

Potential Solution to Global Warming: Influences of Plant Hormones on the Growth of *Arabidopsis thaliana* under Drought and Mild Heat Stresses

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Nowadays, global warming induces growing environmental stresses, such as heat and drought, which significantly inhibit plant growth and threaten global food security. This study investigated the potential of plant hormones to enhance stress resilience in *Arabidopsis thaliana* ecotypes *Landsberg erecta* (*Ler*) and *Columbia-0* (*Col-0*). The plant responses under simulated drought (50 mM and 250 mM D-mannitol) and mild heat (26° and 30°) stresses were studied, separately, by measuring the growth of the roots and some of the shoots. The influences of abscisic acid (ABA), 6-benzylaminopurine (6-BA), gibberellins (GA), and (2,4-Dichlorophenoxy) acetic acid (24D) were first assessed under 250 mM D-mannitol induced drought. The influence of GA on *Ler* and *Col-0* was further investigated and compared under drought and mild heat stresses. In addition, advanced 3D micro-Computed Tomography (micro-CT) technology was employed and demonstrated to be effective in plant research as a high-resolution, non-destructive tool. In addition, quantitative real-time polymerase chain reaction (PCR) analyzed the expression level of key genes ent-kaurene oxidase (KO) and ent-kaurenoic acid oxidase (KAO), and further provided evidence to confirm the effect of the GA. Our results demonstrated the importance of hormone GA in stress adaptation of the *Arabidopsis thaliana*, though its effect may vary between ecotypes and plant types. These findings still highlight the potential of hormones to improve plant resilience under climate change and plant tolerance to global warming.

Keywords: Drought stress, mild heat stress, plant hormone, gibberellins, gene expression, root, shoot

Introduction

Global warming refers to the gradual increase in Earth's overall temperature due to the cumulative greenhouse gases¹. Research indicates that with the continuous rise of temperature, plants would have to grow under elevated atmospheric temperature and prolonged drought stresses. Concurrently, global warming is projected to cause a continued reduction in precipitation. It is predicted to negatively impact the global crop productivity².

Both mild heat and drought conditions can significantly inhibit plant growth and may lead to global food shortages³. Study has found that elevated temperature decreases biological activity of wheat directly, thereby reducing yields and posing challenges to agricultural development⁴. Drought is another main abiotic stress, which causes loss of crop yields⁵. Numerous studies have been conducted to address this critical issue. However, an effective and low-cost solution remains challenging. Recently, new approaches have been explored, such as plant growth regulators or plant hormones, whose knowledge could be transferred from the laboratory to the real crop production. This work studied how plant hormones stimulate *Arabidopsis thaliana* growth under drought or mild heat stresses and compared the impact of four major hormones on two different ecotypes, which haven't

been studied systematically before.

Plant hormones, also called phytohormones, are organic compounds synthesized by plants. Though those compounds exist in the plants at an extremely low concentration (ppm level), they play crucial roles in controlling plant growth, development, and their stress response. They function in complex ways by coordinating the physiological processes such as seed germination, root and shoot elongation, flowering, fruit ripening, and defense mechanisms against stresses. Also, different hormones influence plant growth differently, and their impacts also vary with concentration⁶. Among them, abscisic acid (ABA), 6-benzylaminopurine (6-BA), gibberellins (GA), and (2,4-Dichlorophenoxy) acetic acid (24D) were widely studied and have been demonstrated to be effective in promoting plant growth but with different mechanisms⁷⁻¹⁰. Abscisic acid (ABA) is a key plant hormone to help plants adapt the environmental challenges. It affects not only seed dormancy, cell growth, and leaf abscission, but also regulates stomatal closure and root development under unfavorable conditions⁷. The cytokinin 6-Benzyladenine (6BA) modulates plant growth and development by stimulating cell division and promoting cell enlargement⁸. Gibberellins (GA), a large family of tetracyclic diterpenoid phytohormone, has also been demonstrated to be crucial for promot-

ing stem elongation, seed germination, and fruit development, etc.⁹. 2,4-dichlorophenoxyacetic acid (24D), a synthetic auxin, promotes plant growth and wound healing at low concentrations^{9,10}. Although the influence of those four hormones has been studied, a systematic comparison of those hormones on the same plant is absent. In addition, *Arabidopsis thaliana* was selected as the model plant for two reasons. First, it has a short life cycle, small size, and hardiness, making it excellent to serve as a model plant species. Second, the completion of its genome sequencing in 2000 by the international Arabidopsis research community established a solid foundation for in-depth studies of this model organism and further application of results to other plants¹¹. *Landsberg erecta* (*Ler*) and *Columbia-0* (*Col-0*) are two typical ecotypes of *Arabidopsis thaliana* with the same life cycle. However, *Col-0* is a standard, reference model as a wild type, while *Ler* is the results of an erecta mutant. It has been previously reported that *Ler* shows an escape strategy while *Col* shows a tolerance strategy to drought stress¹². This difference allows the study of understanding how the phytohormones stimulate the two ecotypes' response to stresses. The results will provide important evidence on how the gene variation impacts the plant response differently to the environment.

This work primarily investigated the root growth of the plants as it controls the total nutrition and water uptake of the plant and are particularly vulnerable to stressful environments, thereby inhibiting the overall plant growth. Hence, a detailed investigation of root development under stressful environment and exploration of improvement strategies are essential for maintaining and elevating plant productivity.

Nevertheless, the underground nature of roots brings substantial observational challenges compared to aboveground tissues, making root studies more difficult and less common. Therefore, the use of novel methods and technologies for root observation is crucial for further advancing root growth research. The micro-CT (Micro Computed Tomography) technique employed in this study represents a groundbreaking 3D non-destructive imaging technology with submicron resolution for the root study¹³. This method enables observation of root systems without disturbing the plant growth, signifying a milestone in study of root development.

Overall, this research compared the influence of four major different hormones on the growth of two *Arabidopsis* ecotypes (*Ler* and *Col-0*) under simulated drought or mild heat stresses and revealed the effectiveness of gibberellin (GA) in enhancing plant growth under such stresses. The effects of GA were further confirmed by studying the relationships between the gene KO and KAO expression levels, and plant development. It provided a solution in addressing plants' adaptation disabilities to drought or mild heat stress. As the research was conducted on a model system of *Arabidopsis thaliana*, our results are valuable for studying how the hormone regulates plants and for investigating potential solutions to future crises in possible food shortage.

However, because of the observed slight difference in the influences of phytohormone on *Ler* and *Col-0* originating from the gene variation, more research is required to achieve its final application on other plants. This work also has two primary limitations. First, only two different concentrations of hormones were investigated, and the two concentrations were selected based on previously literature¹⁴⁻¹⁷. Second, all experiments were conducted under controlled environment. No experiments were conducted in the fields or other plants.

Methods

Seed treatment and plant cultivation

Arabidopsis thaliana Columbia-0 (*Col-0*) and *Landsberg erecta* (*Ler*) were planted as following. Approximately 200 seeds for each type were surface sterilized by 1 mL 75% ethanol for 15 min and then washed three times by deionized water and kept at 2-4° for 2 days. The seeds were planted in a half-strength Murashige & Skoog (1/2 MS) medium, which contains 2.2 g/L MS salt, 0.5 g/L 2-morpholinoethanesulfonic acid, and 10 g/L sucrose with a pH value of 5.8, adjusted by 1M NaOH. 10 g agar was added to the mixture. The mixture was autoclaved at 121° for 20 min and marked as 1/2 MS. Under sterile conditions, the 1/2 MS medium was poured into Petri dishes and solidified. When the D-mannitol and hormones were applied, they were mixed with the 1/2 MS medium at the denoted concentrations before the medium was poured into the dishes. All ingredients used above were purchased from Sigma Aldrich. The surface-sterilized seed was planted in two rows at 10 seeds/row on the medium. Sealed plates were vertically incubated under 4000-5000 Lux light intensity under conditions as below:

All seeds were grown on 1/2 MS medium for 14 days with a light/dark cycle of 16 hours/8 hours per day. Drought stress was simulated by adding D-mannitol into the medium at 22°, and the concentration of mannitol in the medium were controlled at 50 mM or 250 mM (9.1 g/L or 45.5 g/L, respectively)¹⁸. Mild heat stress was simulated by growing the plant at 26° or 30° without adding mannitol in the medium. The control samples grew at 22° on the 1/2 MS medium. The concentration of the hormone in this work corresponds the final hormone concentration in the medium. Four biological replicates were conducted and placed randomly in the chamber.

Measurement of shoots and roots

In the experiment, the longest root was defined as the primary root. The other roots with a length not shorter than 1 mm were counted and defined as lateral roots. The length of the primary roots and the number of lateral roots were measured on the plants after 14-day growth. The morphology of the shoots and roots was recorded by photos. The fresh weight of the shoots was

measured with a balance. All results are the average of 20 plants total, which were randomly selected from 4 biological replicates, 5 plants each. Three technical replicates were conducted.

Measurement of KAO and KO gene expression level

For quantitative reverse transcription-polymerase chain reaction (qRT-PCR), the extraction of RNAs and reverse transcription was conducted as being described previously¹⁹. The procedures were as following: the total RNA was extracted from *Arabidopsis thaliana* tissues using Trizol reagent. liquid nitrogen frozen tissue (50-100 mg) was homogenized in 1 mL Trizol and then sat for 5 min, followed by centrifugation at 12,000 rpm under 4° for 5 min. The supernatant was mixed with 200 μ L of chloroform and centrifuged again. The liquid was mixed with 500 μ L of isopropanol. The mixture was stabilized for 20-30 min at -20°. Then, the precipitate was mixed with 1.5 mL of 75% ethanol, centrifuged at 10000 rpm for 5 min. The precipitate was collected and dissolved in 25 μ L RNase-Free water (DERC-treated water)^{20,21}.

The complementary DNA (cDNA) was synthesized by mixing the reverse transcription solution and the genomic DNA (gDNA) removal solution, followed by treatments at 42° for 15 min and 93° for 3 min. The reverse transcription solution was prepared through mixing 2 μ L 10 Fast RT Buffer, 1 μ L RT Enzyme Mix, 2 μ L FQ-RT Primer Mix, and 5 μ L RNase-Free ddH₂O. The gDNA removal solution is obtained by mixing 2 μ L 5x gDNA Buffer and 8 μ L RNA mixture as prepared above and treated at 42° for 3 min.

KAO and KO gene expression was quantified by Bio-Rad CFX96 using 20 μ L PCR reagent, TOYOBO SYBR® Green Real-time PCR Master Mix (Cat # QPK-201) containing 10 μ L Master Mix, 0.8 μ L 10 μ M Forward Primers, 0.8 μ L 10 μ M Reverse Primers, and 8.4 μ L the synthesized cDNA²². The qRT-PCR results are shown as relative transcript levels, which were normalized by the ACTIN primers. The ACTIN genes were ACTIN-QF: TGGCATCAYACTTTCTACAA and ACTIN-QR: CCACCACTDAGCACAATGTT. The reference gene used for KO measurements are LW1662-KO-QRT-F1: ATGAAACGTCAGATCTTCAC and LW1664-KO-QRT-R1: TGATGCAATCTCAGAGGAT. The reference gene used for KAO measurements are LW1665-KAO-QRT-F1: ATGATCTG-GCTAAGCTCGA and LW1667-KAO-QRT-R1: AACAGAG-TATGTAAGTCTG. All experiments included three biological replicates were analyzed and three technical replicates. The tested cycle threshold (C_T) for each measurement was then normalized by the C_T of the corresponding reference genes, marked as $\Delta\Delta C_T$, and the gene expression level was the average of $2^{-\Delta\Delta C_T}$.

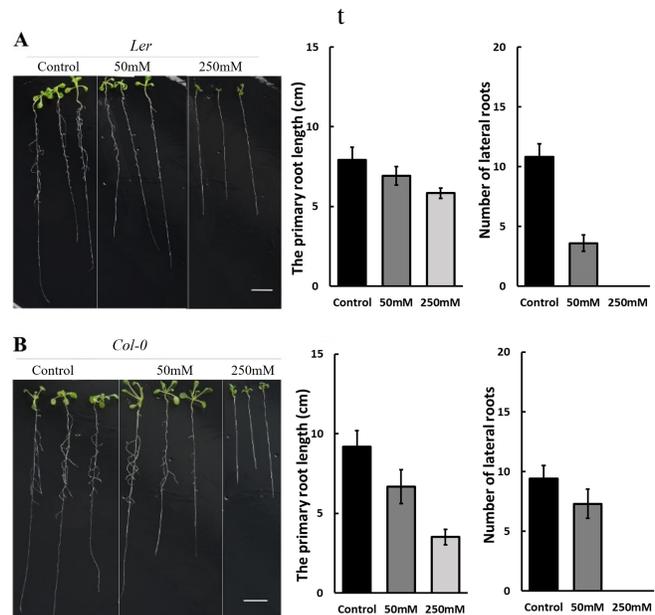


Fig. 1 Comparison of the morphologies and root developments of *Arabidopsis thaliana* A) *Ler* and B) *Col-0* after 14-day growth under drought stresses as simulated by the 1/2 MS mediums with 0 mM (control), 50 mM and 250 mM D-mannitol. The values of the y-axis are the average \pm standard deviation. The comparison showed significant difference at the $p < 0.05$ level. The scale bar in the photos on the left represents a length of 1 cm. Further data analysis was listed in Appendix, Table A-1.

Micro-computed tomography scanning of *Arabidopsis thaliana*

High-resolution micro-computed tomography (micro-CT; Xradia 520 Versa) was employed to analyze the *Arabidopsis thaliana* growth, using a voltage of 50 kV, a current of 100 μ A and exposure time of 0.4 mS. It is a non-destructive 3D imaging technique with a submicron resolution. This approach enabled in situ scanning of plants in soil while maintaining the plant structural integrity, thereby yielding comprehensive 3D morphological data for multiaxial growth analysis. In this work, the 1/2 MS medium was solidified in a 50 mL centrifugal tube. The seeds were planted on the medium and the roots grew in the tube for observation.

Results

Arabidopsis thaliana growth under simulated drought or mild heat stress

D-mannitol (mannitol) has been demonstrated to be an effective chemical to simulate drought stress by its osmotic effect²³. The stress level can be controlled by the mannitol concentration. This work studied the growth of *Arabidopsis thaliana* under

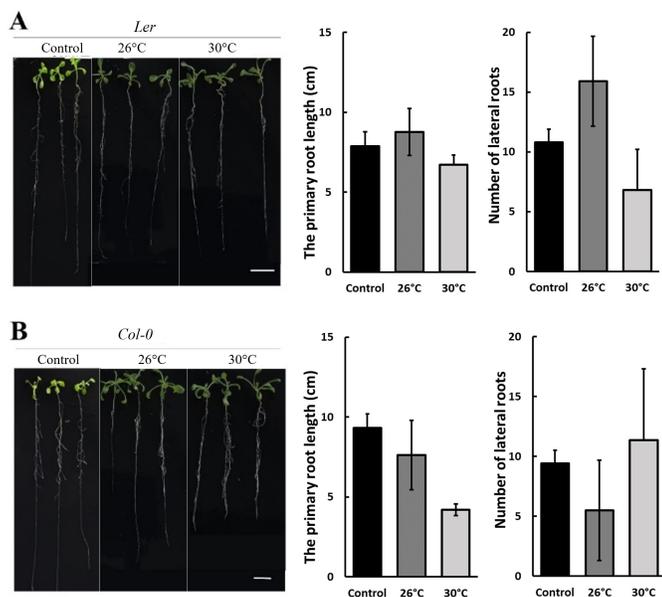


Fig. 2 Comparison of the morphologies and root developments of *Arabidopsis thaliana* A) *Ler* and B) *Col-0* after 14-day growth under mild heat stresses as simulated by 26° and 30° with control at 22°. The values of the y-axis are the average \pm standard deviation. The comparison showed significant difference at the $p < 0.05$ level. The scale bar in the photos on the left represents a length of 1 cm. Further data analysis was listed in Appendix, Table A-2.

drought induced by 50 mM and 250 mM mannitol in the 1/2 MS medium. Figure 1 compares the growth of *Ler* and *Col-0* under concentrations of 0 mM (control), 50 mM, and 250 mM D-mannitol at 22°, suggesting that the primary root length and number of lateral roots were inhibited with the increasing concentration of mannitol. The inhibition is more significant on *Col-0* than *Ler* in the primary root length, while more significant on *Ler* in the number of lateral roots. The photos on the left in Figure 1 further show that the shoots grown under 50 mM mannitol have similar size as the control and is significantly larger than that under 250 mM-induced stress, suggesting a more severe growth inhibition by 250 mM mannitol.

The mild heat stress was studied by growing the *Arabidopsis thaliana* in a constant temperature incubator. *Ler* and *Col-0* grew at temperatures of 22° (control), 26°, and 30° with no mannitol. Figure 2 shows that compared to the control, the primary root length of *Ler* increases slightly at 26° but decreases about 20% at 30°, while the number of lateral roots increases by 30% at 26° and decreases by 40% at 30°. The shoots grow stronger at 26° than control, suggesting 26° is favored by *Ler*. The primary root length of *Col-0* reduced to about 75% and 50% at 26° and 30° compared to 22°, but no clear temperature dependence was observed for the number of lateral roots. The shoot of *Col-0* is larger when growing at elevated temperatures, as shown by the photos on the left in Figure 2B.

Hormone influences on the growth of *Arabidopsis thaliana* under drought simulated by 250 mM D-mannitol

250 mM D-mannitol simulated drought was selected as the stress for the hormone screening. Abscisic Acid (ABA), 6-Benzylaminopurine (6BA), 2,4-Dichlorophenoxyacetic acid (24D), and Gibberellins (GA) were selected because they have been demonstrated to be effective in enhancing plant growth by other studies 6-11. The growth of the primary roots and lateral roots of *Ler* and *Col-0* were further inhibited by 1 μ M and 10 μ M ABA (Figure 3A & 4A) or 0.1 μ M and 1 μ M 24D (Figure 3C & 4C) under 250 mM D-mannitol induced drought. The growth of the shoots was inhibited significantly by ABA and 24D. In addition, the 0.1 μ M and 1 μ M of 6BA also inhibited the primary root growth of *Ler* and *Col-0* ($p < 0.05$), but it stimulated the lateral root growth of *Ler* ($p < 0.05$) (Figure 3B). In addition, the 6BA maintained the shoot growth of *Ler* but inhibited the shoot growth of *Col-0*.

1 μ M of GA slightly increased the primary root length of *Arabidopsis* and notably enhanced the number of lateral roots in comparison with the control (Figure 3D & 4D). 10 μ M of GA has no significant effects ($p > 0.05$) on their growth except for about 25% reduction of the primary root length in *Col-0*. We concluded that low concentrations of GA (1 μ M) effectively improved the root length and lateral root quantity, together with an improved growth of the shoots under simulated drought conditions (250 mM D-mannitol).

Non-destructive 3D micro-CT to monitor the growth of *Ler* under stress

3D X-ray micro-CT technology has been an important tool in medical and biological studies²⁴. It records 2D project images by transmitting X-rays through the sample and processes the 2D into 3D models. It quantitatively measures the morphology of a living organ and enables in-vivo monitoring of the sample growth at a sub-micron meter scale. However, it has been rarely used in plant and agricultural research. In this study, we have demonstrated its effectiveness in measuring the growth of *Arabidopsis thaliana* without disturbing its growth.

The *Arabidopsis thaliana Ler* was cultivated under 30° mild heat or drought simulated by 50 mM mannitol. We compared its root growth with and without 1 μ M GA, as shown in Figure 5. After adding GA, *Ler*'s root volume significantly increased under either simulated drought or mild heat stresses, and more lateral roots grew than those having no GA added. The effect of GA was more effective for plants under mild heat than drought. The results agree well with what shown in section 3.2 and confirmed that the plant hormone is capable to boost the growth of the plant. In addition, the micro-CT technology has also been demonstrated to be effective on studying the growth of plants with no destruction.

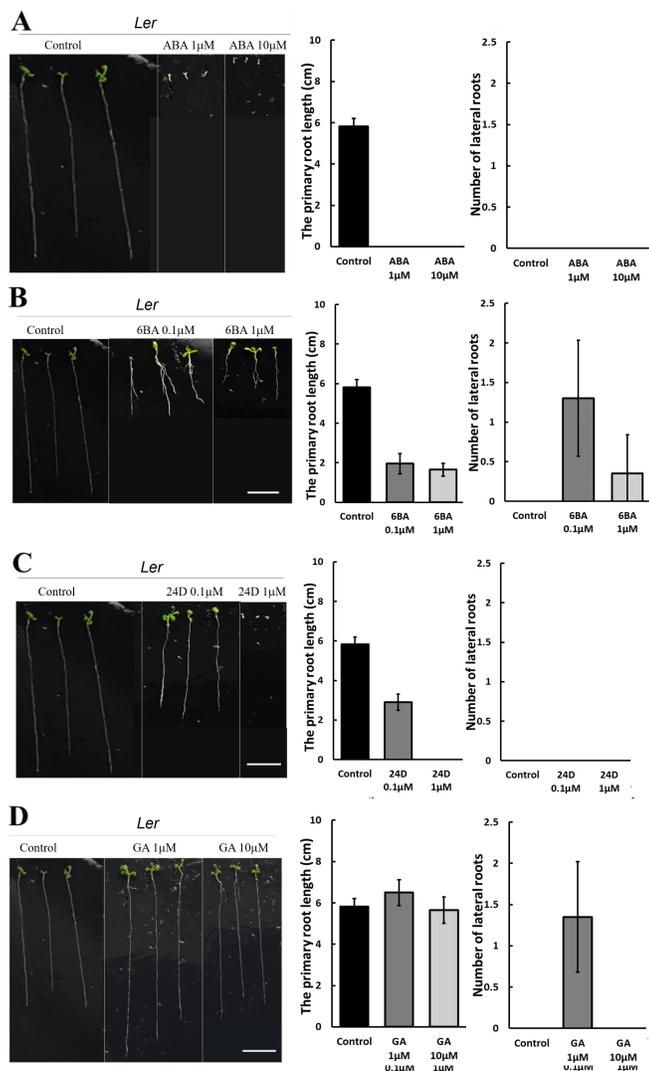


Fig. 3 Hormone influence on the morphologies and root developments of *Ler* as a function of its concentration in the 1/2MS medium. The plants were measured after 14-day growth under drought stresses as simulated by the 1/2 MS mediums with 250 mM D-mannitol. The hormones studied were A).ABA, B).6BA, C).24D, D).GA. The values of the y-axis are the average \pm standard deviation. The scale bar in the photos on the left represents a length of 1 cm. Further data analysis was listed in Appendix, Table A-2.

Influence of GA on the growth of *Ler* and *Col-0* under stress

Sections above have proved that 1 μ M of GA effectively enhanced both the root and shoot growth of *Arabidopsis thaliana* under 250 mM D-mannitol-induced drought. Its influence was further studied under 50 mM D-mannitol-induced drought and 30° stresses, respectively.

Figure 6 showed that 1 μ M GA enhanced the growth of *Ler* more effectively than *Col-0* under 50 mM D-mannitol simulated

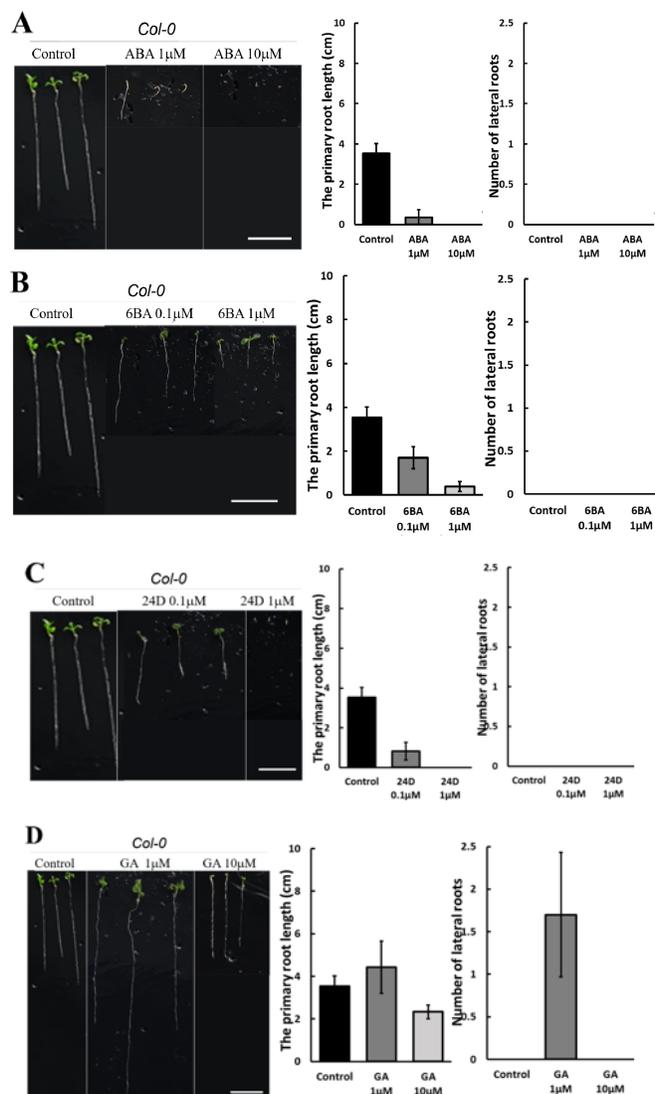


Fig. 4 Hormone influence on the morphologies and root developments of *Col-0* as a function of its concentration in the 1/2MS medium. The plants were measured after 14-day growth under drought stresses as simulated by the 1/2 MS mediums with 250 mM D-mannitol. The hormones studied were A). ABA, B). 6BA, C). 24D, D). GA. The values of the y-axis are the average \pm standard deviation. The comparison showed significant difference at the p_i 0.05 level except those having no lateral roots. The scale bar in the photos on the left represents a length of 1 cm. Further data analysis was listed in Appendix, Table A-4.

drought. The primary root length of *Ler* increases about 40%, and the number of lateral roots increases more than 10 times, which is much higher than the increase in *Col-0*, 20% and 4 times. The photos on the left in Figure 4 also suggest a significant increase in the *Ler* shoot, with a weight increase of three times. Though the shoot dimension of the *Col-0* seems

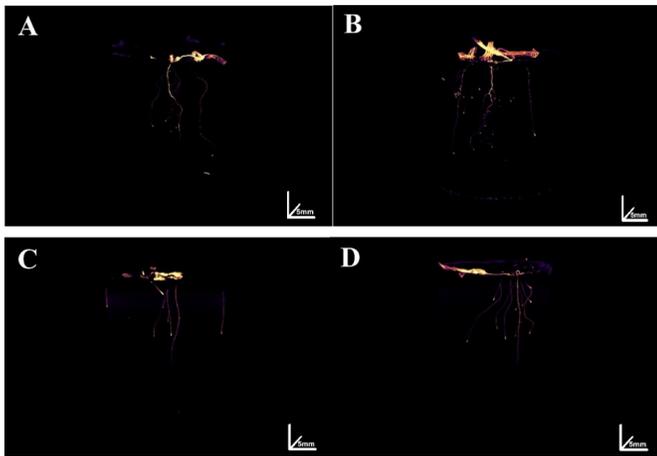


Fig. 5 3D micro-CT image to compare the influence of 1 μM GA on the morphologies and root developments of *Ler* after 14-day growth under: A). at 30° with no hormones in the 1/2 MS mediums; B). with 1 μM GA in the medium at 30°, C). under drought simulated by 50 mM mannitol in the 1/2 MS medium, D). with 1 μM GA and 50 mM mannitol in the medium.

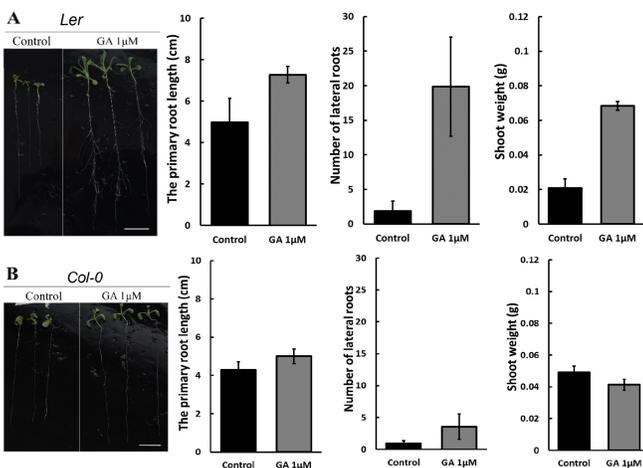


Fig. 6 Comparison of the influence of 1 μM GA on the morphologies and root developments of *Ler*(A) and *Col-0* (B) after 14-day growth under 50 mM D-mannitol simulated drought stress at 26°. The values of the y-axis are the average \pm standard deviation. The comparison showed significant difference at the $p < 0.05$ level for *Ler* and not difference of the primary root length and shoot weight of *Col-0* ($p > 0.05$). The scale bar in the photos on the left represents a length of 1 cm. Further data analysis was listed in Appendix, Table A-5.

larger with GA on the photo, the shoot weight remained after adding 1 μM GA, suggesting GA has no impact on the *Col-0* shoot growth.

The impact of 1 μM GA on the growth of *Ler* and *Col-0* under 30° heat stress is also studied as summarized in Figure 7. 1 μM of GA enhanced the root growth of *Ler* and *Col-0* similarly, with an increase in the primary root length by 20-

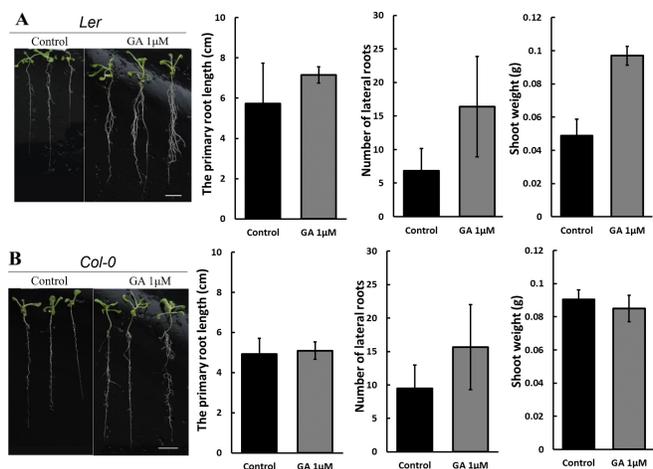


Fig. 7 Comparison of the influence of 1 μM GA on the morphologies and root developments of *Ler* (A) and *Col-0* (B) after 14-day growth under 30° stress. The values of the y-axis are the average \pm standard deviation. The comparison showed significant difference at the $p < 0.05$ level except the shoot weight of *Col-0*. The scale bar in the photos on the left represents a length of 1 cm. Further data analysis was listed in Appendix, Table A-6.

30%, the number of lateral roots by about 2 times, and a similar increase in the shoot morphology visually. However, the lateral roots were observed to be stronger and longer in *Ler*. The shoot weight of *Ler* grown under 1 μM GA is about two times larger than the control, while GA didn't show an increase in the weight of *Col-0* shoot, although its morphology seems larger than the control in Figure 7B.

Overall, the addition of GA enhances the growth of *Ler* and *Col-0*. This is consistent with the established model which suggests that GA promotes the plant growth by triggering the degradation of the DELLA repressor proteins. The DELLA proteins were accumulated when the plant experienced stress conditions. Therefore, the proteins inhibited the plant growth. Its degradation recovered the plant growth again²⁵.

The KO and KAO gene expression levels of *Arabidopsis thaliana* under stresses

We also measured the gene expression level of the KO and KAO in *Ler* and *Col-0* grown under drought or mild heat stress, respectively. Both KO and KAO genes are key synthetic enzyme genes involved in the GA (gibberellin) synthesis in plants. The results, as shown in Figure 8, indicate that the KAO gene expression level of *Ler* is doubled and KO is tripled compared with the control when growing under drought stress. Under mild heat, the expression level of the KAO gene was also doubled, while the KO gene expression level increased about seven times. For *Col-0*, the expression levels of the KAO and KO genes also showed significant increases under drought stress, which are

about 6 times and 3 times, respectively. Under heating conditions, the KAO gene exhibited less than a two-fold increase in expression level, whereas the KO gene showed a slightly more than two-fold increase. The increase in KO and KAO expression level is an indication of the improved amount of GA in the plant, which improves the plant resilience to the stress²⁶. Our results confirmed that the production of GA is an important process for plant adaption to stress and provided evidence for the statement that the GA enhances the growth of *Arabidopsis thaliana* under stresses effectively. The work also indicated the different response of *Ler* and *Col-0* to GA under that same concentration. It could be attributed to the phenotypic variation, which results in the variations in the response of hormone concentration and signaling in plants²⁷. This finding provides a theoretical explanation for GA in enhancing the plant adaption to stressful environments and the differences in various plants.

Discussion

Global warming become a worldwide problem today. Studies have predicted that in about 100 years, the global average temperature would increase by 1.0 to 3.9 °; and in approximately 300 years, the increase could reach 0.6 to 7.8 ° or even higher. If greenhouse gas emissions rise further, the increase in the average temperature would become more significant²⁸.

Global warming is regarded as a major threat to agriculture as it will bring abiotic stresses to plant growth. The threat from drought stress and mild heat stress to crop growth has attracted much attention²⁹, as they would substantially limit the crop growth and reduce their yields, therefore affecting the global food supply³⁰.

In this work, we explored the possibility of using phytohormone to alleviate this issue and concluded that GA should be one solution to help crops adapt to the stresses and resume their productivity.

Plants are considered to grow under stress when the environment is not ideal. The adverse conditions include drought, mild heat, cold, salinity, and flooding, etc. This research studied the growth of *Arabidopsis thaliana*, a model plant, under simulated drought and mild heat stresses. The results showed that drought stress simulated either 50 mM or 250 mM mannitol inhibited the growth of *Ler* and *Col-0* significantly. The inhibition becomes more severe at a higher mannitol concentration, representing a harsher stress. There are no lateral roots under 250 mM mannitol. This could be understood by a higher osmotic stress induced by the higher mannitol concentration³¹.

The growth of *Arabidopsis thaliana* was also observed at 26° and 30° to simulate the mild heat stress with a control at 22°. At 26°, there was a light increase in the primary root length or the number of lateral roots for *Ler*, suggesting that 26° is favorable for *Ler*. At 30°, the root growth of *Ler* was significantly suppressed, suggesting that 30° is a stressful environment

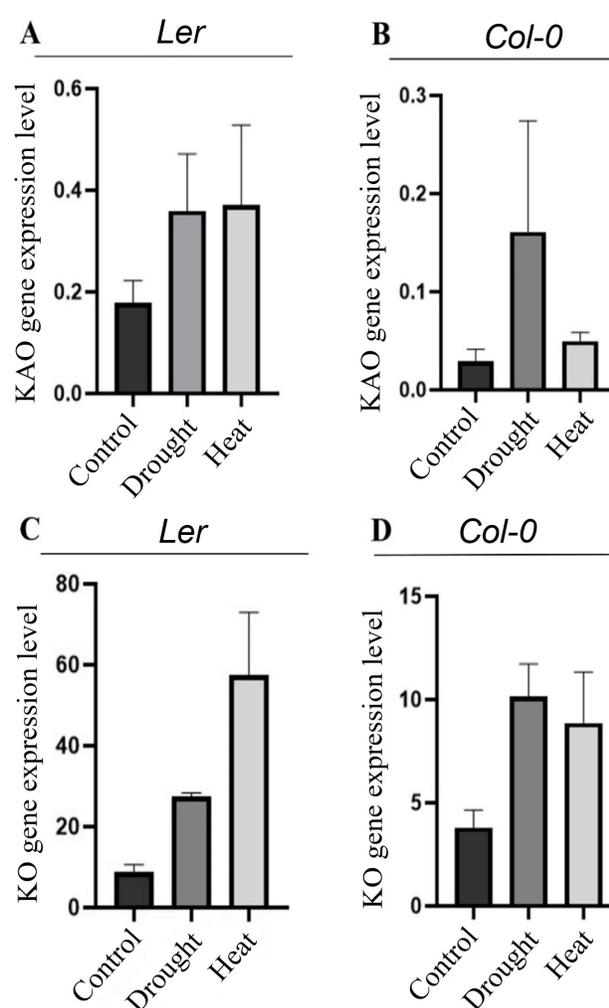


Fig. 8 Comparison of the 1 μ M GA influence on the KO and KAO gene expression level of *Ler* and *Col-0* grown under drought and mild heat. A). the KAO gene expression level of *Ler*, B). the KAO gene expression level of *Col-0*, C). the KO gene expression level of *Ler*, D). the KO gene expression level of *Col-0*. The reported values are measured based on four biological duplicates. The values of the y-axis are the average \pm standard deviation. The comparison showed significant difference at the $p < 0.05$ level.

for *Ler*. The average root length of *Col-0* grown at 26° and 30° was reduced by 25% and 50%. No obvious inhibition was observed on the lateral root growth. The results indicate that the mild heat stress caused by global warming negatively impacts plant root growth, and the impact becomes more severe at higher temperatures. Meanwhile, as *Arabidopsis thaliana* is a model plant, the response of *Ler* and *Col-0* shared the same trend but still with little difference.

To explore solutions to accelerate the plant adaptation to stress and improve their productivity, four plant hormones were investigated, including GA (gibberellin), ABA (abscisic acid), 6-BA

(a synthetic cytokinin), and 24D (2,4-dichlorophenoxyacetic acid). Bharath et al. have found that ABA, as a stress hormone, accumulates in plant under stress to defend against the environment³². However, this study found that the 1 μM and 10 μM ABA completely inhibited the root growth of *Arabidopsis thaliana Ler* and *Col-0* and reduced the shoot growth significantly while *Ler* and *Col-0* still grew under control conditions. This is different from what Miao et al. observed, that the addition of ABA improves the root growth of *Col-0* under water strength³³. Hormone ABA functions as either a promoter or an inhibitor, depending on the type of stress, its concentration, and other influencing factors^{34,35}. Our results suggested that under drought or mild heat stress, several μM ABA inhibits the growth of *Ler* and *Col-0*. 0.1 μM and 1 μM 6BA hormone also inhibit the shoot and the primary root growth of *Ler* and *Col-0* under drought or mild heat stress, as shown in Figure 3 and 4. The 6BA did stimulate the lateral root of *Ler* grow more than control under drought as the function of 6BA observed in tortisepalum, where the 6BA promotes the growth of the lateral buds³⁶. The effect is more prominent at 0.1 μM , suggesting that an overwhelming high concentration of phytohormone may suppress the plant growth.

Hormone 2,4-dichlorophenoxyacetic acid (24D) is a synthetic auxin, used to promote root and shoot growth at low concentration and is normally used to regulate the plant response to environmental stresses. Comparing to control in Figure 3 and 4, the 0.1 μM and 1 μM hormone 24D didn't show any impact on the lateral root growth of *Ler* and *Col-0* under simulated drought conditions. They even inhibited the primary root growth by 50% at 0.1 μM and 100% at 1 μM . The results indicate that though 24D is used as an herbicide-free growth enhancer for crops like wheat and rice³⁷. It doesn't help to stimulate the *Ler* and *Col-0* growth under drought stress at similar concentration. That means when we select plant hormone, both the hormone concentration, type and the plant species need to be considered.

In contrast, GA is proved to be very helpful for root growth under stresses. Figure 3 and 4 showed that 1 μM GA significantly promoted both the shoot, the primary root length and the number of lateral roots of *Ler* and *Col-0*, while 10 μM GA inhibits their growth comparing to control under drought when simulated by 250 mM mannitol. Results suggested 1 μM GA is an appropriate concentration to stimulate the plant grow under stresses. 1 μM GA is also demonstrated to be effective to enhance the root growth of *Ler* and *Col-0* under stresses as simulated by 50 mM mannitol and 30° mild heat. It is also effective for the shoot growth of *Ler* but the shoot growth of *Col-0* at 1 μM GA under drought and mild heat is like the control. The positive effects of GA on the growth of *Arabidopsis thaliana* is further confirmed by the increase in the expression levels of KO and KAO. KO and KAO are two critical gene existing in the GA synthesis pathway³⁸. The increase in the KO and KAO gene expression level indicates an improved amount of GA is synthe-

sized. Our study showed that under simulated drought and mild heat stresses, the expression levels of KO and KAO genes in both *Col-0* and *Ler* significantly increased, indicating to adapt the stress, the plant synthesized more GA. This means more GA would help the plant to grow better under stress. Moreover, the results agree well with our results that the introduction of GA promotes the growth of plant under drought and mild heat stresses.

Among all four hormones measured in this study, only GA greatly enhanced the growth of *Col-0* and *Ler* under simulated stresses. Comparing to control, the addition of GA improves a significant amount of the root growth and the aboveground shoot biomass. It restores the growth of *Ler* and *Col-0* under stresses to that in normal environments.

In addition, the Micro CT technology was confirmed our conclusion further by scanning the vivo-plants grown under simulated mild heat or drought stresses. The *Arabidopsis thaliana* was compared with and without the addition of 1 μM GA, resulting in complete 3D views of the roots. The results clearly showed that plants treated with GA had better root growth, and both the number of lateral roots and root length increased significantly, with more pronounced effects under mild heat stress.

The hormone ABA, 6BA, 24D and GA worked differently on the growth of *Arabidopsis thaliana*, and the same hormone also worked differently on *Ler* and *Col-0*, indicating the importance of the natural variation. The results revealed that only GA improves the *Arabidopsis thaliana* growth significantly under the drought or mild heat stresses. The enhancement in the *Ler* and *Col-0* ecotype growth are slightly different, possibly due to the different genetic background. The improved KO and KAO gene expression levels further confirmed the positive effect of adding GA on the plant growth as they are key gene for GA biosynthesis in plants. The finding provides valuable insights for studying the function of hormones in plant growth. Appropriate utilization of GA could provide a potential solution to mitigate the risk of reduced plant growth under environmental stresses.

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Appendix

Ler	Condition	Mean	SD	95%confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	7.91	0.81	7.53	8.29
	50mM	6.92	0.58	6.64	7.19
	250mM	5.84	0.33	5.68	5.99
Lateral root number	Control	10.80	1.11	10.28	11.32
	50mM	3.60	0.68	3.28	3.92
	250mM	0.00	0.00	0.00	0.00
Col-0	Condition	Mean	SD	95%confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	9.19	1.02	8.71	9.66
	50mM	6.68	1.07	6.17	7.18
	250mM	3.52	0.49	3.28	3.75
Lateral root number	Control	9.40	1.10	8.89	9.91
	50mM	7.30	1.22	6.73	7.87
	250mM	0.00	0.00	0.00	0.00

Table A-1. The calculated mean, standard deviation (SD) and 95% confidence interval for data in Figure 1.

<i>Ler</i>	Condition	Mean	SD	95%confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	7.88	0.89	7.46	8.29
	26°C	8.77	1.47	8.08	9.45
	30°C	6.72	0.59	6.44	7.00
Lateral root number	Control	10.80	1.11	10.28	11.32
	26°C	15.90	3.77	14.14	17.66
	30°C	6.80	3.41	5.20	8.40
<i>Col-0</i>	Condition	Mean	SD	95%confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	9.32	0.87	8.91	9.73
	26°C	7.63	2.17	6.61	8.65
	30°C	4.20	0.38	4.02	4.37
Lateral root number	Control	9.40	1.10	8.89	9.91
	26°C	5.50	4.17	3.55	7.45
	30°C	11.35	5.95	8.57	14.13

Table A-2. The calculated mean, standard deviation (SD) and 95% confidence interval for data in Figure 2.

<i>Col-0</i>	ABA	Mean	SD	95%confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	3.53	0.49	3.30	3.76
	ABA 1mM	0.35	0.39	0.17	0.54
	ABA 10µM	0.00	0.00	0.00	0.00
Lateral root number	Control	0.00	0.00	0.00	0.00
	ABA 1µM	0.00	0.00	0.00	0.00
	ABA 10µM	0.00	0.00	0.00	0.00
<i>Col-0</i>	6BA	Mean	SD	95%confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	3.53	0.49	3.30	3.76
	6BA 0.1µM	1.70	0.50	1.47	1.93
	6BA 1µM	0.39	0.22	0.29	0.49
Lateral root number	Control	0.00	0.00	0.00	0.00
	6BA 0.1µM	0.00	0.00	0.00	0.00
	6BA 1µM	0.00	0.00	0.00	0.00
<i>Col-0</i>	24D	Mean	SD	95%confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	3.53	0.49	3.30	3.76
	24D 0.1µM	0.82	0.45	0.60	1.03
	24D 1µM	0.00	0.00	0.00	0.00
Lateral root number	Control	0.00	0.00	0.00	0.00
	24D 0.1µM	0.00	0.00	0.00	0.00
	24D 1µM	0.00	0.00	0.00	0.00
<i>Col-0</i>	GA	Mean	SD	95%confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	3.53	0.49	3.30	3.76
	GA 1µM	4.43	1.23	3.85	5.00
	GA 10µM	2.33	0.32	2.18	2.49
Lateral root number	Control	0.00	0.00	0.00	0.00
	GA 1µM	1.70	0.73	1.36	2.04
	GA 10µM	0.00	0.00	0.00	0.00

Table A-3. The calculated mean, standard deviation (SD) and 95% confidence interval for data in Figure 3

<i>Ler</i>	ABA	Mean	SD	95%confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	5.82	0.38	5.65	6.00
	ABA 1µM	0.00	0.00	0.00	0.00
	ABA 10µM	0.00	0.00	0.00	0.00
Lateral root number	Control	5.82	0.38	5.65	6.00
	ABA 1µM	0.00	0.00	0.00	0.00
	ABA 10µM	0.00	0.00	0.00	0.00
<i>Ler</i>	6BA	Mean	SD	95%confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	5.82	0.38	5.65	6.00
	6BA 0.1µM	1.95	0.52	1.71	2.20
	6BA 1µM	1.65	0.33	1.49	1.80
Lateral root number	Control	0.00	0.00	0.00	0.00
	6BA 0.1µM	1.30	0.73	0.96	1.64
	6BA 1µM	0.35	0.49	0.12	0.58
<i>Ler</i>	24D	Mean	SD	95%confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	5.82	0.38	5.65	6.00
	24D 0.1µM	2.89	0.41	2.70	3.09
	24D 1µM	0.00	0.00	0.00	0.00
Lateral root number	Control	0.00	0.00	0.00	0.00
	24D 0.1µM	0.00	0.00	0.00	0.00
	24D 1µM	0.00	0.00	0.00	0.00
<i>Ler</i>	GA	Mean	SD	95%confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	5.82	0.38	5.65	6.00
	GA 1µM	6.50	0.63	6.21	6.79
	GA 10µM	5.64	0.64	5.34	5.94
Lateral root number	Control	0.00	0.00	0.00	0.00
	GA 1µM	1.35	0.67	1.04	1.66
	GA 10µM	0.00	0.00	0.00	0.00

Table A-4. The calculated mean, standard deviation (SD) and 95% confidence interval for data in Figure 4.

<i>Ler</i>	GA	Mean	SD	95% confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	4.98	1.146	4.45	9.43
	GA 1µM	7.27	0.399	7.09	14.36
Lateral root number	Control	1.85	1.461	1.17	3.02
	GA 1µM	19.85	7.169	16.49	36.34
Shoot weight (g)	Control	0.021	0.005	0.02	0.04
	GA 1µM	0.068	0.002	0.07	0.14
<i>Col-0</i>	GA	Mean	SD	95% confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	4.29	0.430	4.09	8.38
	GA 1µM	5.01	0.381	4.83	9.84
Lateral root number	Control	0.95	0.394	0.77	1.72
	GA 1µM	3.55	1.986	2.62	6.17
Shoot weight (g)	Control	0.049	0.004	0.05	0.10
	GA 1µM	0.041	0.003	0.04	0.08

Table A-5. The calculated mean, standard deviation (SD) and 95% confidence interval for data in Figure 6.

<i>Ler</i>	GA	Mean	SD	95% confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	5.74	2.010	4.79	10.53
	GA 1µM	7.15	0.406	6.96	14.10
Lateral root number	Control	6.85	3.329	5.29	12.14
	GA 1µM	16.40	7.500	12.89	29.29
Shoot weight (g)	Control	0.049	0.010	0.04	0.09
	GA 1µM	0.097	0.006	0.09	0.19
<i>Col-0</i>	GA	Mean	SD	95% confidence interval	
				Low limit	Up limit
Primary root length (cm)	Control	4.93	0.783	4.56	9.49
	GA 1µM	5.10	0.432	4.89	9.99
Lateral root number	Control	9.50	3.472	7.88	17.38
	GA 1µM	15.65	6.343	12.68	28.33
Shoot weight (g)	Control	0.090	0.006	0.09	0.18
	GA 1µM	0.085	0.008	0.08	0.17

Table A-6. The calculated mean, standard deviation (SD) and 95% confidence interval for data in Figure 7.