Light perception by the root is essential for gibberellin-induced primary-root elongation in tomato

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Summary

- The promoting effect of gibberellin (GA) on primary-root elongation was demonstrated in several species, including Arabidopsis (*Arabidopsis thaliana*). However, in some other species its effect is less clear.
- We have used the tomato (*Solanum lycopersicum*) GA-deficient mutants *gib-1* and *ga 20-oxidase* (*ga20ox1*) to study the role of GA in primary-root elongation and examined the involvement of light in this response.
- Application of GA to *gib-1* following germination, promoted primary-root elongation, but later, when roots were deeper in the dark, GA had no effect. RNA-seq analysis revealed a typical GA transcriptional response in dark-grown roots, but the output toward cell-expansion was blocked. When dark-grown roots were illuminated deep in the ground, the hormone promoted their elongation. We demonstrated that direct red light to the root is essential for GA-induced elongation. A similar light dependent GA-elongation response was found in wheat (*Triticum aestivum*) roots, but not in Arabidopsis.
- We suggest that in tomato GA promotes root elongation following germination when roots are exposed to low light intensity underground, and this contributes to seedling establishment. As root grow deeper into the soil, the insensitivity to GA may be important for continues root growth under fluctuating water availability, as water deficiency suppresses GA accumulation.

Key Words: gibberellin, *gib-1*, red light, primary root, tomato

Introduction

The plant hormone gibberellin (GA) regulates numerous developmental processes, including organ elongation (Yamaguchi, 2008; Hedden & Sponsel, 2015). Bioactive GAs bind to the GIBBERELLIN-INSENSITIVE DWARF1 (GID1) receptor, leading to the interaction of GID1s with the master growth suppressor, the DELLA protein (Locascio *et al*., 2013). This facilitates the degradation of DELLA by the 26S proteasome, a process mediated by the F-box SLEEPY1 (SLY1). DELLA degradation leads to transcriptional reprogramming and activation of GA responses, including elongation (Hauvermale *et al*., 2012).

The role of GA in primary root elongation has been studied mainly in Arabidopsis (*Arabidopsis thaliana*) and its central role was demonstrated (Ubeda- Tomas *et al*., 2008, 2009; Achard *et al*., 2009; Rizza *et al*., 2017). It was shown that GA regulates both cell division in the root meristem (Shtin *et al*., 2022), and cell elongation in the root elongation zone (Ubeda-Tomas *et al*., 2008). The Arabidopsis GA deficient mutant *ga1-3* has shorter primary root, and normal elongation can be restored by GA application or loss of DELLA activity (Fu & Harberd, 2003). Using the GA perception sensor nlsGPS1, Rizza *et al*. (2017) showed that active endogenous GA accumulates in the root elongation zone. Ubeda-Tomas *et al*. (2008) showed that inhibition of GA signaling specifically in the endodermis of Arabidopsis roots is sufficient to disrupt root elongation, indicating that the endodermis is the key site for GA action in the regulation of root elongation. This conclusion was supported by Shani *et al*., (2013) that found accumulation of exogenous bioactive tagged-GAs in the endodermis of the root elongation zone. While root elongation of *ga1-3* was induced by low concentration of GA, this treatment had no effect on shoot growth (Arizumii *et al*., 2008), suggesting that roots are very sensitive to GA. Moreover, it was shown that very low concentrations of GA are required to saturate root elongation response (Tanimoto & Hirano, 2013).

The importance of GA in the regulation of root elongation in other plant species is less clear, with numerous conflicting reports (Torrey, 1976; Feldman, 1984; Phinney, 1985;

Tanimoto, 2005; Tanimoto & Hirano, 2013; Ramon *et al*., 2020). While Whaley and Kephart (1957) show that GA application to maize (*Zea mays*) promotes root elongation, Svensson (1972) reported that the hormone has no effect. In Medicago (*Medicago truncatula*), GA treatment suppressed, and the GA-biosynthesis inhibitor paclobutrazol (PAC) increased primary root elongation (Fonouni-Farde *et al*., 2019), suggesting that GA inhibits Medicago root elongation. Gou *et al*. (2010) showed in *Populus* trees that reduced GA activity promotes lateral root elongations and has no effect on primary-root length. Butcher & Street (1960) found that elevating GA concentrations in tomato (*Solanum lycopersicum*), progressively promote root elongation, whereas Tognoni *et al*. (1967), showed that application of GA to tomato plants inhibits root elongation in a concentration-dependent manner.

In a previous study we showed that PAC inhibited primary root elongation in tomato seedlings (Ramon *et al*., 2020). Application of low GA concentrations to the PAC-treated plants slightly promoted root elongation, but higher concentrations, inhibited elongation, exhibiting a bell-shaped curve of response. In contrast, in the shoots, GA strongly promoted elongation and the effect was saturated only at very high concentrations and exhibited a typical saturated-type curve of response. We also found that application of GA to the wild-type (WT) M82 tomato plants, slightly inhibited primary root elongation, suggesting that in WT tomato roots, GA levels are saturated. Genetic evidence, support a role for GA in tomato primary-root elongation; the extreme tomato dwarf mutant *gid1*^{TRI}, which lack the activity of all GA receptors (Illouz-Eliaze *et al*., 2019) and the strong GA deficient mutant *gib-1* (Koornneef *et al*., 1990; Bensen & Zeevaart, 1990) have shorter primary roots (Barlow *et al*., 1991; Ramon *et al*., 2020).

Most studies on GA and root elongation in Arabidopsis were performed with light-grown seedlings on agar plates (Arizumii *et al*., 2008; Ubeda-Tomas *et al*., 2008; Shani *et al*., 2013; Rizza *et al*., 2017). Roots however, normally grow in the soil, in the dark and escape from light by negative phototropism (Zhang *et al*., 2013). Using the D-root system, Silva-Navas *et al*. (2015) showed that roots respond differently to many stimuli under light or dark conditions. Here we have studied how GA affect tomato root elongation, using the *gib-1* and *ga20ox1* mutants. We found that direct red light to the root is essential for GA-

induced elongation. The results suggest that penetration of light to the upper layers of the soil drives the effect of GA on primary root elongation, but later, when the roots grow dipper into the soil, to the dark, GA has no more effect. This phenomenon is probably conserved in some, but not all plant species, as similar light dependent GA-induced root elongation was found in wheat, but not in Arabidopsis.

Materials and Methods

Plant materials, seed germination, growth conditions and hormone treatments

Tomato cv. M82 (sp/sp), *gib-1* (Koornneef *et al*., 1990; Bensen & Zeevaart, 1990) and *ga20 oxidase1* (*ga20ox1*, Shohat *et al*., 2021) in M82 background were used this study. Plants/seedlings were grown in vermiculite in a growth room set to a photoperiod of 12 h/12 h, day/night, light intensity of 150µmol m^{-2} s^{-1} and 25°C. 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 tomato seeds were sterilized with ethanol 70% for 2 minutes, and then with sodium hypochlorite 3% for 10 minutes. The seeds washed thoroughly by sterilized double distillated water (DDW). For germination of the strong GA deficient mutant *gib-1*, embryos were rescued as described by Illouz-Eliaz et al., (2019) and for germination of *ga 20-oxidase1* (*ga20ox1*, Shohat *et al*., 2021) mutant seeds were scarified.

Arabidopsis Columbia (Col-0) and *ga1-3* mutant in Col-0 background were also used in this study. The seeds were sterilized with 6% sodium hypochlorite and 4% hydrochloric acid for 2 h and then placed in the dark at 4°C for 48 h. Arabidopsis plants were grown in a growth room, under a controlled temperature (22°C) and long photoperiod (16-h light/8-h dark) provided by cool-white lamps (150 µmol m^{-2} s^{-1}) in pots with vermiculite. *ga1-3* seeds were soaked with 1µM GA_3 (Sigma-Aldrich, St. Louis, USA) shake in the dark for 7 days and then washed thoroughly with sterilized DDW several times to remove all GA_3 residues.

Wheat cv. ‘Abu Fashi’ (GA-responsive line, Amram *et al*., 2015) seeds were germinated in rolled papers in flasks filled with water (Watt et al., 2013). The seeds were germinated

in the dark at 16°C. Following germination, the rolled papers were transferred to new flasks filled with water (mock), 5 mg/L PAC or PAC and 10μM GA_3. Half of the papers for each treatment were covered with aluminum foil to block the light (Supporting information Fig. S1). The papers were placed in a growth room with a photoperiod of 16h/8h, light/dark, a light intensity of 150μmol m^{-2} s^{-1} provided by cool-white lamps, and a temperature of 22°C.

GA_3, and PAC) were applied by spraying, drenching or incorporated into the growth medium.

Root elongation assay in Petri dishes (agar plates)

The tomato seeds/embryos were grown in Petri dishes filled with 4.4 g/L Murashige & Skoog (MS) medium (Sigma-Aldrich), 8 g/L plant agar (Duchefa Biochemie, RV Haarlem, The Netherlands), pH adjusted to 5.7 with NaOH. Arabidopsis seeds were placed on 1\2 MS plates (2.2 g/L) containing 5 g/L sucrose. The plates were placed vertically under continuous white light (100μmol m^{-2} s^{-1}, provided by cool-white lamps). For dark condition the plates were covered with aluminum foil after germination. In some experiments, following germination, the location of the root tip was marked on the plate and after 5-7 days the plates were photographed (Canon PowerShot SX60 HS, Melville, NY, USA) and primary root length, from the mark to the root tip, was measured using ImageJ software (http://rsb.info.nih.gov/ij).

D-root and D-shoot growth system

To grow seedlings on agar plates (see above) and keep the root in the dark and the shoot in the light, we have used a modified Dark-Root (D-root) growth system (Silva-Navas et al., 2015; Supporting information Fig. S2). We used black plastic comb that was fitted into the plates separating the shoot and the root system and covered the lower part (root part) of the plate with aluminum foil. To keep the shoot in the dark while exposing the root to the light we used Dark-shoot (D-shoot) growth system. We used the same system as for D-root, but covered the upper part (shoot part) of the plate with aluminum foil. The plates were placed vertically under white light (100μmol m^{-2} s^{-1}, provided by cool-white lamps).

Light treatments

The tomato *ga20ox1* mutant seeds were scarified and then placed on agar D-shoot plates and transferred into a growth chamber (Percival, Perry, Iowa, USA), at 25°C, under the specified constant light conditions: blue light (19μmol m^{-2} s^{-1}), red light (8μmol m^{-2} s^{-1}), and far-red light (17μmol m^{-2} s^{-1}). Light for all experiments was provided by light-emitting diodes (LED30-HL1). The wavelengths and light intensity were measured by LI-180 Spectrometer (LI-COR Biosciences, Lincoln, NE, USA) (Supporting information methods S3).

Molecular Cloning/Constructs and Plant Transformation.

To generate p35S:GID1a construct, GID1a (Illouz-Eliaz *et al*., 2019) cDNA was inserted into the pART7 vector downstream to the 35S promoter into the KpnI and XbaI sites. The construct was subcloned into the pART27 binary vector by NotI site and introduced into *Agrobacterium tumefaciens* strain GV3101 by electroporation. The construct was transferred into M82 cotyledons using transformation and regeneration methods described before (McCormick, 1991). Kanamycin-resistant to plants were grown and at least seven independent transgenic lines were selected and self-pollinated to generate homozygous transgenic lines.

Library Constructions and RNAseq analysis

Total RNA was extracted from root tips and leaves using a RNeasy Plant Mini Kit (Qiagen). RNA-seq libraries were prepared at the Crown Genomics institute of the Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, Israel. Libraries were prepared using the INCPM-mRNA-seq protocol. Briefly, the polyA fraction (mRNA) was purified from 500ng of total input RNA followed by fragmentation and the generation of double-stranded cDNA. After Agencourt Ampure XP beads cleanup (Beckman Coulter), end repair, a base addition, adapter ligation, and PCR amplification steps were performed. Libraries were quantified by Qubit (Thermo Fisher Scientific, Waltham, MA 15 USA) and TapeStation (Agilent, Santa Clara, CA, USA). Sequencing was done on a NextSeq instrument (Illumina, San Diego, CA, USA) using a

HO 75 cycles kit, allocating approximately 18 million reads per sample (single read sequencing).

Sequence data analysis

Stretches of Poly-A/T and Illumina adapters were trimmed from the reads using cutadapt (Martin *et al*., 2011); resulting reads shorter than 30bp were discarded. Reads were mapped to the Solanum lycopersicum reference genome; release SL3.00 using STAR *(*Dobin *et al.,* 2013*)* (with End To End option and out Filter Mismatch Nover Lmax set to 0.04). Annotation file was downloaded from SolGenomics, ITAG release 3.2. Expression levels for each gene were quantified using htseq-count (Andres et al., 2014), using the gtf file above (using union mode). Differentially expression analysis was performed using DESeq2 (Love *et al*., 2014) with the betaPrior, cooksCutoff and independent Filtering parameters set to False. Raw P values were adjusted for multiple testing using the procedure of Benjamini and Hochberg (Bejamini & Hochberg, 1995). The RNA-Seq data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (Edgar *et al*., 2002):

https://dataview.ncbi.nlm.nih.gov/object/PRJNA1002623?reviewer=m2cghdn5fcve5npf4qpqd8es63.

RNA Extraction, cDNA Synthesis and RT-qPCR Analysis

Total RNA was extracted with a RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). 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 was performed using an Absolute Blue qPCR SYBR Green ROX Mix kit (AB-4162/B; Thermo Fisher Scientific, Waltham, MA, USA). Reactions were performed using a Rotor-Gene 6000 Cycler (Corbett Research, Sydney, Australia). A standard curve was obtained using dilutions of the cDNA sample. The expression was quantified using the Rotor-Gene's 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 target-gene-to-*ACTIN* ratio was then averaged.

Confocal Imaging and cell length measurement

Root tips were stained with Propidium iodide (PI) solution (0.01μg ml^{-1}). Laser beam of 552 nm were used for excitation of PI. The samples were placed on a glass slide with a drop of water and sealed with coverslip. Fluorescence images were acquired using Leica SP8 confocal microscope (Boston, MA, USA). The cells lengths were measured by ImageJ software (http://rsb.info.nih.gov/ij).

Statistical analysis

All assays were conducted with three or more biological replicates and analyzed 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), Student's t-test (for one comparison) and Dunnett's test (for comparison against control) ($P < 0.05$). For assays without normal distribution Mann-Whitney -Wilcoxon test was conducted. The experiments were performed three times.

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; GA20ox1, Solyc03g006880; GA20ox3, Solyc11g072310; GID1a, Solyc01g098390.

Results

We previously found that GA levels in WT tomato roots are saturated (Ramon et al., 2020), therefore, we have used the strong tomato GA-deficient mutant *gib-1* (Bensen & Zeevaart, 1990) to study the effect of GA on primary-root elongation. *gib-1* exhibited very low percentage of germination even after seed scarification, thus, we had to rescue *gib-1* embryos. *gib-1* and WT (M82) embryos were grown on vermiculite in pots and after

producing four expanded leaves, the length of the primary roots was measured. Similar to *gid1*^{TRI} (Ramon *et al*., 2020), primary roots of *gib-1* were shorter than those of WT (Fig. 1a). Microscopic analysis revealed that epidermal cells of *gib-1* primary roots are shorter than those of WT roots (Supporting information Fig. S4). We then examined the effect of GA application on *gib-1* primary-root elongation. Two weeks-old *gib-1* seedlings, grown in vermiculite, were treated twice a week for one month with 10μM GA_3 and then the length of leaves, stems and primary roots was measured. While the hormone strongly promoted shoot growth, it had no effect on primary root elongation (Fig. 1b,c). To exclude the possibility that the limiting factor for root elongation in *gib-1* is shortage of assimilates (Stafen *et al*., 2022), due to the very small leaf area and not a direct effect of GA, we measured the elongation rate of the primary roots of *gib-1* and WT immediately after embryo rescue, on agar plates. In this early developmental stage, differences in foliage area between WT and *gib-1* seedlings are neglected (Supporting information Fig. S5). We found that elongation rate of *gib-1* roots was ca. three times lower than that of WT (Fig. 1d), suggesting a direct effect of GA on root elongation.

Since long-term treatment with GA had no effect on *gib-1* root elongation, we hypothesized that GA is not active in the roots deeper in the growth medium. We therefore, examined GA transcriptional responses in the roots. To this end, we grew WT and *gib-1* for two weeks on vermiculite, and then treated *gib-1* plants with 10μM GA_3. A previous study in Arabidopsis showed that after a short period from the GA treatment (1–3 h), growth-related genes were only slightly affected by the hormone, but after 24 h, these genes exhibited clear upregulation (Park et al., 2017). We therefore collected, for RNAseq analysis, shoots and primary root tips, including the elongation zone, 24 h after the GA treatment. We first compared non-treated *gib-1* and WT shoot and roots. In shoots we found ca. 3000 differentially expressed genes (DEGs) between the two genotypes and in roots approximately 1900 DEG (Fig. 2a, Supporting information Fig. S6a, Supporting information Dataset S1). *gib-1* roots exhibited clear transcriptional responses to GA application (Fig. 2b, Supporting information Fig. S6b, Supporting information Dataset S1), including a typical negative feedback responses of *GA20ox1* and *GA20ox3*, whose expression was strongly inhibited both in *gib-1* shoots and roots by GA application (Fig. 2c,). We also analyzed the expression of cell-expansion genes (Park et al., 2017).

While many of these genes were upregulated by the GA treatment in *gib-1* shoots, in the roots they were hardly affected (Fig. 2d). We previously speculated that in tomato, root elongation is less responsive to GA than the shoot due to lower expression of the dominant GA receptor *GID1a* (Ramon et al., 2020), whose activity is responsible for the strong stem-elongation response to GA (Illouz-Eliaz *et al*., 2019). *GID1a* exhibited similar expression in shoots and roots (Fig. 2e). The other two receptors, *GID1b1* and *GID1b2*, exhibited much higher expression in the roots. The F-box *SLY1* showed similar expression in shoot and roots and the tomato *DELLA*, *PROCERA,* exhibited higher expression in the shoot (Fig. 2e). These suggest that all canonical GA signaling components are normally expressed in the root. Moreover, overexpression of *GID1a* in M82 roots slightly inhibited root elongation and eliminated completely the effect of GA (Supporting information Fig. S7), probably due to super-optimal GA activity in the root (Ramon *et al*., 2020). Taken together, these results suggest that GA signaling is active in the root, but the output toward cell elongation is blocked.

Many studies that showed clear effect of GA on primary root elongation were performed on agar plates and the roots were exposed light (Ubeda- Tomas *et al*., 2008, 2009; Achard *et al*., 2009; Rizza *et al*., 2017; Qin *et al*., 2022). We therefore tested if GA could promote elongation of *gib-1* primary root on agar plates in the light. Rescued *gib-1* embryos were grown on agar plates with or without GA_3 and the plates were placed vertically in the light or under dark conditions for five days. Both in light and dark, GA induced hypocotyl elongation (Fig. 3a and b). However, the hormone promoted the elongation of *gib-1* primary roots only in the light (Fig. 3a and c). We also examined microscopically the length of the primary-root epidermal cell above the elongation zone and found that GA increased cell length in the light but had no effect on cell elongation in the dark (Supporting information Fig. S8).

If roots are insensitive to GA in the dark, then under dark conditions the length of M82 roots should be similar to those of *gib-1*. We grew M82 and *gib-1* on agar plates under light or dark conditions. M82 hypocotyls were much longer than those of *gib-1* under light or dark conditions. However, while M82 primary roots were much longer than those of *gib-1* under light conditions, under dark conditions their length was similar (Fig. 3c,

Supporting information Fig. S9). To test if direct light to the root is required for GA-induced elongation, we used the D-root agar plate system (Silva-Navas *et al*., 2015; Supporting information Fig. S2). This system allows growing seedling on agar plates with the shoots exposed to light while the roots grow in the dark, similar to soil-growing seedlings. While GA promoted primary root elongation in the light, it had a very mild effect on root elongation in the D-root system (Fig. 3d). It should be noted that the D-root system does not block completely the light to the root, but just limiting it (Silva-Navas *et al*., 2015). We further examined if a weaker GA-deficient mutant shows similar light-dependent GA-induced primary-root elongation. To this end, we have used the CRISPR-derived mutant *ga20ox1* (Shohat et al., 2021). Primary roots of this mutant are shorter than those of the WT M82 (Supporting information Fig. S10). Scarified *ga20ox1* seeds were placed on agar plates in the light, or covered for the dark treatment, with or without 1 μM GA_3. GA promoted primary root elongation in *ga20ox1* seedlings only under light conditions (Fig. 3e).

We hypothesized that GA induces root elongation right after germination, when light is able to penetrate the upper layer of the soil (Tester & Morris, 1987; Mandoli *et al*., 1990), but later, deeper in the soil, in a complete darkness, the roots cannot respond to GA. To test this hypothesis, we applied 10μM GA_3 immediately after placing the rescued *gib-1* embryos on the vermiculite, or 10 days later, when the roots were deeper (approximately 2 cm long) in the vermiculite. While GA_3 application immediately after sowing promoted primary root elongation, at the later stage the hormone had no effect (Fig. 4a). Moreover, a single application of GA (10μM GA_3) right after germination completely restored *gib-1* root elongation and their primary-root length, three weeks later, was similar to that of the WT (Fig. 4b). To exclude the possibility that only very young roots can respond to GA, but later it becomes insensitive to the hormone, we examined root elongation of *gib-1* seedlings grown on agar plates in the light with or without GA or on plates first without GA and only when primary roots were approximately 2.5 cm long, GA_3 was added. GA promoted elongation when applied at early or later stages (Fig. 4c), ruling out the possibility that GA can induce elongation only at early stages of primary-root development, and further support the hypothesis that perception of light by the root is essential for GA-induced elongation.

We next tested if the effect of GA on root elongation can be induced deep in the soil if we expose the root tip to light. To this end, we removed the bottoms of the pots, and the pots containing vermiculite were placed on LED lighting strips, exposing root tips to light from the bottom (Supporting information Fig. S11). *gib-1* embryos were grown on the vermiculite and two weeks later, when roots were approximately 2.5 cm long, the light was turned on and GA_3 was applied. A week later, we measured the length of the primary roots of all treatments. GA application to LED-lighted *gib-1* roots promoted their elongation, but without bottom lighting it had no effect (Fig 4d), suggesting again that direct light to the root is required for GA to promote their elongation.

We hypothesized that light drives GA-induced primary root elongation in the top layer of the soil. Thus, GA-induced elongation should be activated by low light intensity. We examined the effect of light intensity on GA-induced hypocotyl and primary-root elongation. Rescued *gib-1* embryos were placed on agar plates with or without GA_3 in dark or under 0.1, 1, 10 or 100µmol m^{-2} s^{-1} of white light (WL). The strongest effect of GA_3 on hypocotyls elongation was found in the dark and it was slightly decreased with the increasing light intensity (Fig 5a and b). On the other hand, GA had no effect on primary-root elongation in the dark, but a clear effect was found already at the lowest light intensity (0.1 µmol m^{-2} s^{-1}) (Fig 5a and c).

To study the active light spectrum required for GA-induced root elongation, we first tested in *ga20ox1* the effect of direct WL light to the root on agar plate. For this we have used D-shoot plates (shoot in dark and root in light, Supporting information Fig. S2) and D-root plates (shoot in light and root in dark), with or without 1µM GA_3. GA promoted primary root elongation only when the roots were exposed to light (D-shoot) but not when the root grew in the dark in the D-root plates (Fig. 6a). We then used the D-shoot system to study the active light spectrum. Scarified *ga20ox1* seeds on D-shoot agar plates with or without 1µM GA_3, were vertically placed under blue, red or far-red light (see methods for light spectrum). GA promoted primary root elongation only under red light (Fig. 6b), suggesting that red-light perception by the root is essential for GA-induced primary root elongation.

There is an extensive body of information regarding the promoting effect of GA on primary-root elongation in Arabidopsis. However, most of these studies have been

performed using light-grown roots (Arizumii *et al*., 2008; Ubeda-Tomas *et al*., 2008; Shani *et al*., 2013; Rizza *et al*., 2017). We therefore examined if light has similar effect on GA-induced primary-root elongation in Arabidopsis. WT Col and the GA deficient *ga1-3* seedlings were grown in vermiculite and after three weeks, when *ga1-3* primary roots were approximately 2.5 cm long, the mutant was treated with GA (10μM GA_3). Different from tomato, GA promoted the elongation of *ga1-3* primary roots deep in the soil (Supporting information Fig. S12a). Moreover, GA promoted the elongation of *ga1-3* primary roots also on agar plates under light and dark (full dark, D-root and D-shoot) conditions (Supporting information Fig. S12b).

We also examined the possible interaction between light and GA in the regulation of root elongation in wheat. To this end, the germination paper methods (Watt et al., 2013) was used. Rolled germination papers containing wheat seeds were placed first in Erlenmeyer flasks filled with water. After germination, the rolled papers were transferred to new flasks filled with water (mock), 5 mg/l PAC or PAC and 10μM GA_3. Half of the papers for each treatment were covered with aluminum foil to block the light (Supporting information Fig. S1). The coleoptiles emerged from the paper and further developed in the light in all treatment. After 5 days the length of the primary root was measured. When roots were exposed to light, PAC treatment inhibited the elongation of the primary root and GA restored elongation (Fig. 7). When roots developed in the dark, the primary roots were longer than those developed in the light, but PAC and GA had no effect on their elongation. Taken together, these results suggest that in wheat, similar to tomato, GA-induced primary-root elongation depends on direct light to the root.

Discussion

Rapid elongation of the primary root, following germination, is essential for seedling establishment; it anchorage the seedling and allows water and nutrient uptake. Various factors regulate this rapid, directional elongation, including environmental factors, such as light and gravity that direct the growth downward into the soil (negative phototropism and gravitropism) and growth hormones. Auxin plays a central role in root growth, but other hormones, including brassinosteroids and GA are also known to promote primary

root elongation (Garay-Arroyo *et al*., 2012). While the role of auxin has been studied intensively and is conserved in all tested plant species, the role of GA in different species is less clear (Castro-Camba *et al*., 2022). There are conflicting reports on the effect of GA on root elongation and while numerous studies showed clear promoting effect, some other showed no effect or even inhibitory effect (Tognoni *et al.* 1967; Fonouni-Farde *et al*., 2019). The discrepancy between the reports could be due to application of GA to WT plants with saturated GA levels in the root, in some studies, or application of the hormone to GA deficient mutants, in other studies. It can also be due to application of low GA concentrations that promote elongation in some studies and application of super-optimal GA concentrations that inhibit root elongation, in other studies (Ramon *et al*., 2021). The results of this study show that it can also be due to the experimental setup; growing the plants on plates in the light, or in the soil with roots growing in the dark. Silva-Navas *et al*. (2015) showed that roots behave differently under light and dark conditions. Our results suggest that in tomato and wheat the effect of GA on root elongation depends on light. Thus, the effect of the hormone would be different if it is examined on agar plates in the light or in the soil, in the dark.

The interaction between light and GA was demonstrated in germinating seeds, elongating hypocotyls and stems and developing roots. According to these studies, light affects GA metabolism, transport and responses (Arana *et al*., 2014; Matsuo *et al*., 2019; Lyu *et al*., 2021; Li *et al*., 2020; van Gelderen *et al*., 2023). In soybean (*Glycine max*), blue light, via cryptochrome (CRY), suppresses stem elongation by the induction of *GA 2-oxidase* (*GA2ox*) transcription, leading to GA deactivation (Lyu *et al*., 2021). Low red/far-red ratio, mediated by phytochrome B (PhyB), affects GA accumulation and shoot elongation in *Pinus tabuliformis* seedlings (Li *et al*., 2020). It also affects GA responsiveness in Arabidopsis seeds (Arana *et al*., 2014). Increased far-red light to Arabidopsis shoots promotes GA accumulation and its translocation to the root, where it inhibits lateral root growth (van Gelderen *et al*., 2023). Our results suggest that continuous red, but not blue or far-red light, promotes GA-induced primary root elongation. This implies that activation of PhyB is required for this GA response (Quail, 2002). Our RNAseq data show that both *PhyB1* and *PhyB2* genes are expressed in tomato roots (Supporting information Fig. S13).

How red light and PhyB affect GA elongation responses in tomato roots is unknow. In dark-grown roots, all GA signaling components were normally expressed and GA induced or suppressed the expression of numerous genes and pathways. However, cell expansion genes were not affected by the hormone (Fig. 2). These results suggest that light affects GA-induced root-elongation response downstream of the GA signaling pathway. DELLA regulates down-stream pathways by the interactions with numerous transcription factors (TF) and their inhibition (Locascio et al., 2013). Degradation of DELLA by GA in the root probably releases these TFs, leading to transcriptional activation of many pathways independently of light. These may include the regulation of root architecture (Castro-Camba *et al*., 2022), secondary xylem development, lignification (Wang et al., 2017) and endodermal suberin formation (Binenbaum *et al*., 2023). Several DELLA-interacting TFs are involved in GA regulation of cell elongation (de Lucas *et al*., 2008; Feng *et al*., 2008; Gallego-Bartolome *et al*., 2012; Chaiwanon & Wang, 2015). It is possible that in tomato roots the activity of these TFs depends on the activation of PhyB by red light.

Roots normally grow in the dark and escape from light by negative phototropism (Zhang *et al*., 2013). However, in most cases, shortly after germination, in the upper layer of the soil, the young roots are exposed to low light intensity (Tester & Morris, 1987; Mandoli *et al*., 1990). We suggest that in tomato, at this early stage the light signal interacts with the GA response pathway to accelerate root elongation. Rapid elongation at this early stage of root development contributes to seedling establishment. At later stages however, when the root elongation zone was deeper in the soil, in the dark, GA had no more effect on root elongation. It is possible that the insensitivity of root elongation to GA deeper in the soil, contributes to root growth under changing environment. Under moderate drought conditions, bioactive GA levels in tomato decrease due to the inhibition of GA biosynthesis and increased GA deactivation (Shohat *et al*., 2021). The reduced GA levels suppress shoot growth to reduce water loss. At the same time, under the same dry conditions, root elongation is maintained (xerotromism), enabling roots to grow deeper and find new sources of water (Sharp *et al*., 2004; Miao *et al*., 2021). If root elongation was dependent on GA, this differential growth response between shoot and root, under moderate drought conditions, would not be possible.

Our results show that light is essential for GA-induced root elongation in tomato and wheat, but not in Arabidopsis. If the GA insensitivity deep in the ground in the dark was evolved to allow efficient xerotropism, it can explain the differences between these species. Wild Arabidopsis is a winter annual with a short life cycle, originating from well-watered habitats of the western Eurasia (Kramer, 2015), and normally not exposed to prolonged drought. On the other hand, wild tomato originates from the arid land of the Andean region of South America, and wheat, from the middle east, and both experience periods of drought in their longer life cycle (Chetelat *et al*., 2009; Chetelat *et al*., 2019; Harlan & Zohary, 1966). Thus, changing root to shoot ratio under water limiting conditions may be essential for wild tomato and wheat survival, but not for wild Arabidopsis.

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Author Contributions: UR, IN, YB and DW designed the research; UR, HC, and AA performed experiments; UR, AA and DW analyzed data; UR, IN, YB and DW wrote the manuscript.

Data Availability

All data can be found in the manuscript and in the supporting information.

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Figure Legends

Fig. 1. GA application to *gib-1* had no effect on primary-root elongation. (a). WT M82 and *gib-1* plants. (b and c) Two weeks-old *gib-1* seedlings, grown in vermiculite, were treated twice a week for one month with 10μM GA_3. (b) representative GA-treated and non-treated plants. The average length of the primary roots is presented (n=10 ±SE). (c) *gib-1* leaf (third leaf from the top), stem and primary root length of GA treated or non-treated plants. Data are graphically presented as whisker and box plots (n=10 ±SE). White triangles show the average values (d) Embryos, rescued from *gib-1* and WT seeds, were placed on agar plates and the length of the primary-root was measured each day for six days (n=8 ±SE). Bars in a = 1 cm in b = 2.5 cm.

Fig 2. Transcriptional response to GA in tomato root and shoot. M82 and *gib-1* seedlings were grown for two weeks on vermiculite, and then *gib-1* plants were treated with 10 μM GA_3 and 24 h later, shoots and primary root tips were taken (three biological replications) for RNAseq analysis. (a and b) Violine plot of differentially expressed genes (DEG) between WT M82 and *gib-1* shoot and root (a) and *gib-1* with or without GA (b). (c) Normalized read counts of *GA20ox1* and *GA20ox3* in *gib-1* roots and shoots with or without GA treatment. Values are the average of three biological repeats ±SE. (d) Heatmap of GA regulated cell expansion genes (Park et al., 2017) in *gib-1* shoots and roots. Coloring of the genes is according to the color bar (Log2 fold change). XTH-xyloglucan endotransglucosylase. (e) Heatmap showing normalized read counts of GA-signaling genes in WT M82 shoots and roots. Values are the average of three biological repeats. Coloring of the genes is according to the color bar (normalized read counts).

Fig. 3. Light is required for the promoting effect of GA on primary-root elongation. (a, b and c) Seedlings (*gib-1*) were grown on agar plates with or without 1μM GA_3 and the plates were placed vertically in the light or under dark conditions and after 5 days hypocotyl and primary-root length was measured. (a) Representative seedlings (bars = 1 cm). (b) Hypocotyl length. (c) Primary root length. Data in a and b are graphically presented as whisker and box plots (n=16) ± SE. White triangles show the average values. (d) Representative M82 and *gib-1* seedlings grown on agar plates with or without 1μM GA_3 for 5 days in light or dark (bars = 1 cm). (e) Seedlings (*gib-1*) were grown on agar plates with or without 1μM GA_3 and the plates were placed vertically in the light or under dark or in the light but using the D-root plates. After 5 days the length of the primary roots was measured. Values are mean of 16 seedlings ±SE. (f). *ga20ox1* seedlings were grown on agar plates with or without 1μM GA_3 and the plates were placed vertically in the light or under dark conditions and after 5 days primary-root length was measured. Values are mean of 15 seedlings ±SE. Stars in b, c, e and f represent significant differences between respective treatments by Student's t test (*P < 0.05; **P<0.01; ***P<0.001). ns-not significant.

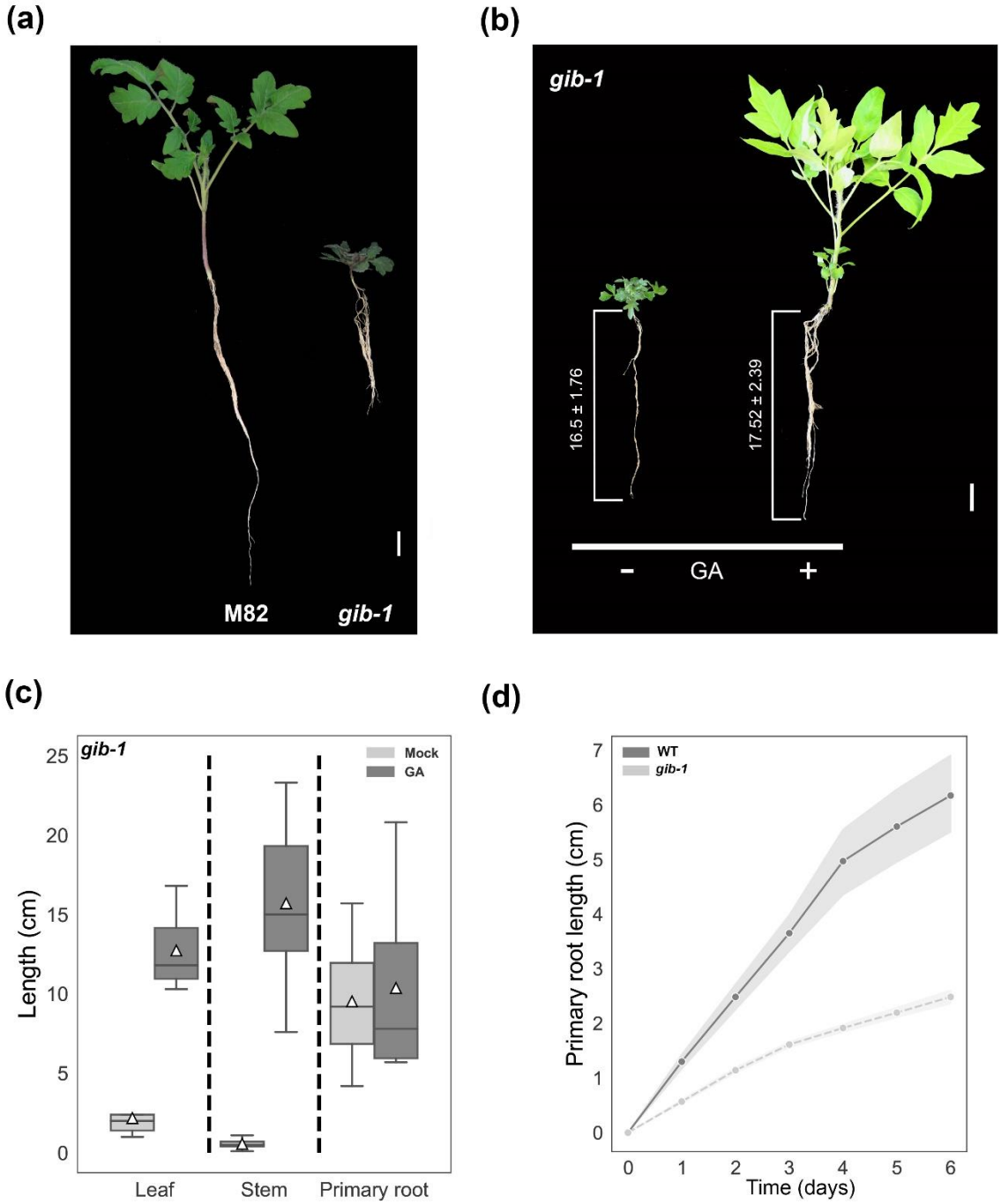
Fig. 4. GA induced primary root elongation right after germination but not deeper in the growth medium. (a) Rescued *gib-1* embryos were placed on vermiculite and treated with 10μM GA_3 immediately, or 10 days later. One week after the GA treatment (for each treatment), the length of the primary roots was measured. Data are graphically presented as whisker and box plots (n= 16 ± SE). (b) Rescued M82 and *gib-1* embryos were placed on vermiculite and 10μM GA_3 was immediately added to *gib-1* and two weeks later the length of the primary roots was measured. Values are mean of 13 seedlings ±SE. (c) Rescued *gib-1* embryos were placed on agar plates without or with 1μM GA_3 *gib-1* or first without GA and only when primary roots were approximately 2.5 cm long, 1μM GA_3 was added. The seedlings were grown in the light for 6 days and then the length of the primary roots was measured. Data are graphically presented as whisker and box plots (n= 12 ± SE). (d) *gib-1* embryos were put on the vermiculite in bottomless pots that were placed (or not) on LED lighting strips and two weeks later, when roots were approximately 2.5 cm long, the light was turned on and part of the plants were treated with 10μM GA_3. A week later, the length of the primary roots was measured for all treatments. Values are mean of 10 seedlings ±SE. Stars represent significant differences between respective treatments or lines by Student's t test (*P < 0.05; **P<0.01; ***P<0.001; ****P<0.0001). ns- not significant. DAG- days after germination. White triangles in a and c show the average values.

Fig. 5. Low light intensity is sufficient for GA to promote primary root elongation. Rescued *gib-1* embryos were placed on agar plates with or without GA_3 in dark or under 0.1, 1, 10 or 100 μmol m^{-2} s^{-1} of white light. After 10 days hypocotyl and primary root length was measured. (a) Representative seedling. Hypocotyls (b) and primary root (c) length. Values are mean of 9 seedlings ±SE. Bars = 1 cm.

Fig. 6. Direct red light to the root is essential for GA-induced elongation in *ga20ox1* mutant. (a) *ga20ox1* seedlings were grown on D-root or D-shoot agar plates with or without 1μM GA_3 and under white light and after 5 days primary-root length was measured. (c) *ga20ox1* seedlings were grown on agar plates with or without 1μM GA_3 under blue, red or far-red light (for light spectrum see Supporting information Fig. S3) for 5 days and then primary-root length was measured. Values in a and b are mean of 16 seedlings ±SE. Stars represent significant differences between respective treatments by Student's t test (P<0.01). ns- not significant.

Fig. 7. Light is essential for gibberellin-induced primary-root elongation in wheat. Wheat seeds were placed between two wet germination papers and the papers were rolled and placed in Erlenmeyer flasks filled with water. Half of the papers for each treatment were

covered with aluminum foil to block the light. After germination, the rolled papers were transferred to new flasks filled with water (mock) or 5mg/l PAC or PAC and 10μM GA_3. After 5 days the length of the primary root was measured. (a) Representative wheat seedlings 5 days after the beginning of the hormonal treatments. (b) Primary root length. Data are graphically presented as whisker and box plots (n= 20 ± SE).. Stars in represent significant differences between respective treatments or lines by Student's t test (**P<0.01; ****P<0.0001). ns- not significant. White triangles in b show the average values. Bar = 1cm



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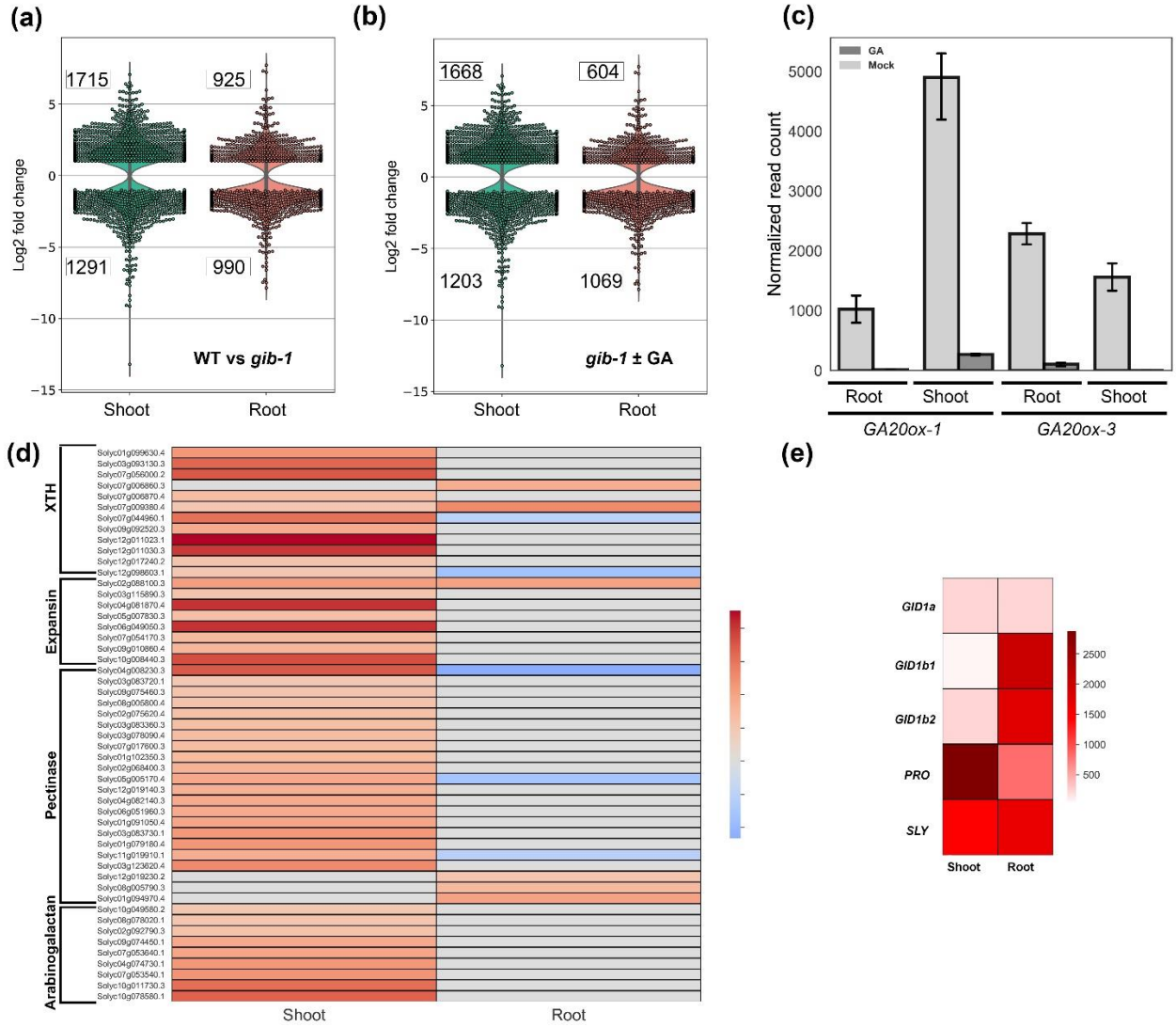

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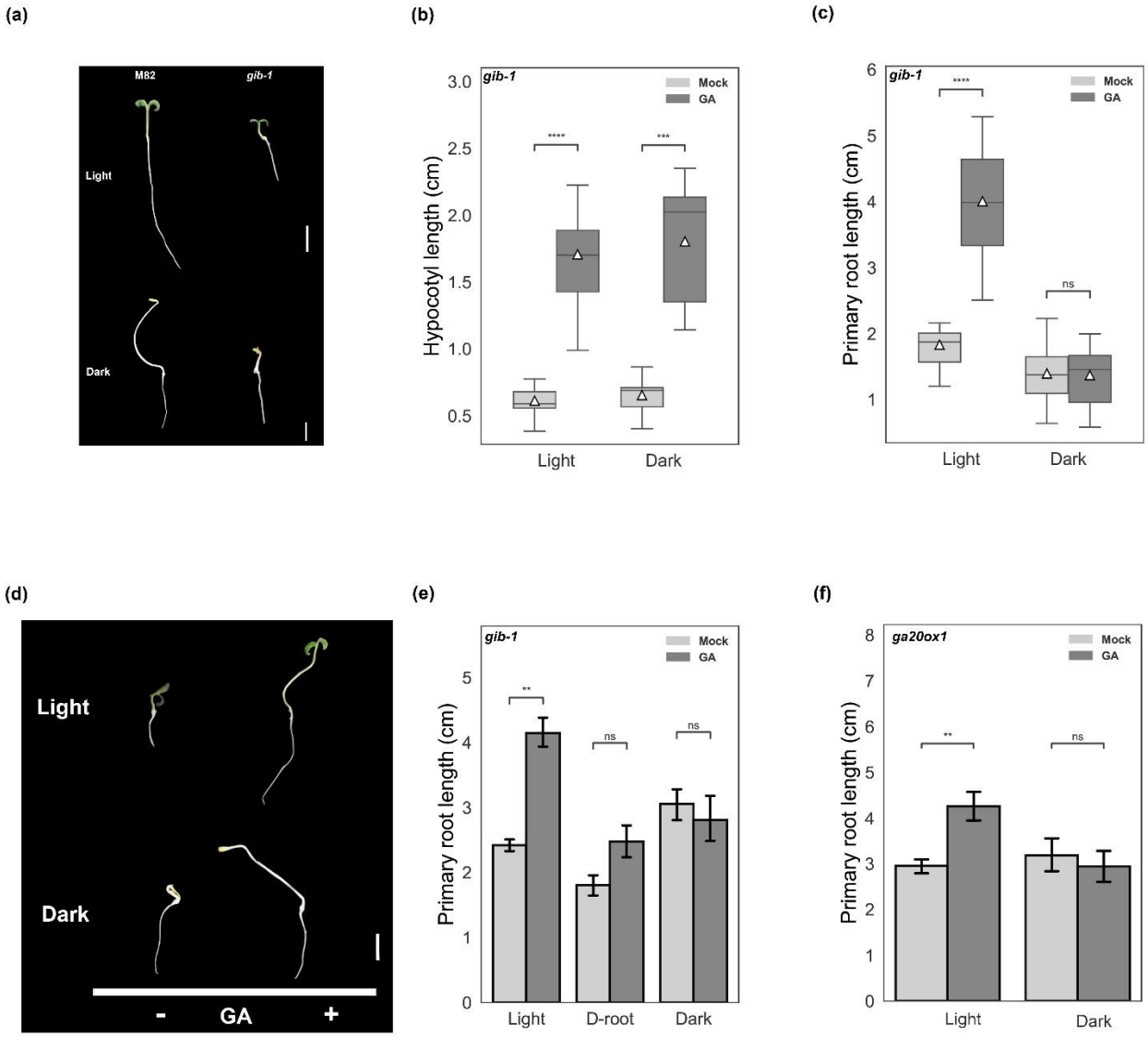
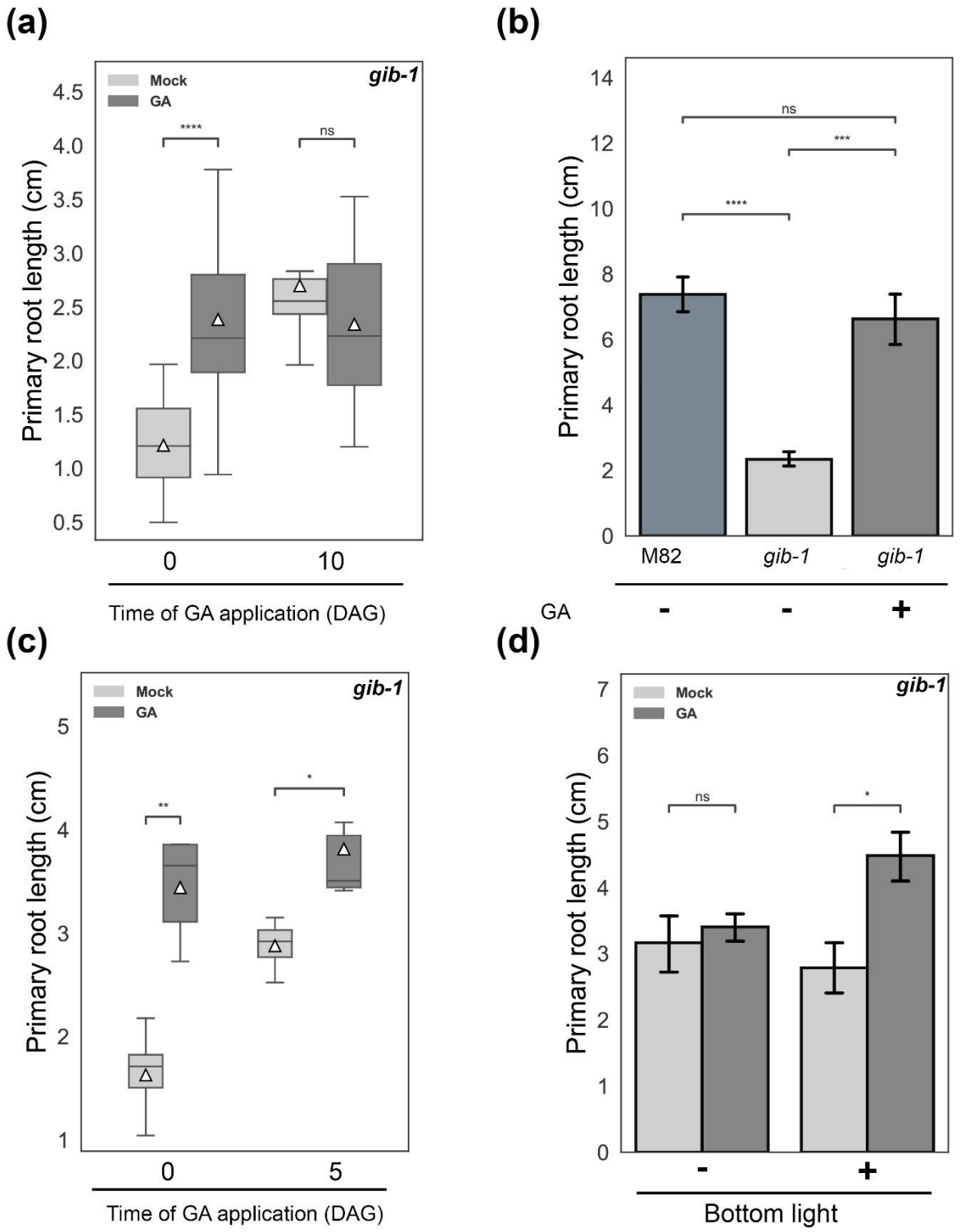


Fig. 3. Light is required for the promoting effect of GA on primary-root elongation. (a, b and c) Seedlings (*gib-1*) were grown on agar plates with or without 1μM GA_3 and the plates were placed vertically in the light or under dark conditions and after 5 days hypocotyl and primary-root length was measured. (a) Representative seedlings (bars = 1 cm). (b) Hypocotyl length. (c) Primary root length. Data in a and b are graphically presented as whisker and box plots (n=16) ± SE. White triangles show the average values. (d) Representative M82 and *gib-1* seedlings grown on agar plates with or without 1μM GA_3 for 5 days in light or dark (bars = 1 cm). (e) Seedlings (*gib-1*) were grown on agar plates with or without 1μM GA_3 and the plates were placed vertically in the light or under dark or in the light but using the D-root plates. After 5 days the length of the primary

roots was measured. Values are mean of 16 seedlings ±SE. (f). *ga20ox1* seedlings were grown on agar plates with or without 1μM GA_3 and the plates were placed vertically in the light or under dark conditions and after 5 days primary-root length was measured. Values are mean of 15 seedlings ±SE. Stars in b, c, e and f represent significant differences between respective treatments by Student's t test (*$P < 0.05$; **$P<0.01$; ***$P<0.001$). ns-not significant.


(a)
Mock
GA
gib-1
Primary root length (cm)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
****
ns
0
10
Time of GA application (DAG)
(b)
Primary root length (cm)
14
12
10
8
6
4
2
0
ns
***
****
M82
gib-1
gib-1
GA
-
-
+
(c)
Mock
GA
gib-1
Primary root length (cm)
5
4
3
2
1
**
*
0
5
Time of GA application (DAG)
(d)
Mock
GA
gib-1
Primary root length (cm)
7
6
5
4
3
2
1
0
ns
*
-
+
Bottom light

817

818

Fig. 4. GA induced primary root elongation right after germination but not deeper in the growth medium. (a) Rescued *gib-1* embryos were placed on vermiculite and treated with 10μM GA_3 immediately, or 10 days later. One week after the GA treatment (for each treatment), the length of the primary roots was measured. Data are graphically presented as whisker and box plots (n= 16 ± SE). (b) Rescued M82 and *gib-1* embryos were placed on vermiculite and 10μM GA_3 was immediately added to *gib-1* and two weeks later the length of the primary roots was measured. Values are mean of 13 seedlings ±SE. (c) Rescued *gib-1* embryos were placed on agar plates without or with 1μM GA_3 *gib-1* or first without GA and only when primary roots were approximately 2.5 cm long, 1μM GA_3 was added. The seedlings were grown in the light for 6 days and then the length of the primary roots was measured. Data are graphically presented as whisker and box plots (n= 12 ± SE). (d) *gib-1* embryos were put on the vermiculite in bottomless pots that were placed (or not) on LED lighting strips and two weeks later, when roots were approximately 2.5 cm long, the light was turned on and part of the plants were treated with 10μM GA_3. A week later, the length of the primary roots was measured for all treatments. Values are mean of 10 seedlings ±SE. Stars represent significant differences between respective treatments or lines by Student's t test (*P < 0.05; **P<0.01; ***P<0.001; ****P<0.0001). ns- not significant. DAG- days after germination. White triangles in a and c show the average values.

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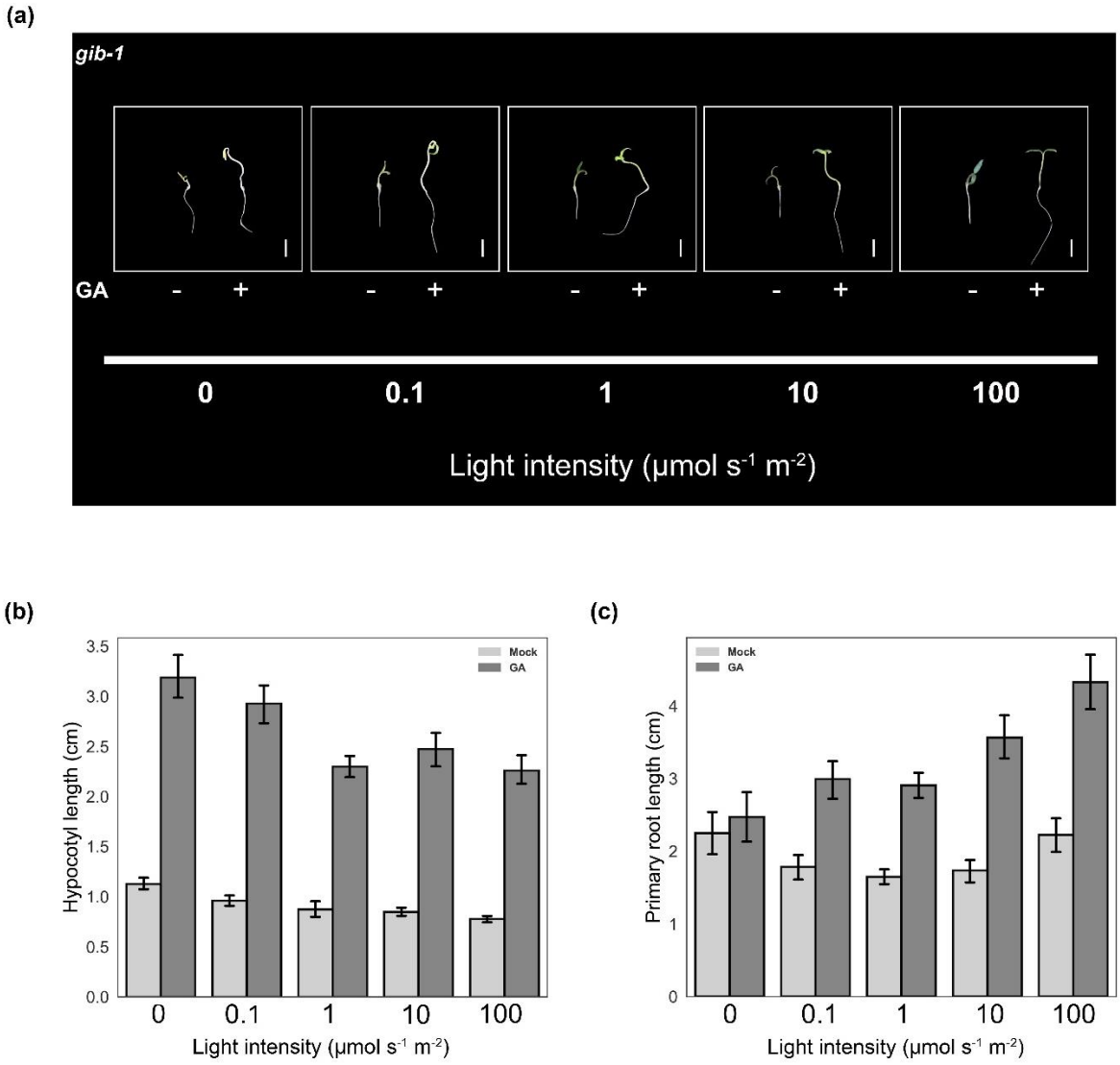

Fig. 5. Low light intensity is sufficient for GA to promote primary root elongation. Rescued *gib-1* embryos were placed on agar plates with or without GA_3 in dark or under 0.1, 1, 10 or 100 µmol m^{-2} s^{-1} of white light. After 10 days hypocotyl and primary root length was measured. (a) Representative seedling. Hypocotyls (b) and primary root (c) length. Values are mean of 9 seedlings ±SE. Bars = 1 cm.

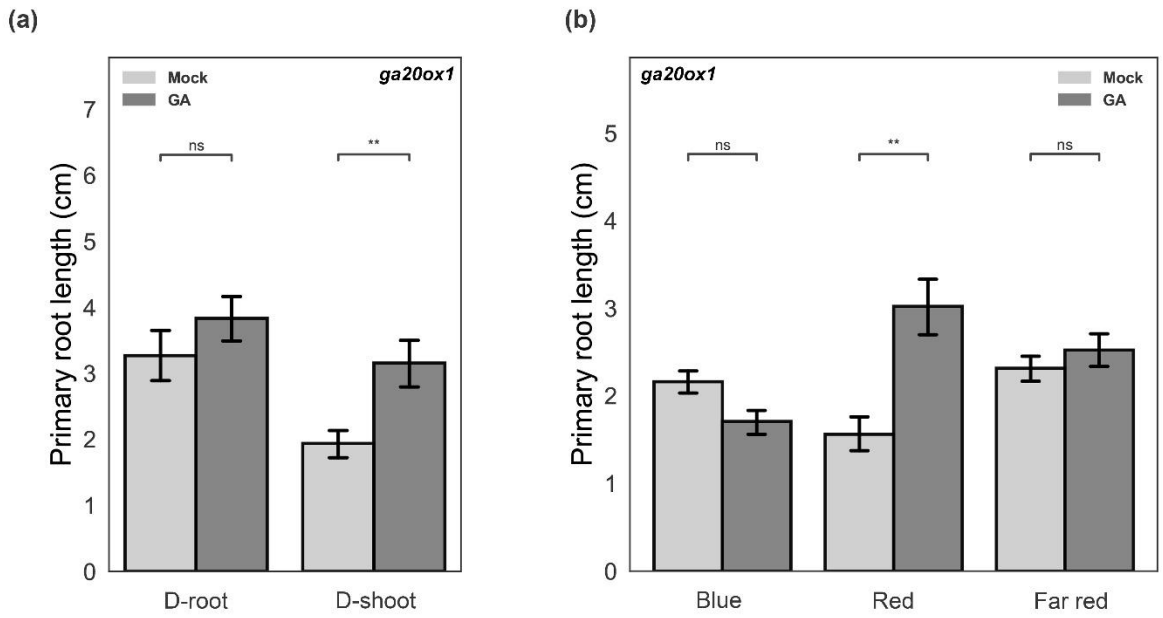

Fig. 6. Direct red light to the root is essential for GA-induced elongation in *ga20ox1* mutant. (a) *ga20ox1* seedlings were grown on D-root or D-shoot agar plates with or without 1μM GA_3 and under white light and after 5 days primary-root length was measured. (c) *ga20ox1* seedlings were grown on agar plates with or without 1μM GA_3 under blue, red or far-red light (for light spectrum see Supporting information Fig. S3) for 5 days and then primary-root length was measured. Values in a and b are mean of 16 seedlings ±SE. Stars represent significant differences between respective treatments by Student's t test ($P<0.01$). ns- not significant.

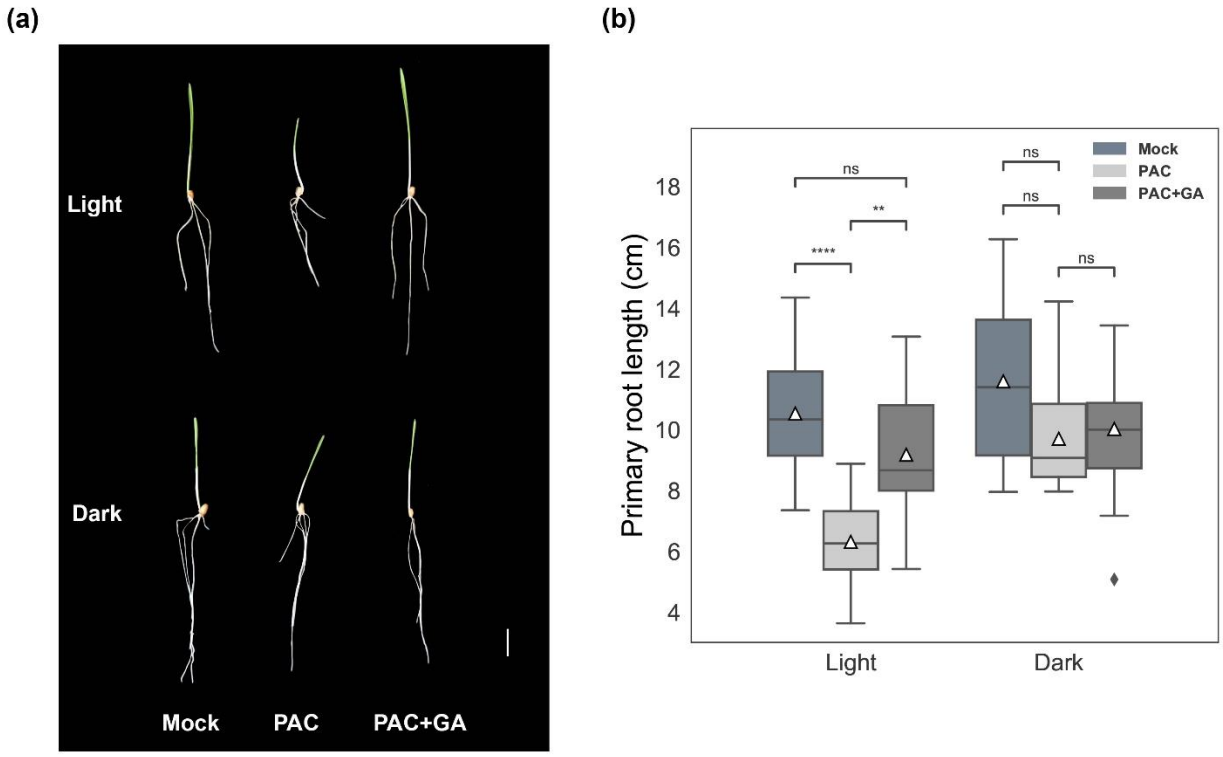

Fig. 7. Light is essential for gibberellin-induced primary-root elongation in wheat. Wheat seeds were placed between two wet germination papers and the papers were rolled and placed in Erlenmeyer flasks filled with water. Half of the papers for each treatment were covered with aluminum foil to block the light. After germination, the rolled papers were transferred to new flasks filled with water (mock) or 5mg/l PAC or PAC and 10μM GA_3. After 5 days the length of the primary root was measured. (a) Representative wheat seedlings 5 days after the beginning of the hormonal treatments. (b) Primary root length. Data are graphically presented as whisker and box plots (n= 20 ± SE). Stars in represent significant differences between respective treatments or lines by Student's t test (**P<0.01; ****P<0.0001). ns- not significant. White triangles in b show the average values. Bar = 1cm

***New Phytologist* Supporting Information**

Article title: Light perception by the root is essential for gibberellin-induced primary-root elongation in tomato

Authors: Uria Ramon, Amit Adiri, Hadar Cheriker, Yogev Borko, Ido Nir and David Weiss

Article acceptance date: Click here to enter a date.

The following Supporting Information is available for this article:

Fig. S1. The paper germination system for wheat root elongation assay (light and dark).

Fig. S2. D-root and D-shoot growth systems.

Fig. S3. The light spectrum of the different light treatment.

Fig. S4. Root epidermal cell length of WT M82 and *gib-1*.

Fig. S5. WT M82 and *gib-1* seedlings

Fig. S6. Differentially expressed genes (DEG).

Fig. S7. Overexpression of GID1a. root elongation.

Fig. S8. GA increases root cell length in the light but not in the dark.

Fig. S9. Primary root length of WT M82 and *gib-1* under light and dark conditions.

Fig. S10. Primary-root length of M82 and *ga20ox1.*

Fig. S11. The bottom lighting growth system.

Fig. S12. GA promoted primary root elongation in Arabidopsis under light and dark conditions.

Fig. S13. Normalized reads counts of the tomato photoreceptors in shoot and root.

Table S1 Primers used in this study

Dataset S1. Differentially expressed genes (DEG) between M82 and *gib-1* shoots and roots and between *gib-1* shoot and roots, treated or not treated with GA (see separate file).

Fig. S1. The paper germination system for wheat-root elongation assay. Seeds were placed between two rolled wet paper, in the outer circle. For dark conditions (to the roots), the rolled papers were covered with aluminium foil.

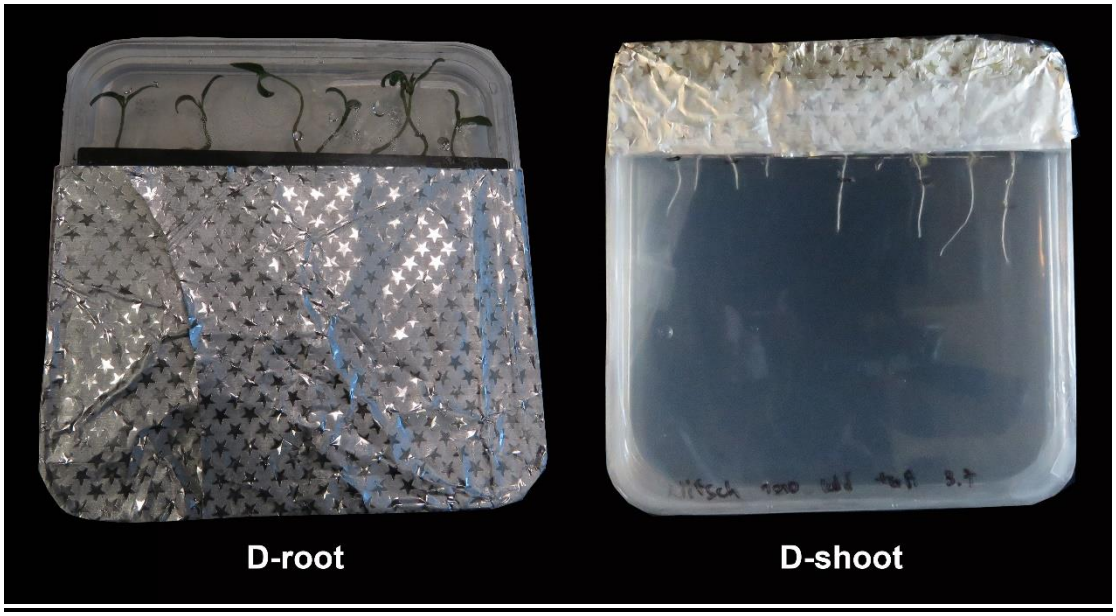

Fig. S2. D-root and D-shoot growth systems.

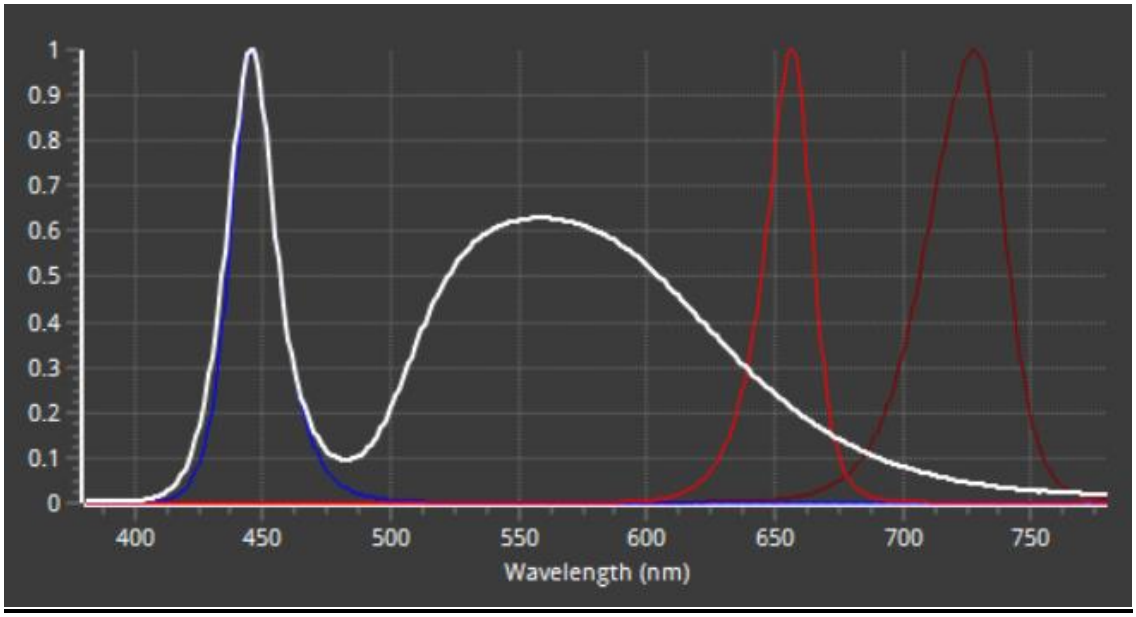

Fig. S3. The light spectrum of the different light treatment. White light (white line), blue light (blue line), red light (red line) and far red light (dark red line).

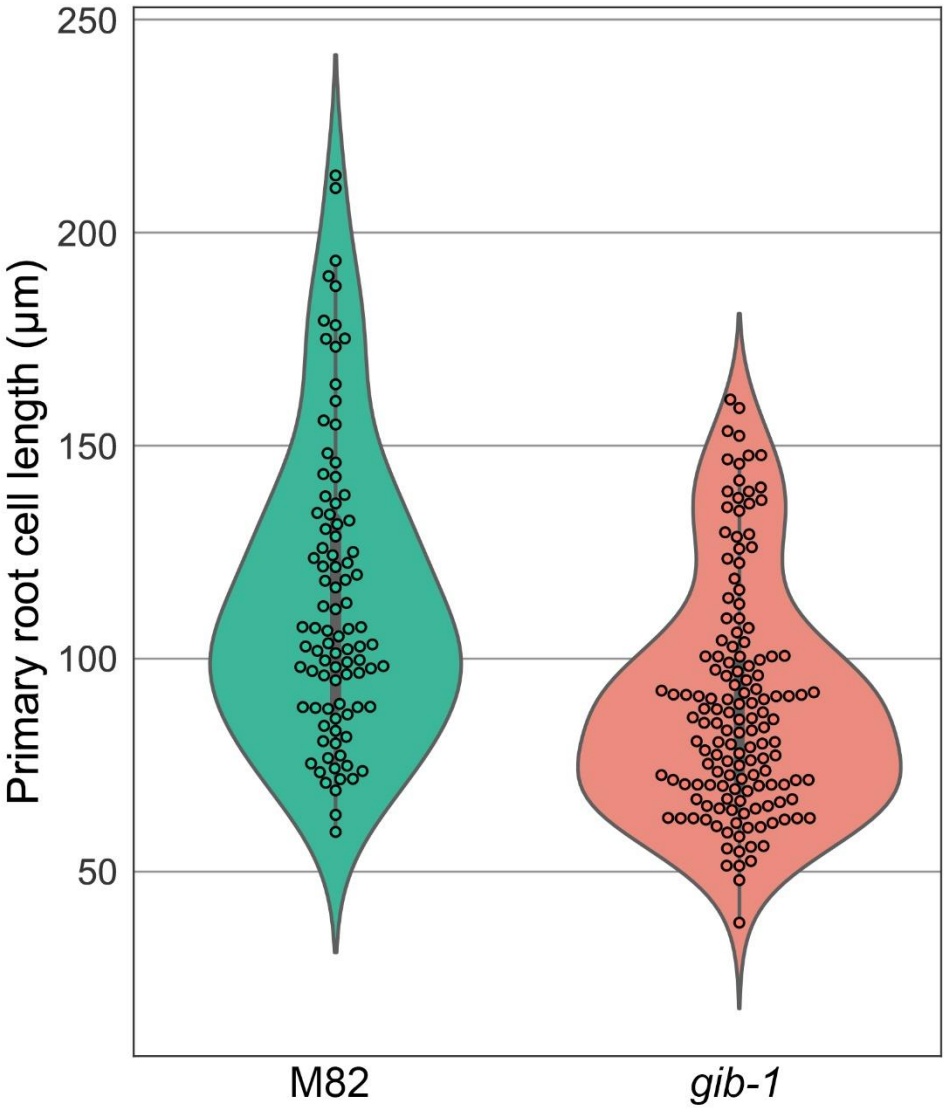

Fig. S4. Cell length of WT M82 and *gib-1* primary roots. Microscopic analysis of epidermal cells of WT M82 and *gib-1* primary roots.

Fig. S5. WT M82 and *gib-1* seedlings. Bar = 1 cm

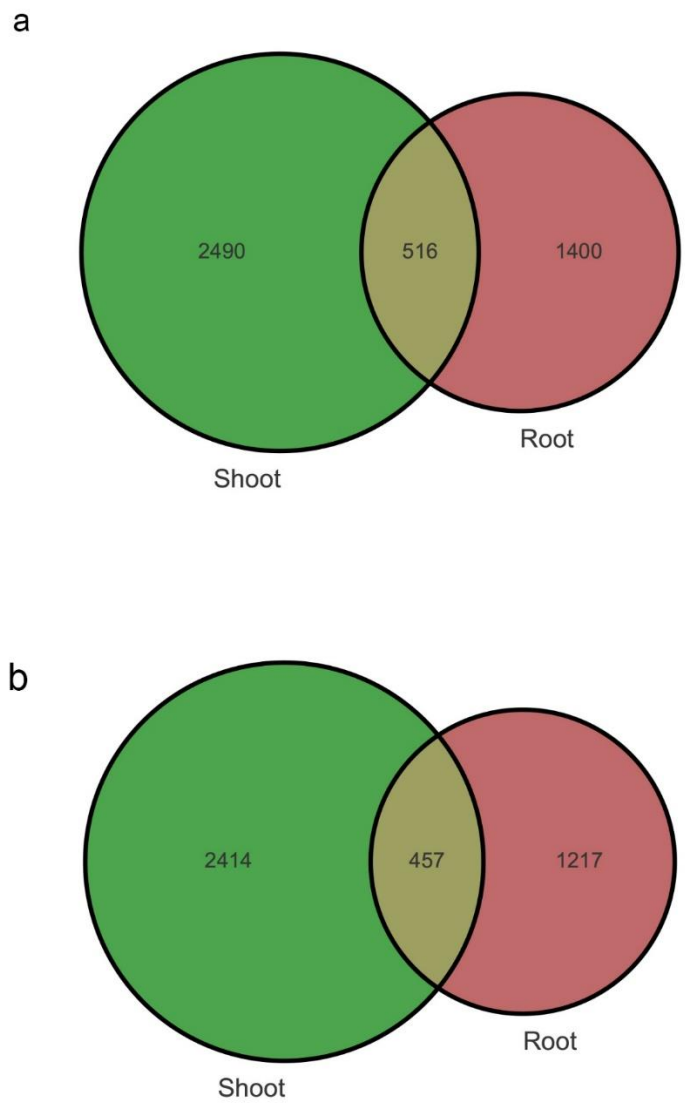

Fig. S6. Differentially expressed genes (DEG). (a) Venn diagram represents DEG between WT M82 and *gib-1* shoots and roots. (b) Venn diagram represents DEG between *gib-1* untreated and GA treated shoots and roots

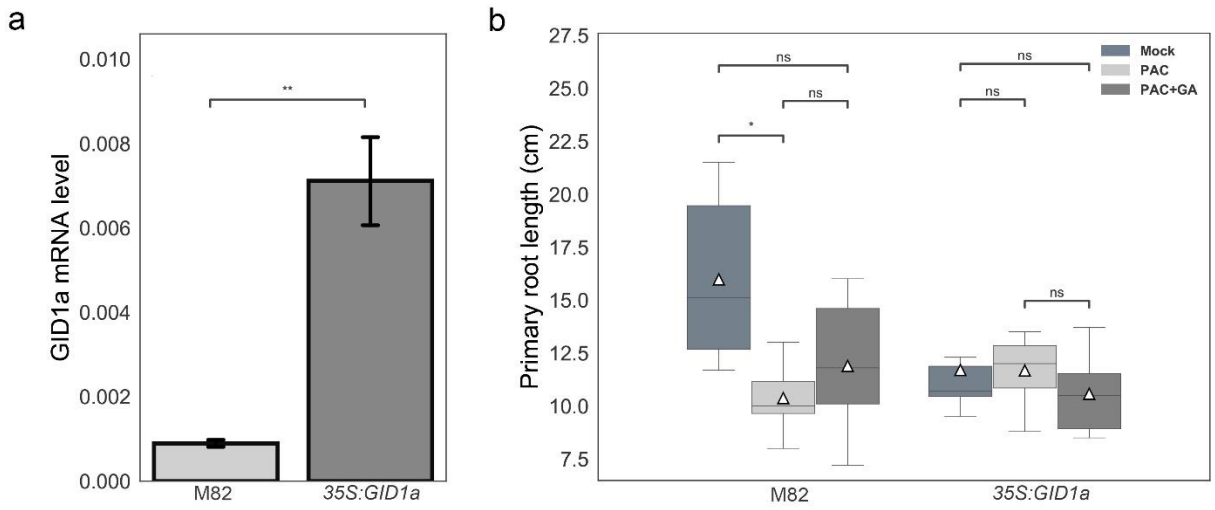

Fig. S7. Overexpression of GID1a. (a) GID1a expression in WT M82 and transgenic 35S:GID1a roots. Values are mean of three biological replicates ± SE. (b) Primary root length in WT M82 and transgenic plants overexpressing GID1a treated with 5mg/l paclobutrazol (PAC) or PAC with 10μM GA_3. Data are graphically presented as whisker and box plots (n= 12 ± SE). White triangles show the average values. Stars represent significant differences between respective treatments or lines by Student's t test (*P < 0.05; **P<0.01).

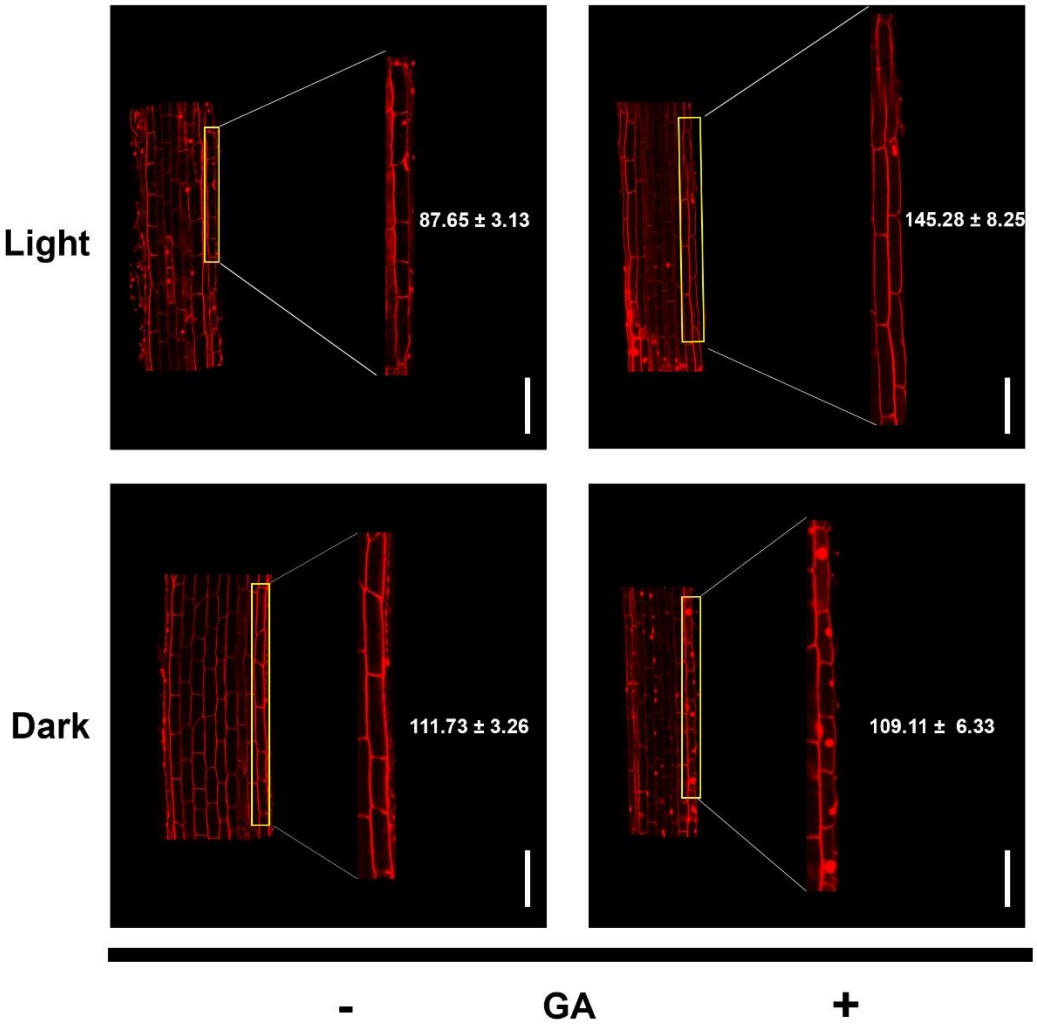

Fig. S8. GA increases root cell length in the light but not in the dark. *gib-1* seedlings were grown on agar plates with or without 1μM GA_3 . The plates were placed vertically in the light or under dark conditions for 5 days. The length of the primary-root epidermal cell above the elongation zone was examined microscopically. Average cell length is presented (n=30 ± SE). Bar = 100 μm

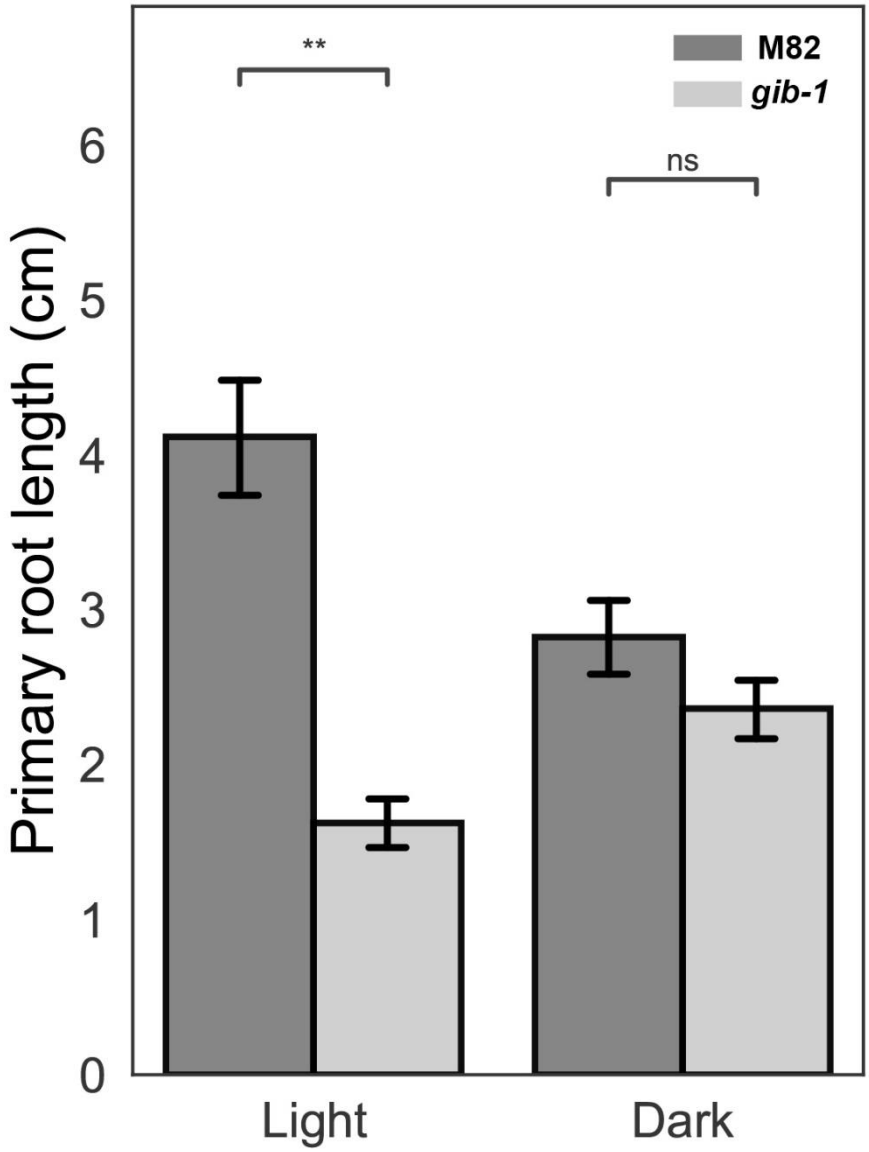

Fig. S9. Primary root length of WT M82 and *gib-1* under light and dark conditions. WT M82 and *gib-1* seedlings were grown on agar plates. The plates were placed vertically in the light or under dark conditions for 7 days and then the length of the primary roots was measured. Values are mean of 12 seedlings ± SE. Stars represent significant differences between respective treatments by Student's t test (P<0.01). ns- not significant.

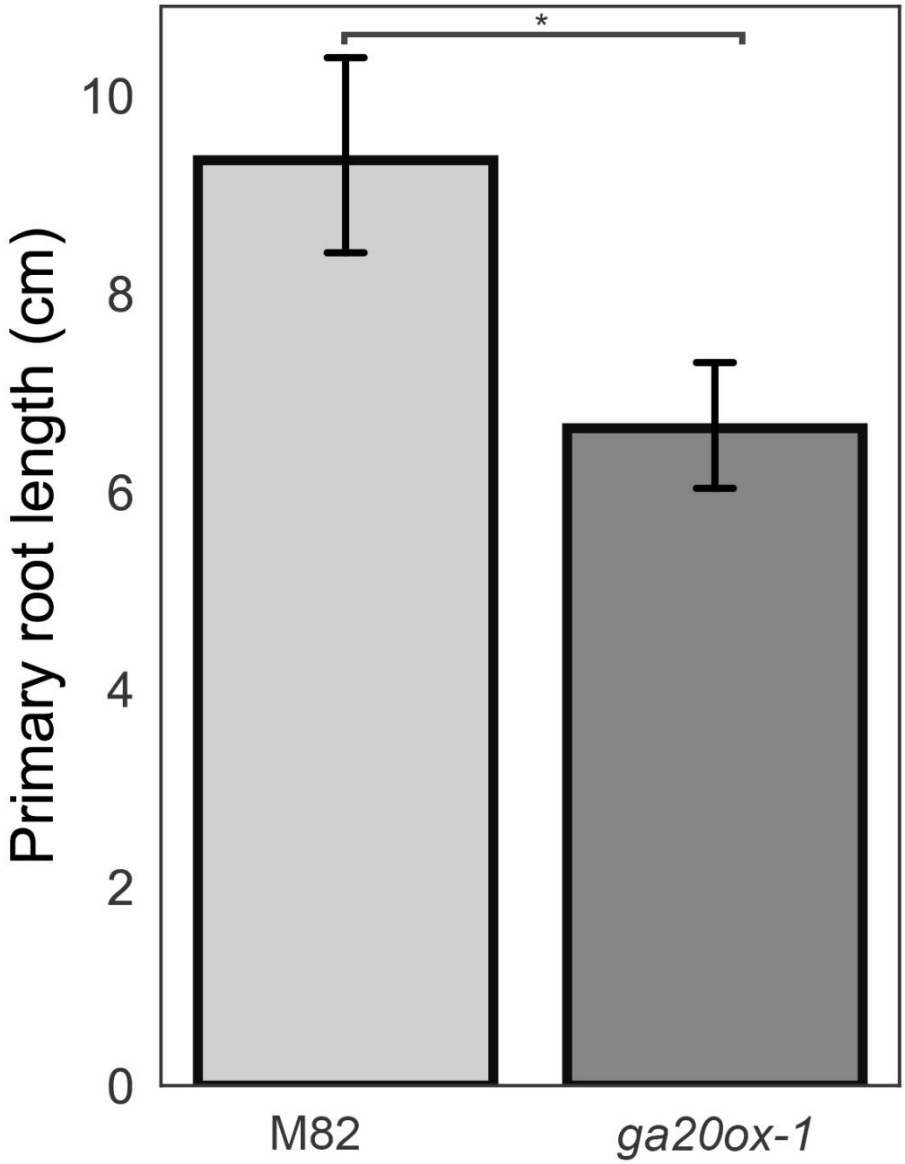

Fig. S10. *ga20ox1* has short roots. M82 and *ga20ox1* seedlings were grown in vermiculite for 10 days and then primary-root length was measured. The average length is presented (n=7 ±SE). Star represents significant difference by Student's t test (P<0.05).

Fig. S11. The bottom-lighting growth system. Left- LED lighting strips fixed to the growing tray. Middle- Try with LED lighting strips and pots without their bottom. Right- Try with LED lighting and pots without their bottom, filled with vermiculite and *gib-1* seedlings.

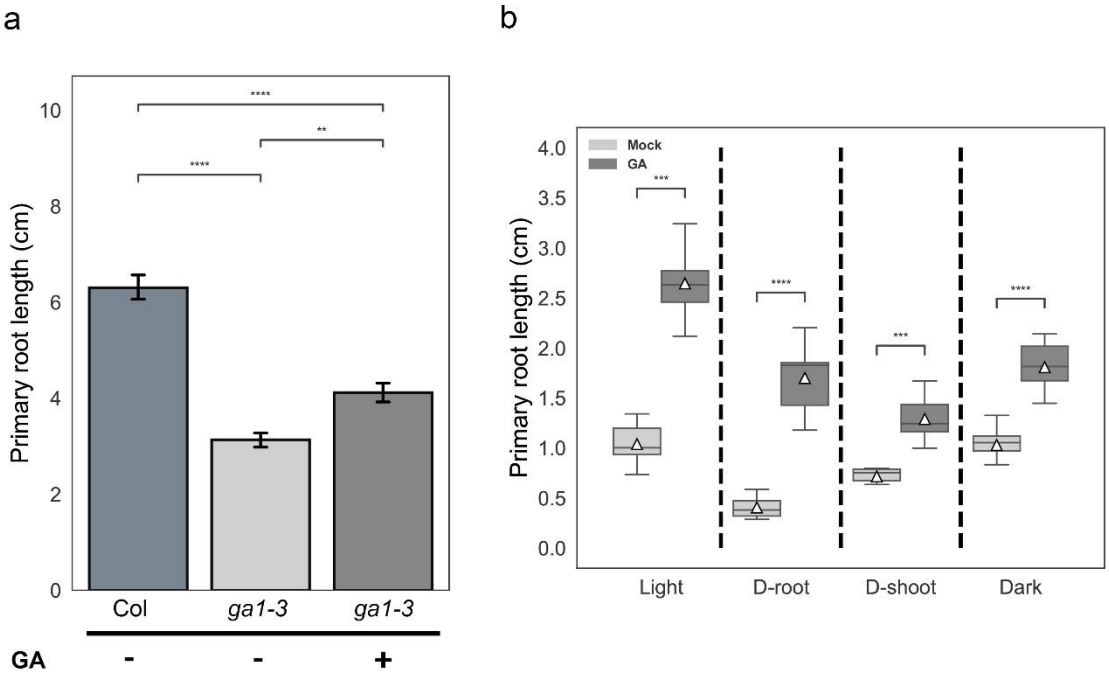

Fig. S12. GA promoted primary root elongation in Arabidopsis under light and dark conditions. (a) WT Col and ga1-3 seedlings were grown in vermiculite and after three weeks, when *ga1-3* primary roots were approximately 2.5 cm long, the mutant was treated with 10μM GA_3. Values are mean of 25 seedlings ± SE. (b) Primary root length of ga1-3 grown for one week on agar plates containing 0.5% sucrose, with or without 1μM GA_3, under light or dark conditions or in the D-root or D-shoot growth systems in the light. Data are graphically presented as whisker and box plots (n= 15 ± SE). Stars represent significant differences between respective treatments by Student's t test (**P<0.01, ***P<0.005, ****P<0.001). White triangles show the average values.

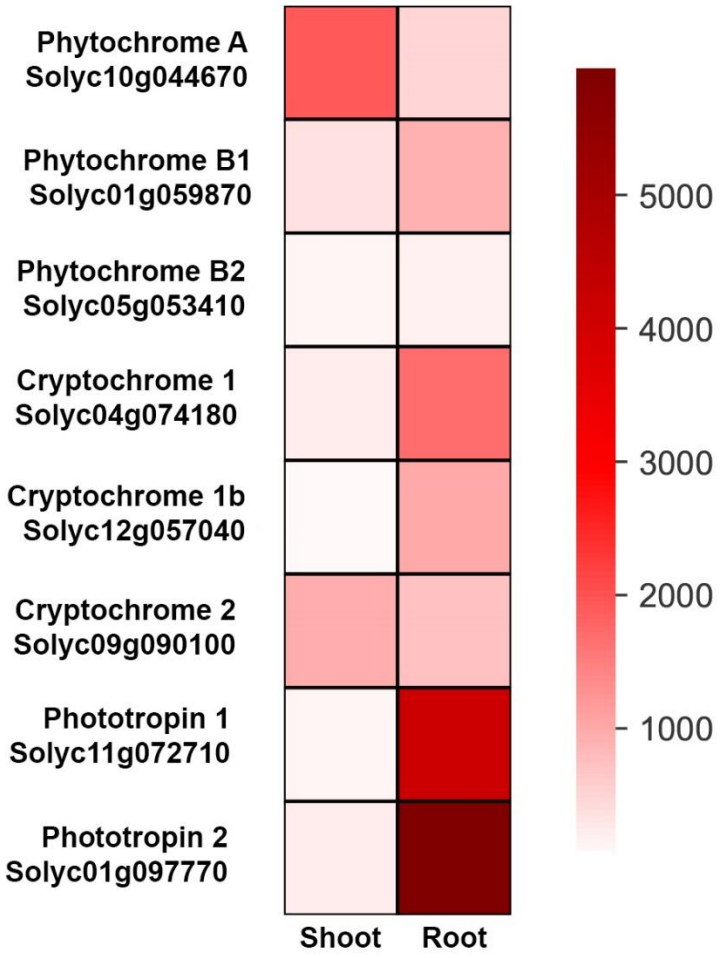

Fig. S13. Normalized reads count of the different photoreceptors (with theie accession numbers) in tomato M82 shoot and root. The data were taken from the RNAseq analysis (see Material and Methods).

Tabl2 S1. Primers used in this study:

Gene	Used for	Sequence (5'-3')
ACTIN	RT-qPCR	Forward- GTCCTCTTCCAGCCATCCAT Reverse- ACCACTGAGCACAATGTTACCG
GID1a	RT-qPCR	Forward- ATGGCAAGAAATAATGAAGCTGT Reverse- CGAAGCTACTTATCTCATCCATG