d) was performed as described previously[24], with the following modifications. After solvent partitioning the samples were directly loaded onto C18 cartridges (Waters), and GA_{51} and GA_{34} were eluted with 50%, GA_9, GA_{24}, GA_4 and GA_{15} with 60%, and GA_{12} with 70% methanol-water, pH 3 (acetic acid). The derivatized samples were analysed by gas chromatography-mass spectrometry (GC-MS) as described elsewhere[24].

GA_{12} content in xylem sap collected from *ga1-3* scions of Col-0/*ga1-3* grafted plants (Figure 1e) was determined by ultra-performance liquid chromatography-tandem mass spectrometry (Thermo Scientific Dionex UltiMate 3000 UHPLC coupled MS Bruker EVOQ Elite) as described previously[9].

K^+ determination

Xylem sap exudation samples were mineralized in concentrated HNO_3 90 min at 160 °C. The potassium concentration in the solution was determined using inductively coupled plasma optical emission spectroscopy (ICP AES Vista MPX).

Gene expression analyses

Total RNA from hypocotyls, roots and grafted shoots were extracted with NucleoSpin RNA Plant kit (Macherey Nagel). RNA samples were treated with DNase I (Promega) and reverse transcribed into complementary DNA (cDNA) with Superscript IV reverse transcriptase (Invitrogen). The cDNA was further quantified with a SYBR Green Master mix (Roche) and gene-specific primers (listed in Supplementary Table 3) on a Lightcycler LC480 apparatus (Roche) as previously described[14]. AT4G34270 (*TIP41-LIKE*) and AT4G26410 genes were used as internal reference genes. Data are means ± s.d. of three biological samples.

Protein gel blot analyses

Protein extraction and immunoblot analyses were as previously described[14]. Total proteins from hypocotyls of 7-d-old seedlings were extracted in 2x SDS-PAGE buffer and fractionated on a 10% SDS-PAGE gel. Immunoblots were performed using a 2000-fold dilution of anti-RGA antibody (Agrisera, product AS11 1630, lot 1511). The blot was subsequently probed with anti-cdc2 (PSTAIRE) antibody (Santa Cruz Biotechnology) for loading control. Quantification of the signals was performed using ImageJ package version 1.48v (www.imageJ.nih.gov). Similar results were obtained in two independent experiments.

GUS analyses

pARSK1:GUS seedlings were fixed for 10 min in 90% (v/v) acetone on ice, washed, then infiltrated in GUS solution (500 µg/ml 5-bromo-4-chloro-3-indolyl-*ß*-D-glucuronide (X-Gluc); 50 mM sodium phosphate pH 7; 1 mM potassium ferricyanide; 1 mM potassium ferrocyanide; 10 mM EDTA; 0.01% triton X-100) for 15 min and incubated at 37°C for 8 h. Then, the GUS solution was replaced with 100% (v/v) ethanol during 6 h at room temperature and kept in 70% (v/v) ethanol at 4°C.

Statistical analysis

Statistical analyses were performed using RSudio package version 1.2.1335 (www.rstudio.com). Phenotypic characterization and qRT-PCR experiments were analyzed using Student's *t*-test ($P < 0.05$) or by analysis of variance (ANOVA). Tukey's Honest Significant Difference (HSD) was used to compare between genotypes using a significance threshold of 5%. T-test and ANOVA analyses can be found in Supplemental Dataset 1.

Reporting Summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All data generated or analyzed during this study are included in the published article and its Supplementary information.

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Author contributions

L.C., T.R., L.S.A., E.C., J.Z., D.H., N.L., M.J.P.L, T.L., J.M.D. and P.A. performed experimental work; L.C., T.R., D.H., N.L., M.J.P.L., T.L., J.M.D. and P.A. designed the experiments; M.S., M.J.P.L., T.L., J.M.D. and P.A. realized the figures and wrote the paper.

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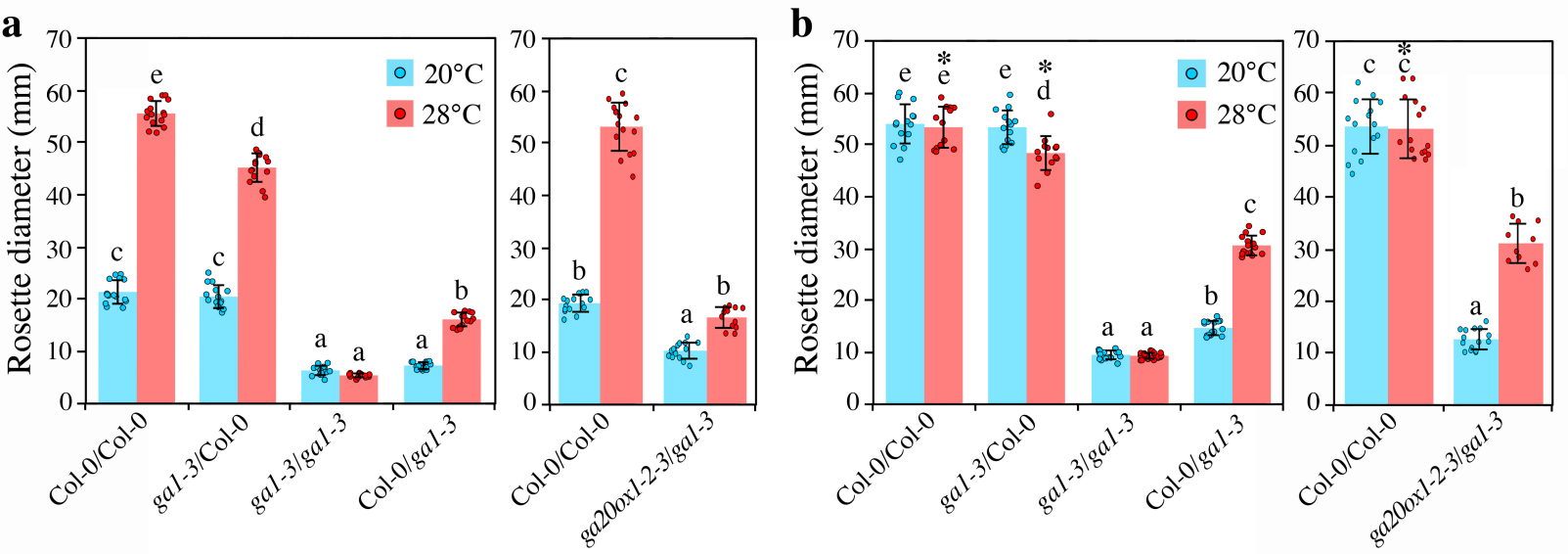
Competing interests

The authors declare no competing interests.

Figure legends

Figure 1. Root-derived GA are essential for the thermal induction of shoot growth. a-b, Feret's rosette diameter (mean ± s.d.) of Col-0/Col-0, *ga1-3*/Col-0, *ga1-3*/*ga1-3*, Col-0/*ga1-3* and *ga20ox1-2-3*/*ga1-3* grafts grown at 20°C (in blue) or 28°C (in red), two- (**a**) and three-weeks (**b**) post-grafting. The exact total number of independent rosette (*n*) for each graft combination is listed in Supplementary Table 1. Different letters denote significant differences ($p < 0.05$) using one-way ANOVA with Tukey's test for multiple comparisons. The exact *p* value for each comparison can be found in Supplementary Dataset 1. Asterisks indicate that plants had already initiated flowering before measuring their rosette diameter. **c,** Concentrations of GAs ($ng.g^{-1}$ dry weight ± s.d.) in the shoots of 12 days post-grafted plants of indicated genotypes, grown at 20°C (highlighted in blue) or 28°C (highlighted in red). Notation of genotype is rootstock/scion. In samples marked n.d. endogenous GA was not detected, but internal standard was recovered. The values are means of three biological replicates except where indicated. [#], Two biological replicates only. Different letters denote significant differences ($p < 0.05$) using one-way ANOVA with Tukey's test for multiple comparisons. The exact *p* value for each comparison can be found in Supplementary Dataset 1. **d**, Concentration of GAs ($ng.g^{-1}$ dry weight ± s.d.) in the roots of Col-0 seedlings grown for 8 days at 20°C (highlighted in blue) or for 6 days at 20°C and 2 days at 28°C (highlighted in red). Values are means of three biological replicates. There is no significant differences ($p < 0.05$) for 28°C versus 20°C by two-way Student's t-test. The exact *p* value for each comparison can be found in Supplementary Dataset 1. **e**, GA_{12} content (nM ± s.d.), K^+ content (mM ± s.d.) and GA_{12}/K^+ ratio (10^{-6} ± s.d.) in xylem exudates of Col-0/*ga1-3* grafted plants grown at 20°C for 5 weeks (highlighted in blue) or for 4 weeks at 20°C and 1 week at 28°C (highlighted in red). Notation of genotype is rootstock/scion. Values are means of three biological replicates. Asterisks indicate significant differences ($p < 0.05$) for 28°C versus 20°C by two-way Student's t-test. The exact *p* value for each comparison can be found in Supplementary Dataset 1.

Figure 2. Root-borne GA_{12} enhances hypocotyl elongation at 28°C via a DELLA-dependent mechanism. a, Schematic of the GA and thermo-responsive growth regulation. **b,** Representative 7-day-old wild-type (Col-0), *kao1 kao2 pARSK1:KAO1-RFP*, *ga20ox1-2-3 pARSK1:GA20ox1-RFP* and *pif4-101* seedlings grown in short day (SD) conditions (30 $\mu mol/m^2/s$) at 20°C or 28°C. Scale bars represent 5 mm. **c,** Hypocotyl length (mean ± s.d.) of 7-day-old Col-0, *kao1 kao2*, *kao1 kao2 pARSK1:KAO1-RFP*, *ga20ox1-2-3*, *ga20ox1-2-3 pARSK1:GA20ox1-RFP* and *pif4-101* seedlings grown at 20°C (in blue) or 28°C (in red), in SD conditions. The exact total number of independent seedling (*n*) for each genotype is listed in Supplementary Table 2. Different letters denote significant differences ($p < 0.05$) using one-way ANOVA with Tukey's test for multiple comparisons. The exact *p* value for each comparison can be found in Supplementary Dataset 1. **d,** Immunodetection of RGA protein in hypocotyls of 7-d-old Col-0, *kao1 kao2 pARSK1:KAO1-RFP*, *ga20ox1-2-3 pARSK1:GA20ox1-RFP* and *pif4-101* seedlings grown at 20°C or 28°C, in SD conditions. PSTAIRE serves as sample loading control. Values indicate RGA signal intensity relative to PSTAIRE signal intensity. Similar results were obtained in two independent experiments (shown in Supplementary Figure 4b). **e**, Expression levels (mean) of *IAA19*, *IAA29*, *YUC8* and *PRE1* in hypocotyls of wild-type (Col-0), *kao1 kao2 pARSK1:KAO1-RFP*, *ga20ox1-2-3 pARSK1:GA20ox1-RFP* and *pif4-101* seedlings grown in short-day conditions (30 $\mu mol/m^2/s$) at 20°C (in blue) or at 20°C and then transferred at 28°C for 4 h under light (in red). Error bars indicate s.d. (n = 3 biologically independent experiments). Different letters denote significant differences ($p < 0.01$) using one-way ANOVA with Tukey's test for multiple comparisons. The exact *p* value for each comparison can be found in Supplementary Dataset 1.



c

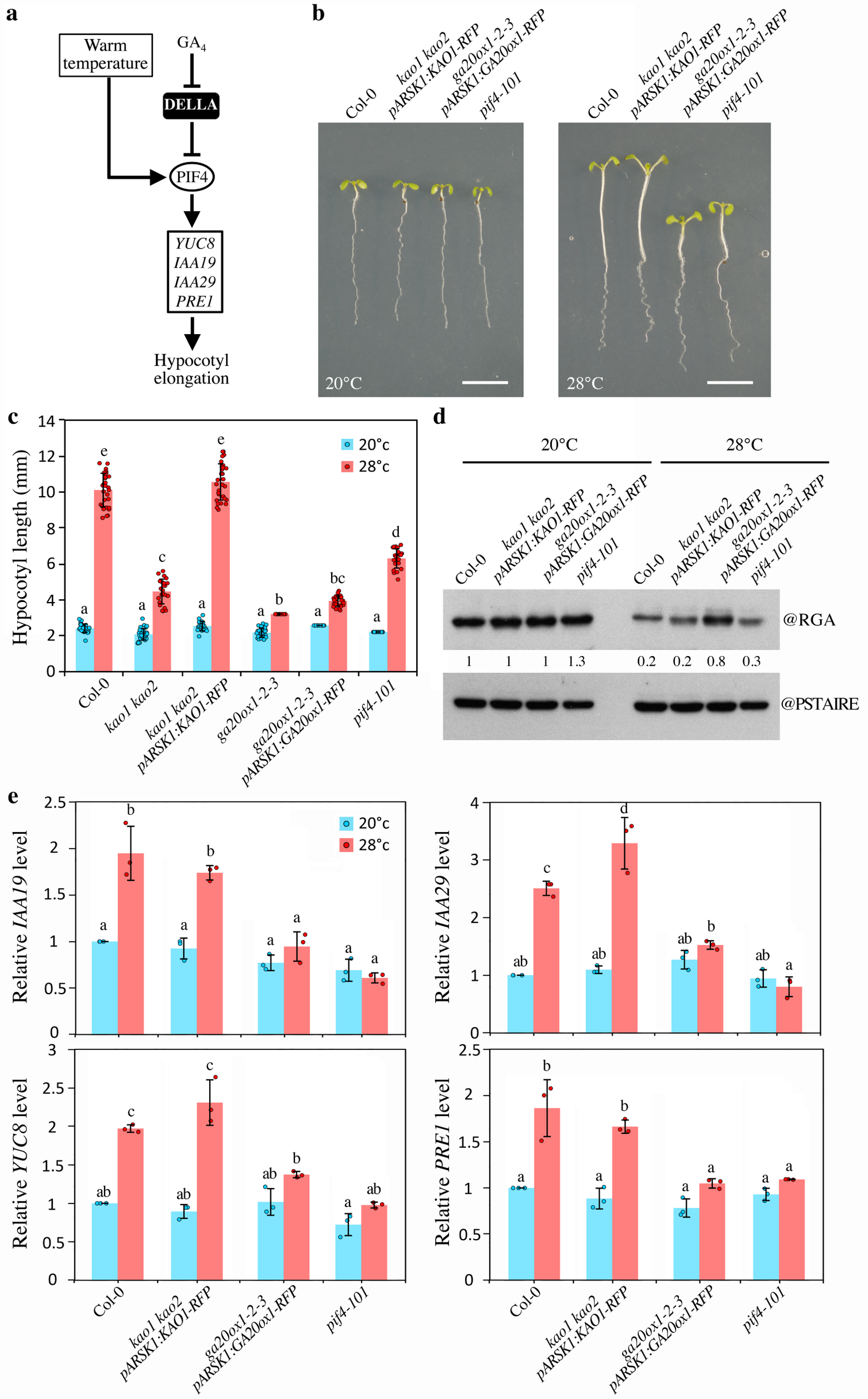
Genotype	GA_{12}	GA_{15}	GA_{24}	GA_{9}	GA_{4}	GA_{34}	GA_{51}
Col-0/Col-0	$62.6^{c} \pm 5.6$	$7.8^{bc} \pm 0.7$	$31.9^{de} \pm 3.6$	$1.7^{bc} \pm 0.2$	$4.3^{b} \pm 0.4$	$8.3^{d} \pm 0.8$	$1.3^{d} \pm 0.3$
ga1-3/*ga1-3*	n.d.	n.d.	$0^{a} \pm 0.1$	$0^{a} \pm 0.0$	$0.1^{a} \pm 0.1$	n.d.	n.d.
Col-0/*ga1-3*	$0^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$2.6^{\#} \pm 0.6$	$0.1^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
ga1-3/Col-0	$47.4^{bc} \pm 10.1$	$8.4^{bc} \pm 1.4$	$37.1^{e} \pm 4.6$	$1.4^{bc} \pm 0.2$	$4.3^{b} \pm 0.7$	$5.3^{cd} \pm 2.0$	$1.3^{cd} \pm 0.3$
Col-0/Col-0	$27.2^{b} \pm 11.1$	$9.5^{c} \pm 2.7$	$18.1^{bc} \pm 5.6$	$2.1^{c} \pm 0.5$	$7.2^{c} \pm 1.1$	$6.5^{cd} \pm 2.1$	$0.8^{b} \pm 0.1$
ga1-3/*ga1-3*	n.d.	n.d.	$0.1^{a} \pm 0.0$	$0^{a} \pm 0.0$	$0.1^{a} \pm 0.1$	n.d.	n.d.
Col-0/*ga1-3*	$26.1^{b} \pm 16.7$	$4.9^{b} \pm 0.9$	$2.2^{b} \pm 2.5$	$0.8^{ab} \pm 0.8$	$4.4^{\#} \pm 0.1$	$1.5^{ab} \pm 0.2$	$0.2^{a} \pm 0.1$
ga1-3/Col-0	$24.2^{b} \pm 4.7$	$7.1^{bc} \pm 3.3$	$26.6^{cd} \pm 4.7$	$1.9^{c} \pm 0.4$	$5.7^{bc} \pm 0.8$	$4.6^{bc} \pm 2.1$	$0.8^{bc} \pm 0.1$

d

Col-0	6.3 ± 1.3	0.5 ± 0.1	0.5 ± 0.2	0.1 ± 0.0	1.0 ± 0.3	0.6 ± 0.0	0.6 ± 0.1
Col-0	6.9 ± 2.0	0.8 ± 0.3	0.3 ± 0.2	0.2 ± 0.1	0.7 ± 0.3	0.6 ± 0.0	0.5 ± 0.2

e

	GA_{12}	K^{+}	GA_{12}/K^{+}
Col-0/*ga1-3*	4.1 ± 0.8	19.5 ± 0.4	0.21 ± 0.04
Col-0/*ga1-3*	$7.6^{*} \pm 0.9$	19.7 ± 0.8	$0.39^{*} \pm 0.06$


a
Warm temperature
GA4
DELLA
PIF4
YUC8
IAA19
IAA29
PRE1
Hypocotyl elongation
b
Col-0
kao1 kao2
pARSK1:KAO1-RFP
ga20ox1-2-3
pARSK1:GA20ox1-RFP
pif4-101
20°C
28°C
c
Hypocotyl length (mm)
20°c
28°c
d
20°C
28°C
@RGA
@PSTAIRE
1 1 1 1.3 0.2 0.2 0.8 0.3
e
Relative IAA19 level
Relative IAA29 level
Relative YUC8 level
Relative PRE1 level

Root-derived GA_{12} contributes to temperature-induced shoot growth in *Arabidopsis*

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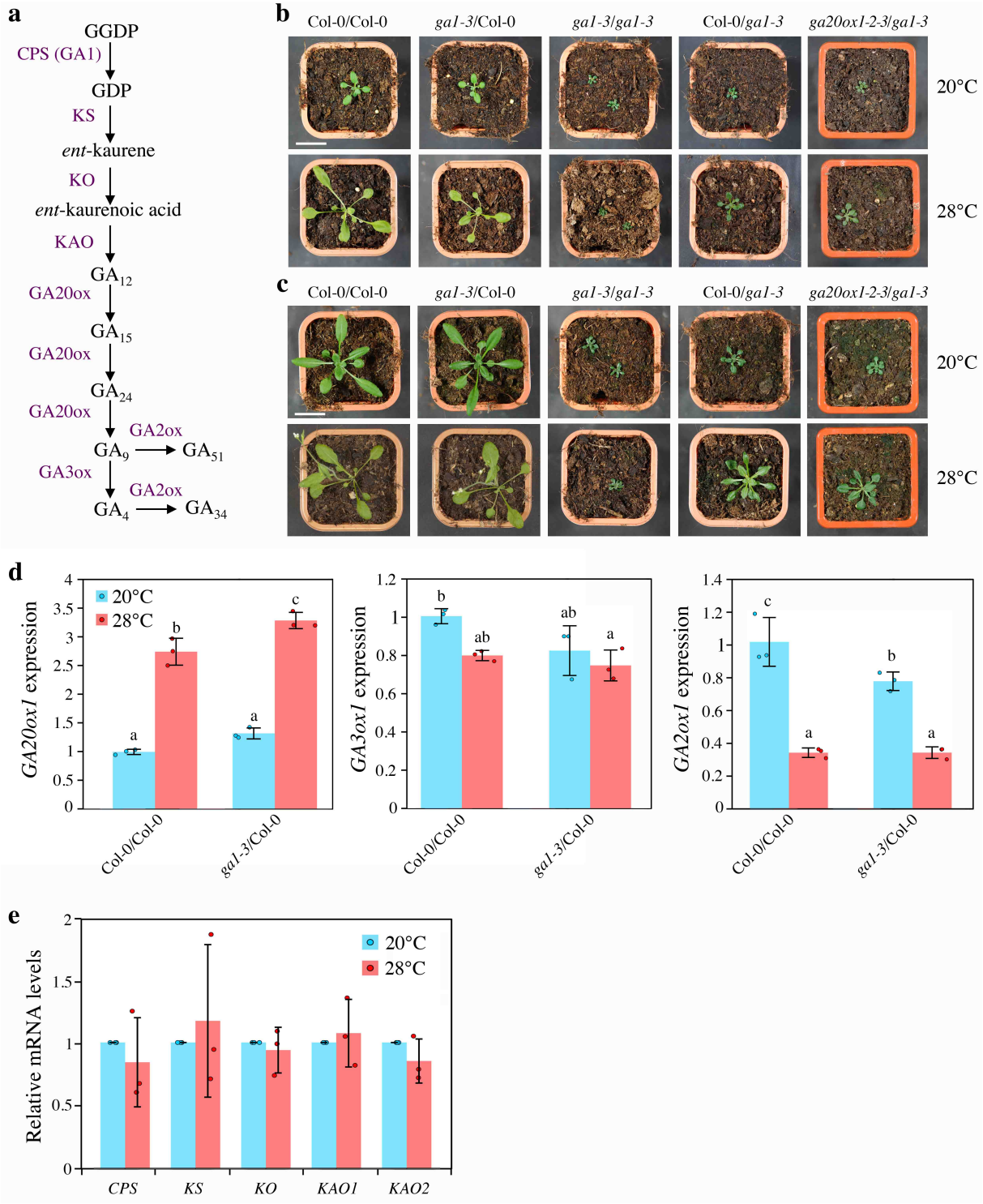
Supplementary Discussion

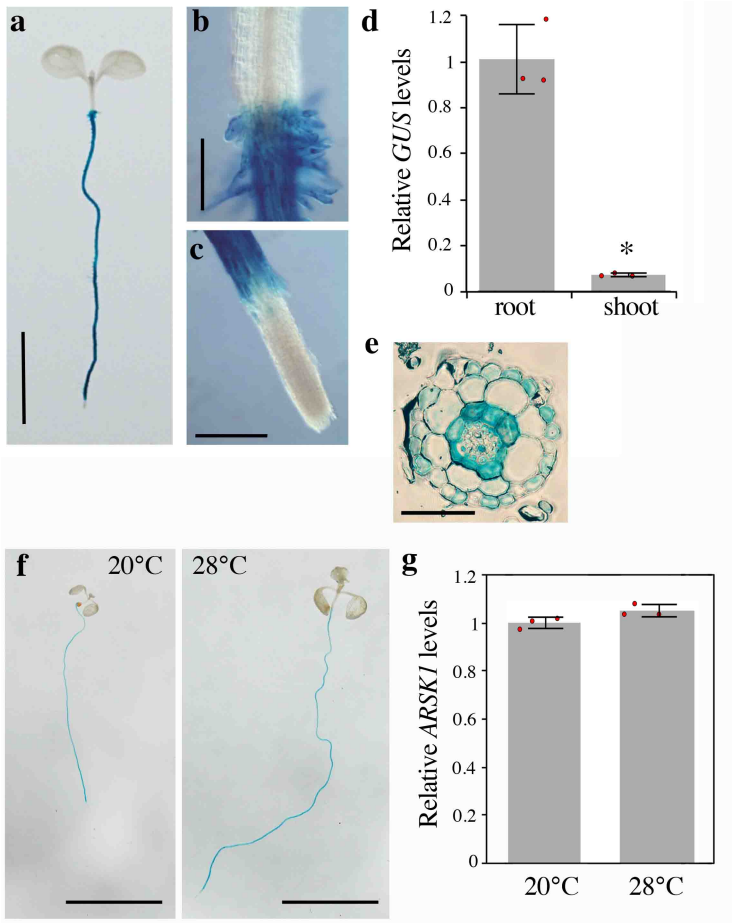
Here, we develop few points that were only shortly discussed in the *Brief Communication* article. In our work, we have shown by using grafting (Col-0/*ga1-3* graft combination; Fig. 1) and transgenic plants (*kao1 kao2 pARSK1:KAO1-RFP*; Fig. 2), which both produce GA solely in the root, that root-derived GA_{12} contributes substantially to temperature-induced shoot growth. We may regret that these experiments were performed at different developmental stages (at adult and seedling stage, respectively), imposed by the leakiness of the *ARSK1* promoter activity in rosette leaves (Fig. S3). Nevertheless, the results obtained with the grafts and the transgenic seedlings clearly support the conclusion that root-borne GA_{12} mediates thermo-responsive shoot growth. Another issue is that there is no straightforward experiment to evaluate the physiological relevance of the root-to-shoot transport of GA_{12} at elevated temperature, in wild-type plants. The concentration of GA_{12} in xylem sap (Fig. 1e) was determined in exudates collected from scions of Col-0/*ga1-3* grafts and not from wild-type plants to ensure that GA_{12} only derives from root, and not the combination of both root-derived and shoot-synthetized GA_{12}. Although we cannot exclude that lack of GA-synthesis in *ga1-3* scions (in Col-0/*ga1-3* grafts) might influence the transport of GA_{12} from source (Col-0 root) to sink (*ga1-3* shoot) organs, we found that wild-type rosettes of Col-0 self-grafts grown at 28°C were larger than those grafted onto *ga1-3* rootstocks (Fig. 1a). Consistent with this result, the hypocotyl length of rootless wild-type seedlings (even though they produce GA in their hypocotyl) was smaller than that of intact wild-type seedlings at 28°C (Fig. S4a). Hence, these results indicate that root-borne GA_{12} contributes substantially to enhanced shoot growth at elevated ambient temperature, in *Arabidopsis*. This adaptive mechanism may have essential role in the development of field grown plants exposed to daily ambient temperature fluctuations.

Supplementary Figures

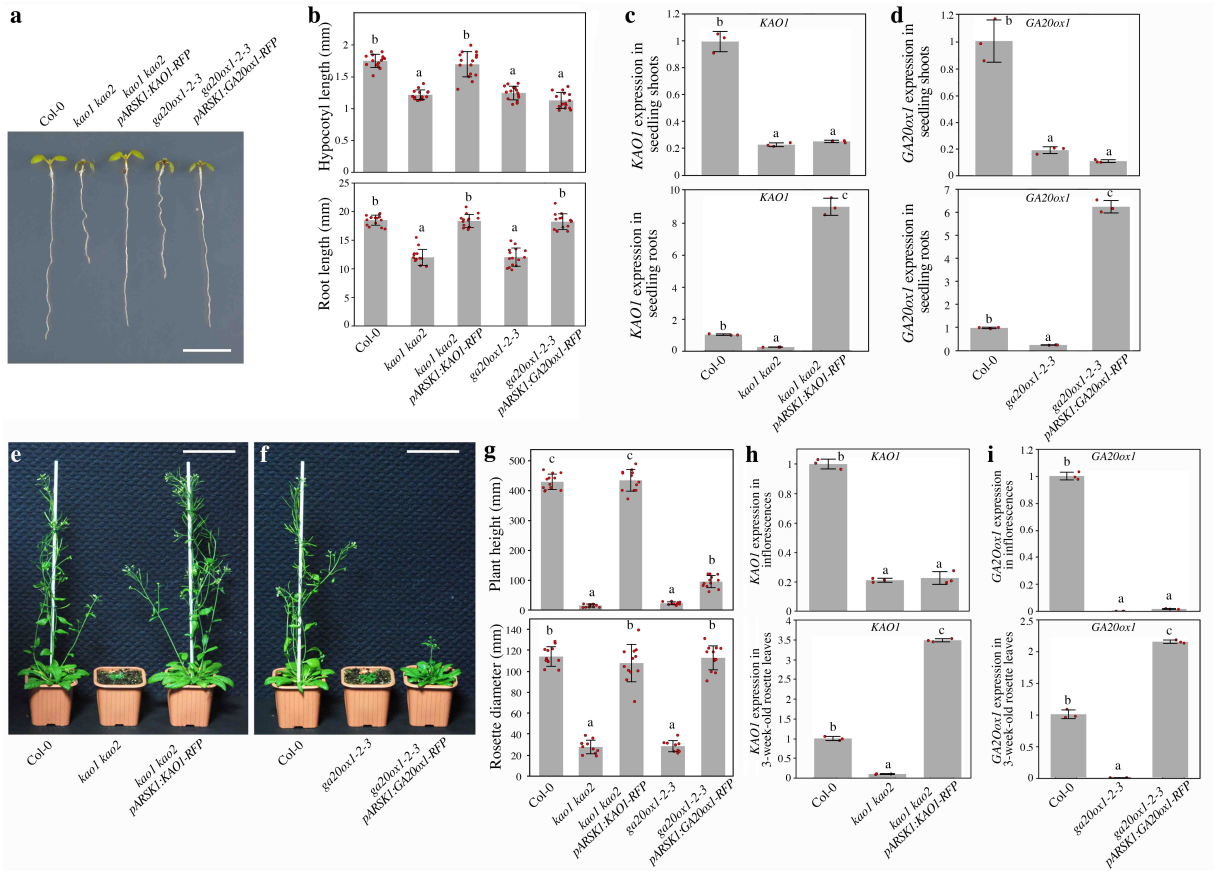


Supplementary Figure 1. Warm temperature modulates growth and GA metabolism. a, Schematic of the GA biosynthetic pathway. Biosynthetic enzymes are indicated in purple. GGDP, geranylgeranyl diphosphate; CDP, *ent*-copalyl diphosphate; CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase; GA20ox, GA 20-oxidase; GA3ox, GA 3-oxidase; GA2ox, GA 2-oxidase. **b-c,** Representative rosette phenotype of Col-0/Col-0, *ga1-3*/Col-0, *ga1-3*/*ga1-3,* Col-0/*ga1-3* and *ga20ox1-2-3*/*ga1-3* grafts grown in continuous light (75 µmol/m^2/s) at 20°C or 28°C, two- (**b**) and three-weeks (**c**) post-grafting. Statistical data are shown in Figure 1a,b. Genotype notation is rootstock/grafted scion. Scale bar represents 2 cm. **d,** Expression levels (mean) of *GA20ox1*, *GA3ox1* and *GA2ox1* in wild-type scions of

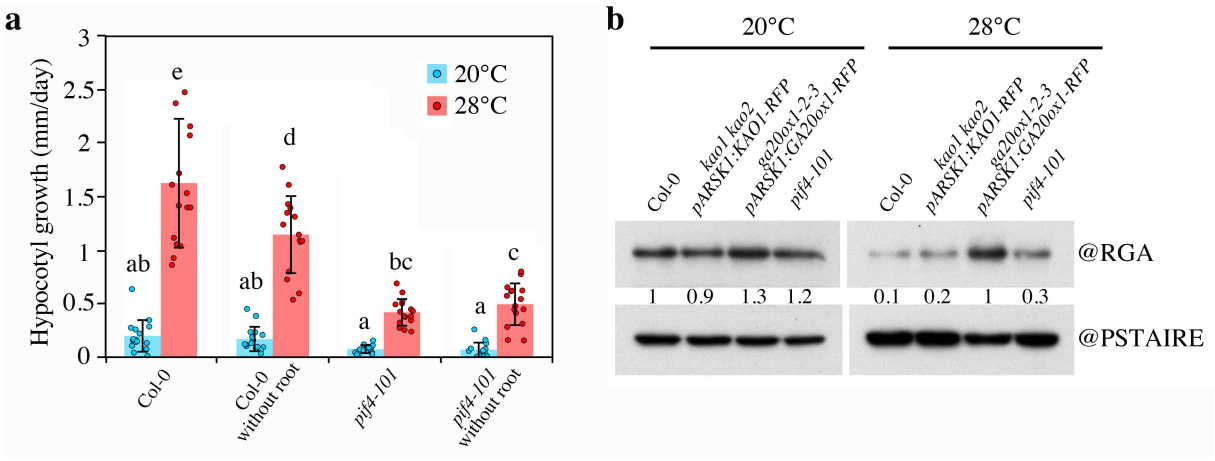
Col-0/Col-0 and *ga1-3*/Col-0 grafts grown in continuous light at 20°C (in blue) or 28°C (in red), 12 days post-grafting. Error bars indicate s.d. (n= 3 biologically independent experiments). Different letters denote significant differences ($p < 0.05$) using one-way ANOVA with Tukey's test for multiple comparisons. The exact p value for each comparison can be found in Supplementary Dataset 1. **e,** Expression levels (mean) of *CPS*, *KS*, *KO*, *KAO1* and *KAO2* in roots of 8-d-old Col-0 seedlings grown in continuous light at 20°C (in blue) or at 20°C for 6 days and then transferred at 28°C for 2 days (in red). Error bars indicate s.d. (n= 3 biologically independent experiments). There is no significant differences ($p < 0.05$) for 28°C versus 20°C by one-way Student's t-test. The exact p value for each comparison can be found in Supplementary Dataset 1.



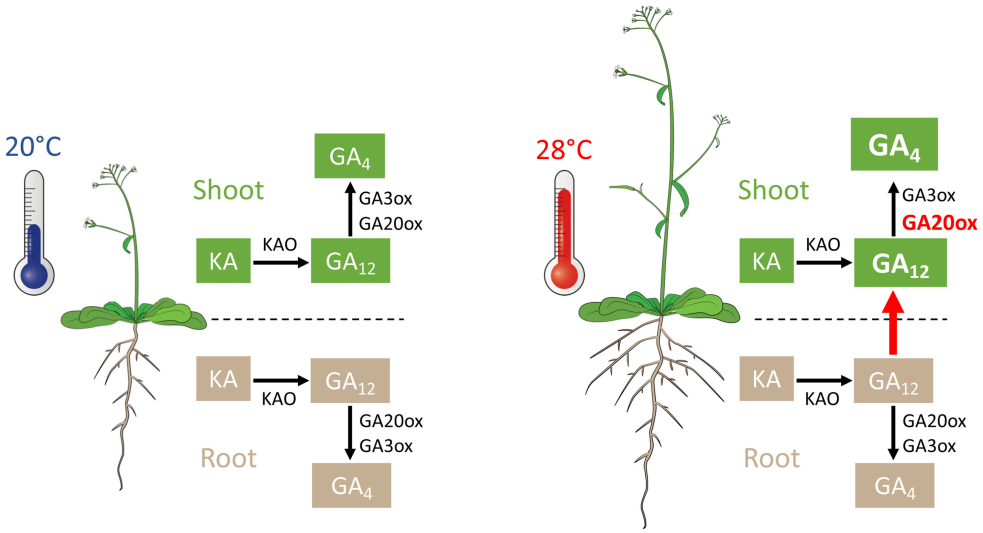
Supplementary Figure 2. *ARSK1* promoter drives a root specific expression. a-c, *pARSK1:GUS* expression in 7-d-old seedling (**a**), root-hypocotyl junction (**b**), root tip (**c**). **d,** Relative *GUS* levels (mean) in root and shoot of 7-d-old *pARSK1:GUS* seedlings. Error bars indicate s.d. (n= 3 biologically independent experiments). Asterisk indicates significant difference (p = 0.0004) for shoot versus root expression by one-way Student's t-test. **e,** *pARSK1:GUS* expression in 7-d-old seedling root; transversal cut in differentiation zone of the root. Similar results were obtained in three independent experiments. **f,** *pARSK1:GUS* expression in 7-d-old seedling grown at 20°C or at 28°C. **g**, Expression level (mean) of *ARSK1* in 7-d-old Col-0 seedling roots grown at 20°C (blue) or at 28°C (red). Error bars indicate s.d. (n= 3 biologically independent experiments). There is no significant differences (p = 0.067) for 28°C versus 20°C by one-way Student's t-test. Scale bars represent 5 mm (**a**), 0.3 mm (**b**), 0.5 mm (**c**), 0.1 mm (**e**) and 1 cm (**f**).



Supplementary Figure 3. *pARSK1:KAO1-RFP* expression rescues the growth of *kao1 kao2* mutant. a, Representative growth phenotype of 7-d-old wild-type (Col-0), *kao1 kao2*, *kao1 kao2 pARSK1:KAO1-RFP*, *ga20ox1-2-3* and *ga20ox1-2-3 pARSK1:GA20ox1-RFP* seedlings grown at 22°C in long day (LD) conditions (100 µmol/m^2/s). **b**, Hypocotyl and root lengths (mean ± s.d.; n = 15) of Col-0, *kao1 kao2*, *kao1 kao2 pARSK1:KAO1-RFP*, *ga20ox1-2-3* and *ga20ox1-2-3 pARSK1:GA20ox1-RFP* seedlings grown in LD conditions. **c,** Relative *KAO1* expression (mean) in root and shoot of Col-0, *kao1 kao2* and *kao1 kao2 pARSK1:KAO1-RFP* seedlings grown in LD conditions. Error bars indicate s.d. (n= 3 biologically independent experiments). **d,** Relative *GA20ox1* expression (mean) in root and shoot of Col-0, *ga20ox1-2-3* and *ga20ox1-2-3 pARSK1:GA20ox1-RFP* seedlings grown in LD conditions. Error bars indicate s.d. (n= 3 biologically independent experiments). **e**, Representative growth phenotype of 6-week old Col-0, *kao1 kao2* and *kao1 kao2 pARSK1:KAO1-RFP* plants grown in LD conditions. **f**, Representative growth phenotype of 6-week old Col-0, *ga20ox1-2-3* and *ga20ox1-2-3 pARSK1:GA20ox1-RFP* plants grown in LD conditions. **g**, Feret's rosette diameter and plant height (mean ± s.d.; n ≥ 15) of Col-0, *kao1 kao2*, *kao1 kao2 pARSK1:KAO1-RFP*, *ga20ox1-2-3* and *ga20ox1-2-3 pARSK1:GA20ox1-RFP* plants grown in LD conditions. The exact total number of independent plant (*n*) for each genotype is listed in Supplementary Table 5. **h**, Relative *KAO1* expression (mean) in rosette and inflorescence of Col-0, *kao1 kao2* and *kao1 kao2 pARSK1:KAO1-RFP* plants grown in LD conditions. Error bars indicate s.d. (n= 3 biologically independent experiments). **i**, Relative *GA20ox1* expression (mean) in rosette and inflorescence of Col-0, *ga20ox1-2-3* and *ga20ox1-2-3 pARSK1:GA20ox1-RFP* plants grown in LD conditions. Error bars indicate s.d. (n= 3 biologically independent experiments). Different letters denote significant differences ($p < 0.05$) using one-way ANOVA with Tukey's test for multiple comparisons (**b,c,d,g,h** and **i**). The exact *p* value for each comparison can be found in Supplementary Dataset 1. Scale bar represents 5 mm (**a**) and 7 cm (**e** and **f**).



Supplementary Figure 4. Elevated temperature enhances hypocotyl growth and GA signaling. a, Hypocotyl growth per day (mean ± s.d.; n = 16) of Col-0 and *pif4-101* seedlings with and without roots. The seedlings were grown in short day conditions at 20°C for 5 days and then transferred at 20°C (in blue) or 28°C (in red) for 3 days. Roots were cut off for one set of the seedlings before the transfer. Hypocotyl length was measured between day 5 and day 8. Different letters denote significant differences ($p < 0.05$) using one-way ANOVA with Tukey's test for multiple comparisons. The exact p value for each comparison can be found in Supplementary Dataset 1. **b,** Root-borne GA_{12} enhances RGA protein degradation in shoots at 28°C. Immunodetection of RGA protein in hypocotyls of 7-d-old Col-0, *kao1 kao2 pARSK1:KAO1-RFP*, *ga20ox1-2-3 pARSK1:GA20ox1-RFP* and *pif4-101* seedlings grown at 20°C or 28°C, in short day conditions. PSTAIRE serves as sample loading control. Values indicate RGA signal intensity relative to PSTAIRE signal intensity. Similar results were obtained in two independent experiments (replicate shown in Figure 2d).



Supplementary Figure 5. Root-derived GA_{12} is required for temperature-induced shoot growth. At 20°C, the growth of root and shoot is regulated by GA_4 in a root- and shoot-autonomous manner. At 28°C, warm temperature activates root-to-shoot transport of GA_{12}, whose action combined with shoot-synthetized GA_{12}, promotes shoot growth, after metabolic conversion into GA_4. KA, *ent*-kaurenoic acid; KAO, *ent*-kaurenoic acid oxidase; GA20ox, GA 20-oxidase; GA3ox, GA 3-oxidase.