scientific reports

Check for updates

OPEN

Microwave pre-stimulation methodology for plant growth promotion

Nobuhiro Suzuki[1], Yasuhiko Hasegawa[1], Kanae Kadomatsu[1], Kazuha Yamakawa[1], Miori Sameshima[1], Atsumi Ando[2] & Satoshi Horikoshi[1]✉

Although positive effects of microwave irradiation on plants have been reported, their underlying mechanisms remain unknown. In this study, we investigated the effects of low microwave irradiation on *Arabidopsis thaliana*. Interestingly, we found low output (23 W) with oscillating condition (not continuous irradiation) promoted plant growth. The microwave irradiation neither raised the plants' temperature nor induced heat responsive gene expression. Furthermore, overall transcriptome profile in microwave irradiation treated plants were significantly different from heat treated plants, suggesting that growth promotion might be attributed to non-thermal effects of microwave. Transcriptome and metabolome analysis indicated that microwave irradiation altered circadian clock as well as hormonal response especially in auxin and gibberellin, which promoted plant growth by inducing amino acid biosynthesis and stress tolerance, and reducing cell wall thickness. This finding potentially contributes to develop new approach to increase food production through accelerating crop yield in environmentally friendly way.

Keywords Microwave stimulation, Electromagnetic field effects, Growth acceleration of plants, Molecular and physiological mechanisms, Microwave pulse irradiation

To meet the food demands of the rapidly growing global population, it is urgent to increase food production by accelerating crop yield. While genetic modification of crops has been proposed as an efficient method to boost crop yield, legal restrictions limit its application in several countries. Therefore, it is essential to establish efficient methods to increase crop yield that do not rely on genetic modifications.

In 1856 James Clerk Maxwell developed the theory that the energy of the electromagnetic field exists not only within the conductors but also in the space surrounding them. By 1864 he had formulated his own electromagnetic theory, predicting that both light and radio waves stem from electric and magnetic phenomena[1]. This groundbreaking insight of electromagnetic waves has deepened our understanding of the interplay between electromagnetic waves (especially radio waves) and biological processes. Microwaves, which are the main focus of this paper, are also electromagnetic waves with frequencies ranging from 300 GHz to 300 MHz (i.e., from a wavelength of 1 mm to 1 m)[2].

Microwaves can be used as an energy source to induce movement (heat generation) in material, and as radio waves or radar for transmitting information. Microwaves serve as a heat source for sintering materials and used in chemical synthesis, referred to as microwave chemistry[3]. On the other hand, early reports that investigated the effects of microwaves on living organisms primarily focused on enzymatic reactions in the latter half of the 20th century, with the first study appearing in the early 1970s. For instance, microwave irradiation has been widely employed to inactivate the enzymes in the brain of animals since Stavinoha and coworkers first pioneered it in 1970[4]. Inactivation of these enzymes allows for sampling and measurement of many brain neurochemicals, reducing the influence of postmortem alterations[5]. Usage of microwaves has succeeded in selectively stopping enzymatic activity with minimal damage to other cells and/or proteins under in vivo conditions, for which equipment is commercially available.

Then how do microwaves affect plants? Most reports addressing this question demonstrated that microwaves from WiFi and mobile phones negatively affected growth of plants. In this regard, Soran and coworkers[6] investigated the influence of microwave irradiation at bands corresponding to a wireless router (WLAN) and mobile devices (GSM) on leaf anatomy, essential oil content, and volatile emissions of *Petroselinum crispum* (Parsley), *Apium graveolens* (Celery), and *Anethum graveolens* (Dill). Their findings suggest that microwave

[1]Department of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, 7-1 Kioicho, Chiyodaku, Tokyo 102-8554, Japan. [2]Sumitomo Chemical Co., Ltd, 2-1 Takatsukasa 4-chome, Takarazuka, Hyogo 665-8555, Japan. ✉email: horikosi@sophia.ac.jp

irradiation resulted in thinner cell walls, and reduced size of the chloroplasts and mitochondria, as well as enhanced emissions of volatile compounds, particularly the monoterpenes and green leaf volatiles. On the other hand, several studies demonstrated the positive effects of microwave on plant growth. For example, Tafforeau and co-workers have shown that irradiation of a 105 GHz microwave at non-thermal levels induced production of meristems with a plateau of around six meristems in plants[7]. Additionally, Saitou et al. reported that irradiation of continuous microwave on spinach during the entire lifecycle resulted in improved germination rate and root elongation rate[8]. Furthermore, it has been also reported that microwave can be utilized to enhance tolerance to abiotic stresses[9], induce mutagenesis for breeding[10], accelerate in vitro regeneration from callus[10] and extend the shelf-life of the tomato fruit[11].

Previous studies have highlighted the pivotal roles of plant hormones in the regulation of plant growth. Notably, auxin and gibberellic acid (GA) play crucial roles in accelerating cellular growth by modulating cell wall extensibility, thereby promoting leaf growth[12,13]. Numerous proteins activated by auxin or GA signals, as well as other plant hormones such as cytokinin and brassinosteroids, have also been identified as contributors to growth acceleration[13]. In addition, GA and abscisic acid (ABA) exert control over seed germination. GA is renowned for its role as a accelerator of seed germination[14], while ABA functions antagonistically to GA, inhibiting seed germination[14]. Furthermore, there is evidence of integration between the circadian clock and hormone signaling. For example, the PHYTOCHROME INTERACTING FACTOR 4 (PIF4) protein, regulated by the circadian clock, function together with auxin, GA, and brassinosteroid signaling to govern plant growth[15]. Transcriptome analysis of Arabidopsis plants has also unveiled the influence of the circadian clock on auxin biosynthesis, perception, and signaling[16].

Several studies have proposed that microwave effects on plants are mainly due to a temperature increase in potting soils, water, and plants thierselves. However, a few research reports hypothesized that non-thermal effects have progressed because the power of the microwave irradiation is weak, and the temperature rise has not been monitored[8]. Some studies suggest that microwaves do not impact plant growth. For instance, Balodis and co-workers conducted a 20-year study of tree growth in and around Skrunda radio location station[17]. They analyzed the dynamics of tree growth based on retrospective tree ring data, but no significant effects on tree growth were observed. The consistency across various these studies is low. One of the possible reasons for such ambiguous results is that these studies employed a microwave oven which has low reproducibility. Such a situation makes it challenging to have a more-in-depth discussion of the mechanism from the perspective of electromagnetic waves.

At some point, we serendipitously discovered that irradiating plants with weak microwaves for one hour without raising the temperature can improve their growth rate[18]. In addition to promoting growth, these plants also demonstrated insect repellent properties and enhanced heat tolerance[19]. Just as we have been researching for over 25 years to elucidate the non-thermal effects of microwaves on chemical reactions[20], we could easily imagine translating this into biology. In this study, we, therefore, analyzed in detail the positive effects of microwave irradiation on growth parameters in plants and their underlying mechanisms. The output of this research can lead to increase in crop yields.

Experimental setup

Plant

Arabidopsis thaliana (ecotype Columbia-0) WT and mutant deficient in PHYB (*phyB-9*[21]; kindly provided by Prof. Ron Mittler) were grown on peat pellet under the controlled conditions (21°C, 16 h light cycle, 50 μmols^{-1} m^{-2}) as previously described[22]. Fourteen or fifteen-d-old plants of similar size were selected and irradiated with microwave for 1 h with the condition described below (see "Microwave irradiation conditions"), and analyzed in growth and molecular characteristics and stress tolerance as described below. Following the microwave irradiation, 14-d-old plants were grown under the control, and diameter, fresh weight and dry weight of rosette were scored every 3–7 days for 22–42 days. After state transition to reproductive growth stage, inflorescent stem length was also measured every 4 days from 16 to 20 days following the microwave irradiation. Weight and number of seeds per 5 plants were also counted at 45, 53, 58 and 63 days after microwave irradiation. For the analyses of these growth parameters.

Microwave irradiation conditions

Commercial microwave irradiation system of Milestone General Co., Ltd., Start SYNTH was used as an apparatus for microwave irradiation to *Arabidopsis thaliana*. The 2.45-GHz magnetrons, serving as microwave generators with a power range of 23 to 120 W (W), respectively, are installed in multimode microwave cavity 370 (depth) × 345 (width) × 335 (height) mm with a mode stirrer. The temperature of plants and peat pellet was measured not only by the optical fiber thermometer and radiation thermometer attached to the Start SYNTH, but also by another optical fiber thermometer (FL-2000, Anritsu Meter Co. Ltd.).

The microwave irradiation conditions were examined using a microwave heating device consisting of a single-mode applicator connected to a semiconductor microwave generator. This device afforded highly accurate microwave irradiation control (Fig. 1). The microwave irradiation setup with the single-mode cavity TE_{103} (transverse electric 103 mode), used to irradiate the 14-d-old *Arabidopsis thaliana* planted in a peat pellet and schematically illustrated in Fig. 1, included a short plunger, an iris, and an *E*/*H*-tuner. The continuous microwave radiation was generated from the 2.45-GHz microwave semiconductor generator containing an isolator (LEANFA Srl; GEAP-A003A01; 2.45 GHz; maximum power, 250 W). The length of the single mode applicator was 49 cm; the length of the cavity was changed moving the short plunger. The resonance of the microwaves was adjusted with the iris and the plunger at 1.5 cycles. *Arabidopsis thaliana* samples were achieved by positioning the samples at positions of maximal *E*-field density (position (i) in Fig. 1A, inset *i*) within the waveguide in the single-mode microwave apparatus of Fig. 1A, B. The incident and reflected waves of the microwaves

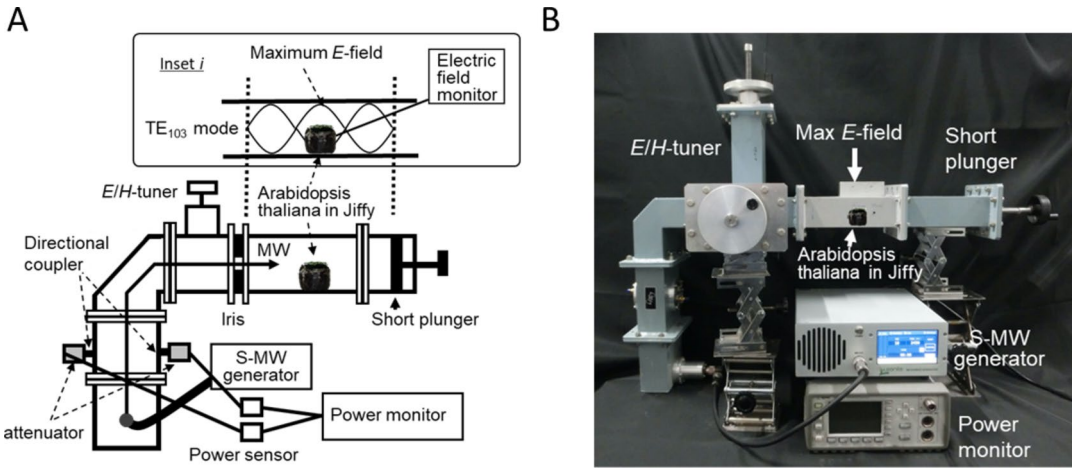


Fig. 1. (**A**) Schematic and (**B**) photograph of the microwave irradiation system consisting of a 2.45-GHz semiconductor microwave generator (S-MW generator), an optical fiber thermometer, a power sensor, a power monitor and a single mode applicator. Inset *i*: distribution of the electric field (*E*-field) inside the single-mode applicator and location of the samples.

were measured precisely with a power meter (Keysight technologies, E4419B EPM series dual-channel power meter) and a power sensor (Keysight technologies, E9300A E-series average power sensor), respectively. These were cabled in the directional coupler (Fuji Electronic Industrial Co. Ltd., E-049 directional coupler) on the waveguide, by inserting an attenuator (Keysight technologies, 8496B manual attenuator) between the directional coupler and the power sensor. The actual microwave output was attenuated and displayed on the power meter. These calibrations were performed by Fuji Electronic Industrial Co. Ltd. Note that the reflected experimental power was less than 0.1 W. To confirm that plants were not exposed to high temperature during microwave irradiation, the temperature of the plants was measured with a radiation thermometer (AR-1500, Anritsu Meter Co. Ltd.) and an optical fiber thermometer (FL-2000, Anritsu Meter Co. Ltd.) through a hole drilled in the top of the single-mode applicator.

The wavelength of propagation of the microwaves in the TE_{103} mode within the waveguide was ca. 14.78 cm, estimated from Eq. (1)[23].

$$\lambda = \frac{\lambda_0}{\sqrt{1 - \left(\frac{\lambda_0}{2b}\right)^2}} \tag{1}$$

where λ is the wavelength in the waveguide; λ_0 (2.45 GHz) = 12.24 cm is the wavelength in vacuum given by c/f, {c being the speed of light, 2.9979×10^{10} cm s^{-1}, and f being the microwave frequency 2.45×10^9 s^{-1}, i.e. 2.45 GHz}; and b is the long side height of the waveguide, 10.92 cm. The maximal position of the *E*-field from the iris was at 1/2 the wavelength of the standing wave in the waveguide, namely 7.39 cm.

The *E*-field intensity (maximum value for V cm^{-1} unit) of an Arabidopsis leaf placed in the microwave cavity was measured by using an optical electric field sensor with SH-10MS sensor head (manufactured by Seiko Giken Co., Ltd.).

Analytical setup

Molecular and physiological analysis

Fourteen-d-old plants were treated with microwave irradiation as above and rosettes of these plants were sampled immediately after the treatment. For comparison, rosettes were also sampled from 14-d-old plants subjected to sub-lethal heat stress (40 °C, 1 h) using a growth chamber (LH-80LED-DT, NK system, Tokyo, Japan). To analyze expression of transcripts involved in the regulation of circadian clock, 14-d-old plants were treated with microwave irradiation as above and grown for additional 14 days under the controlled conditions (16 h light from 10:00 PM to 6:00 AM). These plants were then transferred to the conditions with constant light at 10:00PM and sampled every 4 h. RNA was extracted from the samples as previously described[22]. At least 15 plants were included in one sample. Quantitative real-time PCR (qPCR) analysis was performed in an optical 96-well plate with the Quantstudio 3 system (Applied Biosystems) and the Thunderbird qPCR Master Mix (Toyobo, Tokyo, Japan). Threshold cycle values for *Multiprotein bridging factor 1c* (*Mbf1c*: At3g24500), *Heat shock protein 90* (*Hsp90*: At5g52640) and 101 (*Hsp101*: At1g74310) were calculated with the CT of *Elongation factor 1-a* (*Ef1-a*: At5g60390) as an internal control. For the analyses of *Timing of Cab expression 1* (*Toc1*: At5g61380), *Circadian clock associated 1* (*Cca1*: At2g46830) and *Late elongated hypocotyl 1* (*Lhy1*: At1g01060) transcripts, primers from a previous study were employed[24]. *Protein phosphatase 2 A* (*Pp2A*: AT1G13320) was used as an internal control. Primer pairs used for amplifications are shown in Supplemental Table 1. For the analyses of HSPs or MBF1c protein, rosettes of 14-d-old plants treated with microwave irradiation or sub-lethal heat stress were sampled immediately after these treatments. For the analysis of FT protein, 14-d-old plants were treated with microwave

irradiation or sub-lethal heat stress, and rosettes of these plants were sampled immediately after these treatments and at 4 and 8 days following these treatments. Protein extraction and western blot analysis were conducted as previously described[22]. MBF1c antibody was generated as previously described[25]. Antibodies against HSP70 or FLOWERING LOCUS T (FT) were purchased from Funakoshi Inc. H_2O_2 was measured using Amplex Red reagent as previously described[26]. Anthocyanin was measured as previously described[27]. Total soluble sugar was measured by anthrone method as previously described[28].

Microarray analysis

RNA samples prepared as described above were used for the microarray analysis. One color microarray analysis was conducted by using Arabidopsis Oligo DNA microarray Ver 4.0 in the National Agricultural and Food Research Organization. Following data analysis was conducted by using custom R code. PCA analysis was conducted by using R function "prcomp" (https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/prcomp). To identify differential expression genes (DEGs), we calculated the average expression level and performed Student's *t*-test. Transcripts with p-value < 0.05 were then extracted. Of these, transcripts exhibited the differential expression of more than 1.0 on the log2 fold change-scale were selected. Gene Ontology (GO) analysis was conducted by using R with Bioconductor package clusterProfiler (https://Bioconductor.org/packages/release/bioc/html/clusterProfiler.html). KEGG pathway analysis was conducted by using R with Bioconductor package pathview (https://Bioconductor.org/packages/release/bioc/html/pathview.html; https://pathview.r-forge.r-project.org/pathview.pdf) and enrichplot (https://Bioconductor.org/packages/release/bioc/html/enrichplot.html). Heatmap, dot plot and Venn diagram were generated by using pheatmap (https://www.rdocumentation.org/packages/pheatmap/versions/1.0.12/topics/pheatmap), ggplot2 (https://ggplot2.tidyverse.org/) and VennDiagram (https://cran.r-project.org/web/packages/VennDiagram/index.html), respectively. The raw data of the microarray analysis was uploaded to Gene Expression Omnibus (Accession number: GSE241899).

Untargetted metabolome analysis

Fourteen-d-old plants grown on the peat pellet were treated with microwave irradiation and rosettes of these plants were sampled immediately after the irradiation as described above. Untargeted metabolome analysis of these samples was performed by Human Metabolome Inc. The samples were ground in 700 µL of methanol including 50 mL of internal standard material by using a homogenizer (1000 rpm, 60 s, two times) at 4 °C. Samples were then mixed with 700 µL of double distilled water and centrifuged (2300 g, 4 °C) for 5 min. After centrifugation, 400 µL of the aqueous layer was transferred to an ultrafiltration tube (Ultrafree MC PLHCC, HMT, centrifugal filter unit 5 kDa) and untrafiltration was carried out by centrifugation (9,100×g, 4 °C, 120 min). The filtrate was dried and then dissolved again in 50 µL of double distilled water. The samples were subsequently analyzed by using Agilent CE-TOFMS systems in both cation and anion mode. The conditions of the measurement were indicated in Supplemental Table 2. The peak detected by CE-TOFMS was analyzed by MasterHands ver.2.17.1.11. Peaks with a signal/noise (S/N) ratio more than 3.0 were automatically extracted, and the mass-to-charge ratio (m/z), peak area value, and migration time (MT) were obtained. Based on the m/z and MT values of the detected peaks, all substances registered in the metabolite library of the Human Metabolome Inc. and the Known-Unknown library were collated and searched.

Results and discussions

Microwave effect(s) on plant growth promotion

To quantitatively evaluate the effects of microwave irradiation to plant growth, seedlings of *Arabidopsis thaliana*, a model plant which has been extensively used for the plant biology researches, were subjected to microwave irradiation and subsequent changes in growth characteristics were investigated (Fig. 2, Supplemental Fig. 1). The plants exposed to microwave irradiation exhibited larger rosette diameter compared to those kept under the control condition (Fig. 2A). This trend started to be apparent from approximately 10 days following the microwave irradiation. However, microwave irradiation did not positively affect the fresh and dry weight of rosettes (Fig. 2B, C). Notably, even short period of microwave irradiation to 14-d-old seedlings resulted in accelerated growth of inflorescent stem during later growth stage (Fig. 2D). In addition, microwave irradiation resulted in increased seed weight and a higher number of seeds per 5 plants (Fig. 2E, F). These results suggest that a short period of microwave irradiation on seedlings positively affect growth of Arabidopsis plants both during vegetative and reproductive stages.

Since the growth of inflorescence stems of plants was enhanced by the microwaves, we proceeded to analyze the expression of FT protein which is known for its role in the state transition from vegetative to reproductive stage[29]. Accumulation of FT protein was not affected immediately after the treatment with microwave irradiation or sub-lethal heat stress (Fig. 3A, Supplemental Fig. 2A, D). However, the level of FT protein declined at 4 days following these treatments. After 8 days, the level of FT protein declined in the plants under the control condition but increased in the plants treated with sub-lethal heat stress or microwave irradiation. Notably, after 8 days following treatment, level of FT protein was clearly higher in plants treated with sub-lethal heat stress or microwave irradiation compared to that under the control condition, with the highest expression in plants treated with microwave irradiation. This result suggests that beneficial effects of microwave irradiation on plant growth might be at least partially attributed to altered regulation of the circadian clock. The regulation of flowering by the circadian clock has been extensively studied[30–32]. Harmonized action of the circadian clock and light signaling facilitates the transcription of a gene encoding FT that accelerates flowering via the accumulation of CO protein in the late afternoon under long-day conditions[30,33]. It is necessary to further investigate how the regulation of the circadian clock, the accumulation of FT protein and expression of transcripts involved in inflorescent stem growth are coordinated following the short-term microwave irradiation.

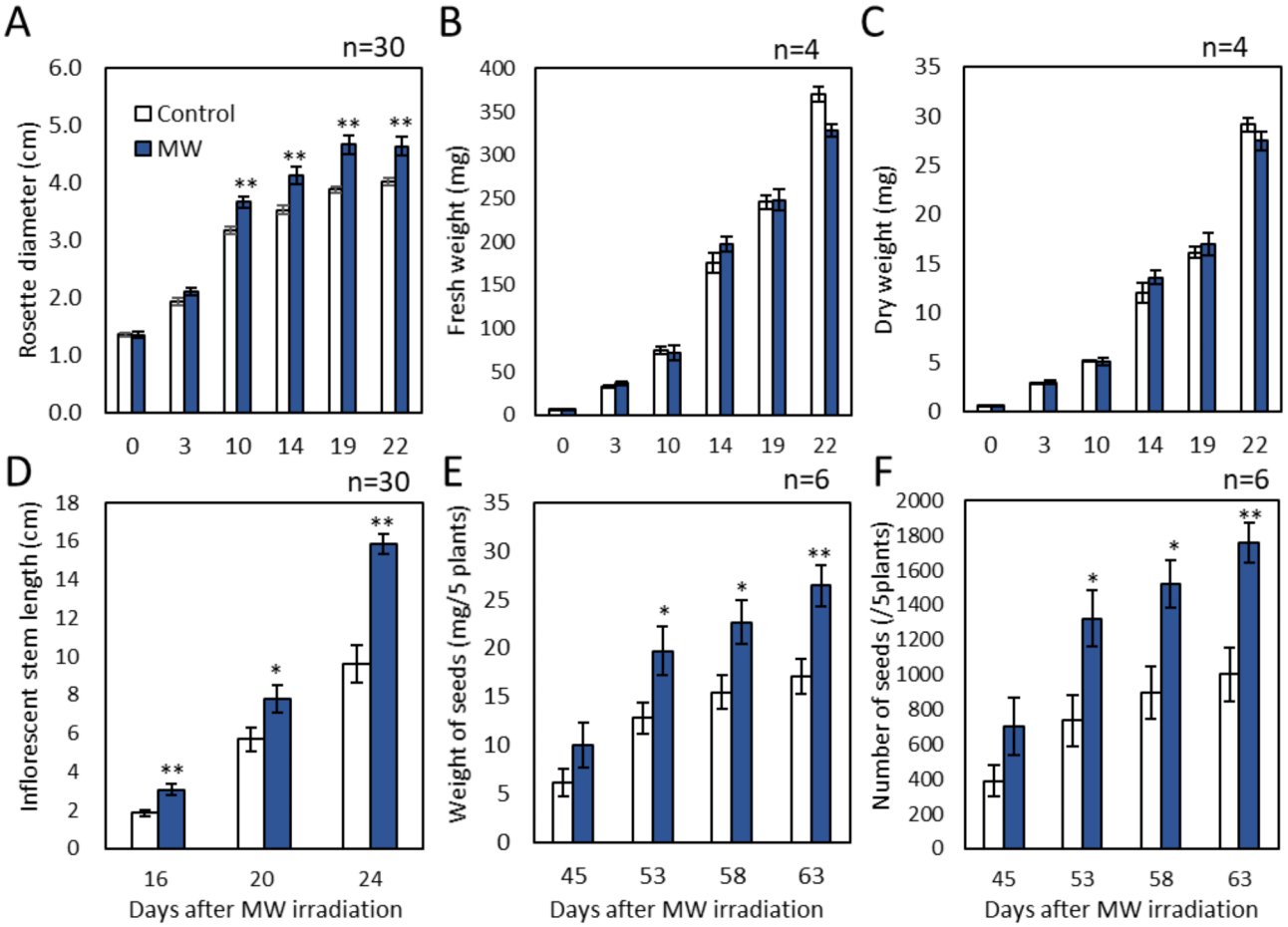


Fig. 2. Effects of microwave (MW) irradiation on growth of Arabidopsis. (**A**–**F**) Effects of MW-irradiation on rosette diameter (**A**), fresh and dry weight of rosette (**B**,**C**), inflorescent stem length (**D**), weight of seed per 5 plants and (**F**) number of seeds per 5 plants. Bars indicate standard error. * and **: Student's *t*-test significant at 5 and 1% compared to control, respectively. "n" indicates the number of plants and replicates. For (**A**) and (**D**), 30 plants were analyzed. For (**B**) and (**C**), 24 plants were used for each treatment, with 4 plants harvested and analyzed for each day. For (**E**) and (**F**), 120 plants were grown for each treatment, and the weight and number of seeds for 6 replicates (total 30 plants) were scored each day.

This result leaves us with one question. Is the role of microwaves on plants simply as an electromagnetic wave source, or is it simply a heat source? To solve this question, we investigated the effects of heat at the microscopic or macroscopic levels.

To investigate effects of microscopic temperature changes, we analyzed the expression of several heat response transcripts and proteins in plants treated with sub-lethal heat stress or microwave irradiation. For sub-lethal heat stress, *Arabidopsis thaliana* plants were exposed to 40 °C for 1 h. As we expected, sub-lethal heat stress dramatically enhanced the expression of *Mbf1c*, *Hsp90* and *Hsp101* transcripts that are known to play crucial roles in plant heat tolerance[34,35] (Fig. 3B–D). The expression of MBF1c and HSP70 proteins was also dramatically increased by this treatment (Fig. 3E, Supplemental Fig. 2B–D). In contrast, the expression of these heat response transcripts and proteins was not clearly altered by microwave irradiation (Fig. 3B–E, Supplemental Fig. 2B–D).

For examining of macroscopic level, the temperature was monitored while *Arabidopsis thaliana* on the 14th day after sowing was irradiated to microwaves for 1 h, we patiently monitored any changes in temperature using a plurality of optical fiber thermometers, and not least by thermography. Especially the finding that temperature of leaves (macroscopic level) did not increase by the conditions of microwave irradiation we employed (Supplemental Fig. 3).

These results suggest that mechanisms contributing to the accelerated growth induced by microwave irradiation might be specifically triggered by microwave electromagnetic effect(s), rather than by microwave thermal effect.

Microwave irradiation conditions and growth promotion

To find out the parameter(s) of microwave that accelerate growth of plants, plants were subjected to microwave irradiation with different conditions and analyzed in growth characteristics. As a standard condition, the plants irradiated with 23 W microwave showed significantly larger rosette diameter and longer inflorescent stem length

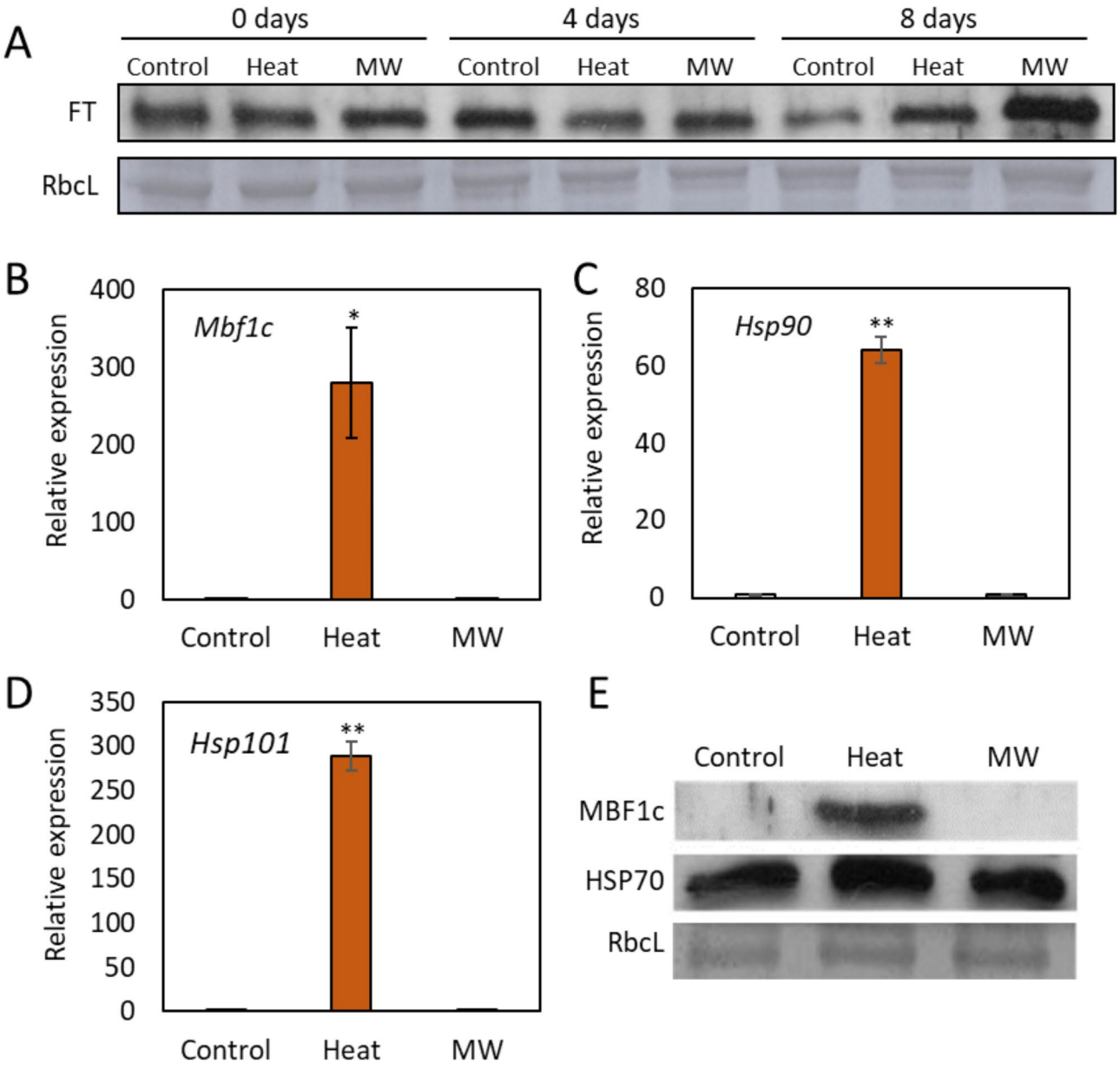


Fig. 3. Expression of proteins and transcripts involved in state transition of growth stages and heat stress responses. (**A**) Expression of FT protein. (**B**–**D**) Expression of transcripts encoding (**B**) MBF1c, (**C**) HSP90 and (**D**) HSP101 during heat stress. Expression of MBF1c and HSP70 during heat stress. * and **: Student's *t*-test significant at 5 and 1% compared to control, respectively ($n = 3$). "n" indicates number of samples used in the experiments. Ten plants were included in one sample. *MW* microwave.

compared to that under the control condition (Fig. 4A, B). It should be noted that the minimum output of microwave irradiation from the StartSYNTH is 23 W. Although microwave irradiation with 26 W resulted in slightly larger rosette diameter, effects of this microwave irradiation are less obvious compared to that with 23 W. Surprisingly, despite not causing a temperature increase in Arabidopsis plants and inducing HSP90 expression, microwave irradiation with 30 W did not alter the rosette diameter and inflorescent stem length. Based on these results, we considered the possibility that varying effects of microwave irradiation could be attributed to the differences in *E*-field intensity that near the surface of Arabidopsis leaves, rather than electric power of microwave. To test this possibility, we conducted in-situ measurement of *E*-field intensity close to the leaf surface of Arabidopsis. There was no significant difference in *E*-field intensity at any output power at 23, 25 and 30 W. However, the *E*-field spectrum revealed that the pattern of the spectrum of microwave with 23 W was different from those with 25 W and 30 W (Fig. 4C). The equipment was used in Tokyo, Japan. Therefore, the power source operates at 50 Hz, and microwaves are oscillated from the magnetron with an average period of 20 ms. Although consistent pulse oscillations were observed at 26 W and 30 W, pulse oscillation at 23 W was irregular. In addition, at 23 W, pulse oscillation paused for as long as 3 min. It might be due to the properties

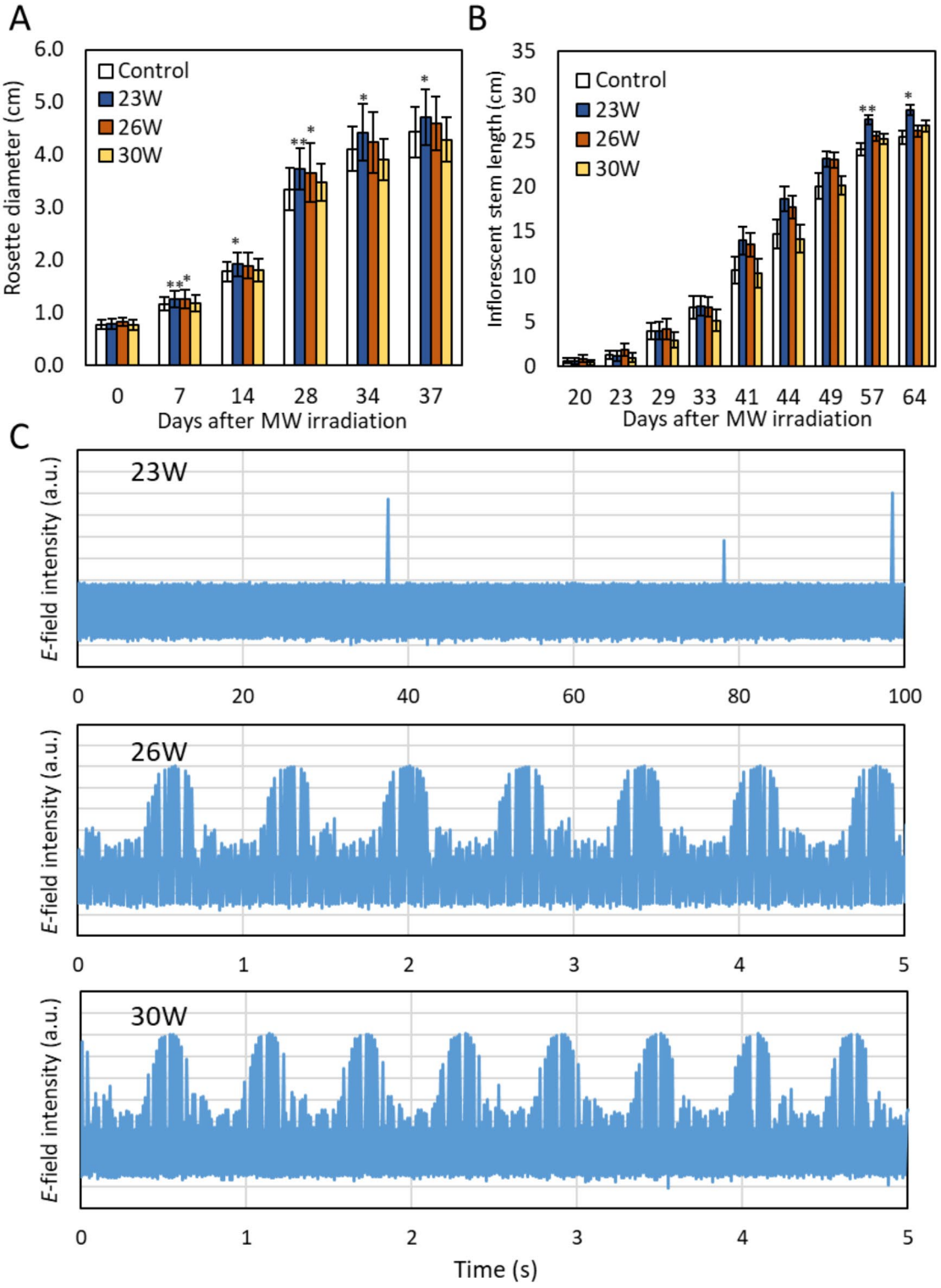


Fig. 4. Temporal change for (**A**) rosette diameter and (**B**) inflorescent stem length for different microwave power. (**C**) Spectrum of microwave pulse oscillation for 23 W, 25 W and 30 W (peaks: electric field intensity). * and **: Student's *t*-test significant at 5 and 1% compared to control, respectively ($n = 17–30$). "n" indicates the number of plants analyzed in the experiments.

of the magnetron. The minimum oscillation power of the magnetron is 23 W, and microwave oscillation is highly unstable at this output. However, despite this instability, positive effects of microwave irradiation at 23 W on growth of Arabidopsis plants were consistently observed. To further understand this phenomenon, we monitored the electric field strength for 1 h to investigate number of oscillations during microwave irradiation.

We found that, on average, the microwaves oscillated 90 ± 6 times across 10 times measurements of electric field. Our monitoring of electric field suggested that there is a regularity in the times of oscillation, even if the period of pulse is unstable. The microwave *E*-field intensity (maximum value) against microwave power irradiated to an Arabidopsis a leaf installed in a single mode in the form of *E*-field intensity, and the *E*-field intensity at 23 W was ca. 972 V m^{-1}, 26 W was ca. 1242 V m^{-1} and at 30 W was ca. 1718 V cm^{-1}.

We investigated whether irregular pulse patterns of *E*-fields spectrum affect plant growth differently. A single mode applicator with a semiconductor microwave generator was utilized to apply continuous or pulse microwave to plants (Fig. 1). Although irradiations of either continuous or pulse microwave at 2.1 W did not affect growth (Fig. 5A), enlargement of rosette diameter was observed when plants were irradiated with pulse microwave at 8.4 W for 1 s (Fig. 5B). As an additional experimental information, it measured the microwave *E*-field intensity (maximum value) against microwave power irradiated to an Arabidopsis leaf installed in a single mode in the form of *E*-field intensity, and the *E*-field intensity at 2.1 W was ca. 753 V m^{-1} and at 8.4 W was ca. 1288 V cm^{-1}. The similar effect was not observed when plants were irradiated with continuous microwave at 8.4 W. These results suggest that pulse microwave as well as certain level of electric energy are required to accelerate the growth of plants. To investigate how different durations of pulse microwave irradiation affect growth of plants, plants were treated with pulse microwave at 8.4 W for 1 s, or 1, 3, 6–10 min with duty cycle of 0.25 (Supplemental Fig. 4). Pulse microwave irradiation for 1 s resulted in enlarged rosette diameter (Fig. 5C). However, irradiation of pulse microwave at 8.4 W for 1, 3–6 min did not alter the rosette diameter. This finding prompted us to investigate how microwave irradiation with different duty cycle affects plant growth. The similar effect on rosette diameter was observed among plants subjected to microwave for 1 min with any duty cycles we tested (0.1, 0.25 or 0.5; Fig. 5D, Supplemental Fig. 4) suggesting that duty cycle is not significant factor to alter the plant growth.

In the pulse microwave irradiation that accelerated plant growth, the total number of irradiations was approximately 90 times. To test whether effects of microwave on plant growth can be altered by total number of irradiations, plants were exposed to microwave with different total numbers of pulse irradiation and scored in rosette diameter (Fig. 5E). Total number of irradiations was adjusted by modulating frequency, duty cycle and duration. In this study, plants were irradiated with the 90 times pulse at 50 Hz frequency with 1 s duration and 0.25 duty cycle, as well as that at 10 Hz frequency with 9 s duration and 0.05 duty cycle. For comparison, plants were also irradiated with the 10 times pulse at 10 Hz frequency with 1 s duration and 0.25 duty cycle. When plants were treated with pulse microwave irradiation at 90 times, enlargement of rosette diameter was observed regardless of frequency or duration of the treatments (Fig. 5E). However, irradiation at 20 times did not result in significant changes in the rosette diameter. These results suggest the significance of the total number of pulse irradiation to accelerated growth of plants. Microwave consists of an electric field and a magnetic field. In the experiments described so far, we focused solely on measuring and discussing the electric field strength without considering the effects of magnetic field. Therefore, we separated electric and magnetic fields by using a single mode applicator to investigate whether either an *E*-field or a magnetic field (*H*-field) affects plant growth. The rosette diameter of plants was largest when irradiated with *E*-field (Fig. 5F). Irradiation of both electric and magnetic field also showed positive effects on growth acceleration. The beneficial effects of magnetic field became evident 18 days following the microwave irradiation.

Comprehensive analyses of transcripts

To investigate the mechanisms underlying effects of microwave irradiation on plant growth, the transcriptome of plants treated with microwave irradiation at 23 W and sub-lethal heat stress for 1 h was investigated by microarray analysis. As shown by the scatter plot of PCA analysis, transcripts significantly altered in their expression by either heat treatment or microwave irradiation exhibited similar behavior and clustered together (Fig. 6A). Sub-lethal heat stress and microwave irradiation enhanced expression of 2271 or 1689 transcripts, respectively (Fig. 6B, Supplemental Tables 3–4). Among these, 1904 or 1322 transcripts were specifically up-regulated by sub-lethal heat stress or microwave irradiation, respectively (Fig. 6B, Supplemental Tables 3, 4), although 367 transcripts were commonly up-regulated in response to both treatments (Supplemental Table 5). Sub-lethal heat stress or microwave irradiation also resulted in down-regulation of 2905 or 294 transcripts, respectively (Fig. 6B, Supplemental Tables 6, 7). Of these, 2810 or 199 transcripts were specifically down-regulated by sub-lethal heat stress or microwave irradiation, respectively (Fig. 6B, Supplemental Tables 6, 7). In addition, 95 transcripts were commonly down-regulated by both treatments (Supplemental Table 8). These results suggest that microwave irradiation might activate specific mechanisms that are not activated by sub-lethal heat stress. Thus, alterations in growth triggered by microwave irradiation might be microwave-specific effects.

Among the transcripts up-regulated by microwave irradiation, those belonging to Gene Ontology (GO) terms, "cellular response to abscisic acid stimulus", "fertilization", "abscisic acid-activated signaling pathway" and "protein localization to cell surface" were specifically enriched (Fig. 6C, Supplemental Tables 9 and 10). Up-regulated transcripts involved in abscisic acid (ABA) responses and signaling included key transcription factors, kinases, phosphatases and ubiquitin ligases (Supplemental Tables 9 and 10). In addition, four transcripts encoding REGULATORY COMPONENTS OF ABA RECEPTORs (RCARs) were up-regulated in response to microwave irradiation (Supplemental Table 10), suggesting that microwave irradiation influences the early stages of ABA signaling and response. The up-regulation of ABA-related transcripts might be linked to the enhanced tolerance to biotic and abiotic stresses suggested in a previous study[19].

Transcripts associated with the GO terms "fertilization" and "protein localization to the cell surface" overlapped (Supplemental Table 10). While previous studies have proposed their significance in egg cell development and sperm cell activation in reproductive organs[36,37], their roles in leaves remain unknown. In this study, we demonstrated that microwave irradiation on seedlings accelerates flowering and increases seed production (Fig. 2). Therefore, further investigation is needed to determine how the enhanced expression of

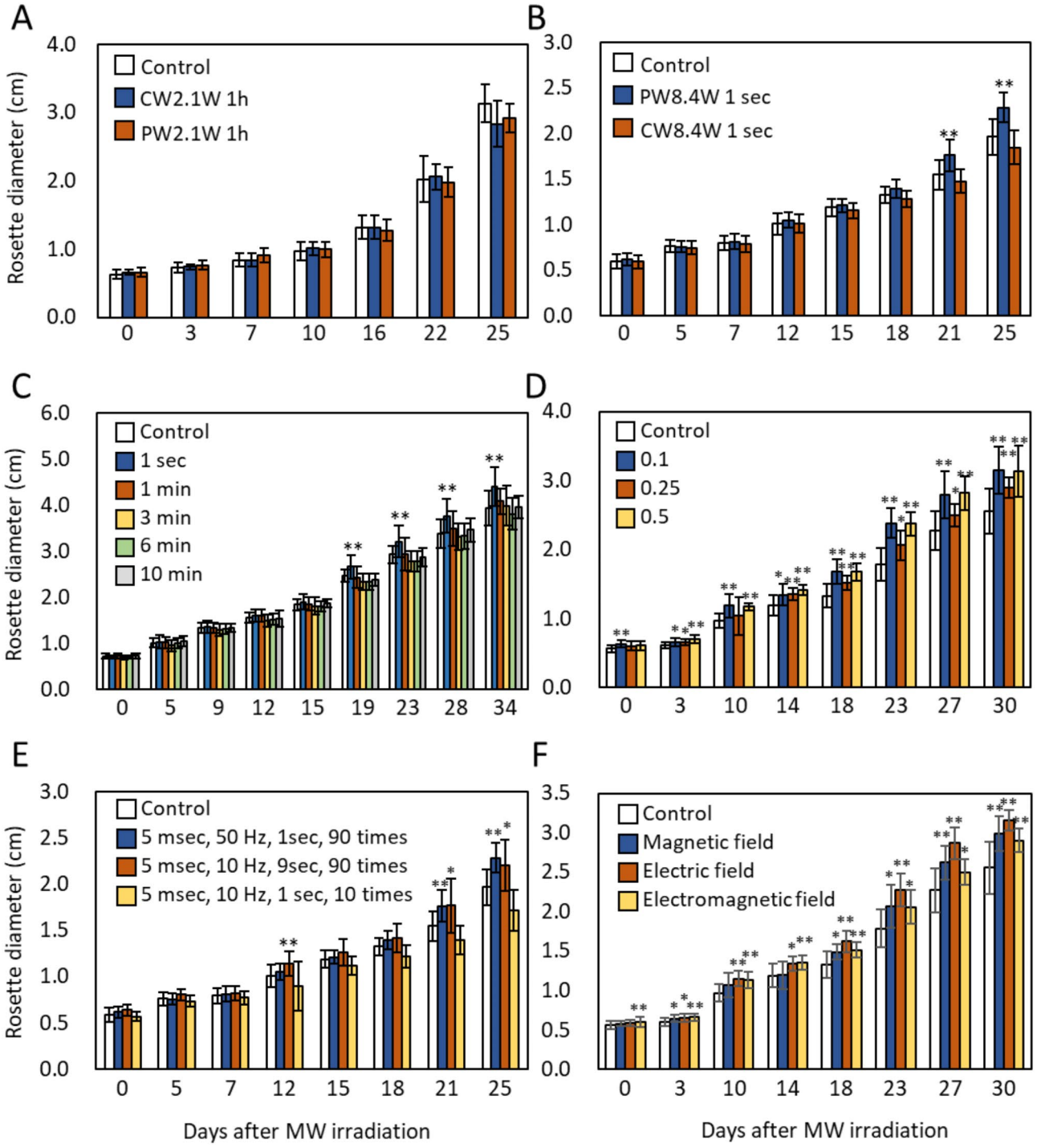


Fig. 5. Effects of microwave irradiation with different conditions on rosette diameter. (**A**) Constant (CW) and pulse (PW) MW-irradiation with 2.1 W for 1 h. (**B**) Constant and pulse MW-irradiation with 8.4 W for 1 s. (**C**) MW irradiation with different durations. Pulse MW with 8.4 W was irradiated on plants for 1 s or 1–10 min. (**D**) MW irradiation with different duty cycle (0.1, 0.25 or 0.5) as indicated in Supplemental Fig. 4. (**E**) MW with different total number of pulse irradiation. Total number of pulse irradiation was adjusted as indicated in Supplemental Fig. 3. (**F**) Magnetic field, electric field or electromagnetic field. * and **: Student's *t*-test significant at 5 and 1% compared to control, respectively ($n = 15$). "n" indicates the number of plants analyzed in the experiments.

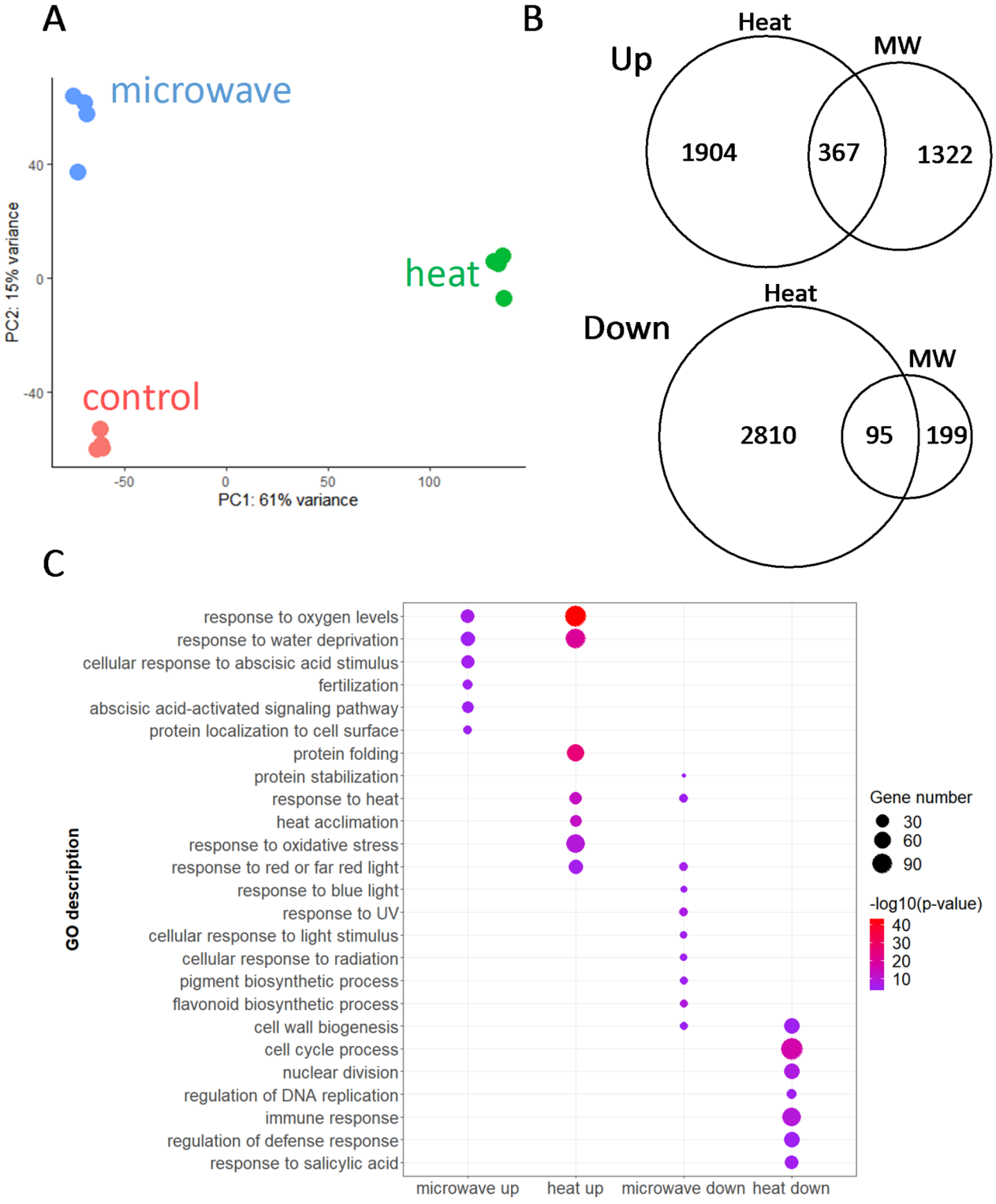


Fig. 6. Microarray analyses of plants exposed to microwave irradiation or sub-lethal heat stress. (**A**) The scattered plot of PCA analysis. (**B**) Venn Diagram showing the expression of transcripts that are significantly up- or down-regulated by microwave irradiation or sub-lethal heat stress. (**C**) Gene Ontology (GO) analysis of transcript indicated in (**B**).

these transcripts in leaves influences plant growth, particularly the transition from vegetative to reproductive stages and reproductive development.

KEGG analyses highlighted the representation of transcripts involved in hormone signaling among the transcripts up-regulated by microwave irradiation (Supplemental Fig. 5, Supplemental Table 11). In the software we employed (pathview), when multiple genes are present, they are grouped under a primary gene name (for example, *Jaz1* to *Jaz12* are collectively represented as "JAZ5"). Hormone signaling might play key roles in the regulation of altered growth triggered by microwave irradiation. In our transcriptome analysis, we detected the upregulation of several transcripts associated with the regulation of auxin and gibberellic acid (GA) including *Iaa*s (*Iaa1*, *2*, *5*, *7*, *11*, *12*, *13*, *14*, *16* and *18*), *Tir1*, *Pif3*, *Gid*s (*Gid1A*, *1B* and *1 C*) and *Saur-like* (AT1G16510) (Supplemental Fig. 5, Supplemental Table 11). Auxin has been known to stimulate cell elongation by increasing the extensibility of cell wall, thereby regulating cell wall properties through the induction of wall loosening[12]. GA is also known to positively regulate leaf growth by affecting the cell size and number and, eventually, the size of the leaf[13]. In addition, it has been demonstrated that GA promotes cell elongation by increasing extensibility of cell wall[38]. Furthermore, expression of transcripts involved in signals associated with other hormones, such as jasmonic acid, salicylic acid, cytokinin, brassinosteroid, abscisic acid and ethylene were also up-regulated by microwave irradiation (Supplemental Fig. 5, Supplemental Table 11). These include *Jaz*s (*Jaz3* and *4*), *Tga*s (*Tga1*, *4*, *6* and *9*), *Ahp*s (*Ahp1* and *3*), *Rr1*, *Gsk1*, *Bes1*, *Rcar*s (*Rcar1* and *3*), *Hai*s (*Hai1* and *2*), *Gbf4*, *Ebf*s (*Ebf1* and *2*) and *Ers*s (*Ers1* and *2*). These plant hormones were also shown to be involved in the regulation of plant growth and development[39–42]. Therefore, these results suggest that microwave irradiation might accelerate plant growth via modulation of various hormone signals.

In contrast, several auxin-response transcripts were significantly down-regulated in response to sub-lethal heat stress (Supplemental Fig. 5). Transcripts associated with GO terms "protein folding", "response to heat", "heat acclimation", "response to oxidative stress" and "response to red or far-red light" were specifically up-regulated by sub-lethal heat stress (Fig. 6C, Supplemental Table 9). These findings align well with previous studies which underscored the significance of reactive oxygen species scavenging systems, phytochrome B and maintenance of protein folding by chaperons in heat response of plants[43]. Transcripts belong to GO terms, "response to oxygen levels" and "response to water deprivation" were commonly represented in those up-regulated in response to microwave irradiation and sub-lethal heat stress (Fig. 6C, Supplemental Table 9).

Among the transcripts down-regulated by microwave irradiation, those involved in responses to heat stress, light and UV as well as biosynthesis of pigment and flavonoid are highly represented (Fig. 6C, Supplemental Table 9). Indeed, level of anthocyanin, total soluble sugar and H_2O_2 also decreased in response to microwave irradiation (Supplemental Fig. 6). It is plausible that microwave irradiation could decrease the absorption of light energy, thereby preventing oxidative stress. In addition, KEGG analysis revealed the significant representation of transcripts involved in the regulation of the circadian clock among those downregulated in response to microwave irradiation and those up-regulated in response to sub-lethal heat stress (Supplemental Fig. 7, Supplemental Table 12). This could be associated with the deactivation of light responses as well as acceleration of reproductive development. Among the transcripts specifically down-regulated by sub-lethal heat stress, those belonging to GO terms, "cell cycle process", "nuclear division", "regulation of DNA replication", "immune response", "regulation of defense response" and "response to salicylic acid" were highly represented (Fig. 6C, Supplemental Table 9). It is possible that plant might consume energy to activate heat response mechanisms, resulting in inhibition of cell cycle processes.

The downregulation of transcripts involved in the regulation of the circadian clock together with the up-regulation of hormone associated transcripts might be a key finding of this study. It was demonstrated that the expression of transcripts involved in auxin biosynthesis, perception, and signaling is controlled by the circadian clock[16]. In addition, it was also found that transcriptional and growth responses to exogenous auxin are coordinated by the circadian clock. Notably, the oscillation of the expression of the transcriptional repressors and activators of auxin responses (i.e. AUX/IAA and ARFs, respectively) are anti-phasic, suggesting that the circadian clock plays a vital role to precisely determine the timing of antagonistic auxin-related components function. Furthermore, PHYTOCHROME-INTERACTING FACTOR 4 (PIF4), a transcription factor known to be regulated by circadian clock, plays key roles in controlling plant growth via the regulation of hormone signaling, including auxin and gibberellins (GA)[15].

Our transcriptome analyses suggest the involvement of the circadian clock in regulating plant responses to microwave irradiation. To further explore this, we analyzed the expression of key circadian clock regulators, *Cca1*, *Lhy1*, and *Toc1* both immediately after and during the period following microwave irradiation. The transcriptome analysis showed that the expression of these circadian clock transcripts was not significantly altered immediately after microwave irradiation (Supplemental Tables 3 and 6). However, FT protein accumulation was found to be altered at 4 and 8 days following microwave irradiation (Fig. 3A). These findings prompted us to investigate whether the effects of microwave irradiation on the circadian clock persist over time after the irradiation. The timing at which *Cca1* and *Toc1* transcripts reached their maximum expression level was altered by the microwave irradiation (Supplemental Fig. 8). Although the timing at which *Lhy1* transcript reached its maximum level remained unchanged, microwave irradiation tended to increase the maximum expression level of this transcript (Supplemental Fig. 8). These results suggest that the circadian clock can be altered by microwave irradiation, with effects persisting even after the irradiation.

Moreover, it is also possible that microwave irradiation enhances mechanisms underlying leaf expansion by stimulating cell growth. Transcripts involved in cell wall biogenesis were highly represented among the transcripts significantly downregulated by microwave irradiation (Fig. 6C). It was demonstrated that cell wall became thinner by exposure to microwave[44]. This could potentially enhance cell expansion as a result of thinner call wall. Therefore, we can speculate that downregulation of transcripts involved in cell wall biosynthesis might contribute to enhanced flexibility of cell walls.

The 20 transcripts most significantly up-regulated in response to microwave irradiation, showing the highest fold changes, included those related to plant hormone signaling involving ABA, ethylene, and jasmonic acid, as well as processes associated with these hormones, such as seed maturation and responses to drought or salinity (Supplemental Table 13). These findings support the hypothesis that microwave irradiation affects plant growth by altering hormone signaling regulation. Although microwave irradiation up-regulated transcripts involved in GA signaling (Supplemental Fig. 5, Supplemental Table 11), other transcripts associated with GA signaling were also among the most down-regulated (Supplemental Table 13), suggesting that hormone signaling is tightly regulated under microwave irradiation. Additionally, microwave irradiation dramatically down-regulated expression of transcripts related to pigment synthesis and light perception (Supplemental Table 13).

It has been reported that signals regulated by a red/far-red light receptor, phytochrome B (PHYB) as well as PIF4 are integrated with circadian clock-dependent signals[45]. We therefore investigated the effects of microwave irradiation on growth of a mutant deficient in PHYB (*phyB-9*[21]) to test if PHYB is involved in the regulation of microwave-dependent growth promotion. Although the expression of transcripts encoding PHYB and PIF4 was not significantly altered immediately after microwave irradiation, the altered circadian clock after the irradiation led us to analyze this mutant. Interestingly, in contrast to WT plants, growth promotion by microwave irradiation was not observed in *phyB-9* plants (Fig. 7). This result suggests that PHYB is required for the growth promotion by microwave irradiation. In addition, we can speculate that microwave irradiation alters the circadian rhythm via affecting functions of PHYB.

Metabolic responses

To further explore the mechanisms underlying acceleration of growth and enhanced heat tolerance induced by microwave irradiation, we conducted metabolome analysis using the rosettes of plants treated with microwave irradiation. Accumulation of 20 metabolites were significantly altered by microwave irradiation, and among these, 14 metabolites were amino acids or their derivatives. Although level of Gly significantly declined, the levels of β-Ala, Asn, His, Ile, Leu, Lys, Phe, Thr, Trp, Tyr, Val, Nω-Methylarginine, S-Methylmethionine and Trimethyllysine increased in response to microwave irradiation (Fig. 8, Supplemental Fig. 9, Supplemental Table 14). Of these, involvement of 9 amino acids (Fig. 8) in the amino acid catabolic pathway network was proposed in a previous study (Supplemental Fig. 9[46]). In the microarray analysis, transcripts involved in protein stabilization were significantly downregulated in response to microwave irradiation (Fig. 6C). Excess amount of amino acid could be used for plant growth. Degradation of unnecessary proteins might contribute to the increase in amino acid level. Allantoic acid and uric acids, metabolites of guanine (G), and diethanolamine, a secondary amine were also up-regulated in response to microwave irradiation. Up-regulation of these metabolites might be also associated with to modulation of amino acid level, as they are proposed to be involved in nitrogen metabolisms[47,48]. Amino acids might contribute to the growth acceleration induced by microwave irradiation. Amino acids are known to be critical for the various biological processes underlying growth and development, functioning as signaling molecules or activators of hormone signaling via amino acid conjugation[49]. The GH3 family of enzymes, for example, was shown to conjugate various amino acids to auxin and jasmonate[50]. Indeed, the transcriptome analysis, along with qRT-PCR and phenotypic analysis of the *phyB-9* mutant, indicates the involvement of the circadian clock and hormone signaling in regulating plant responses to microwave irradiation (Figs. 6 and 7, Supplemental Figs. 3, 5, and 7). Additionally, the circadian clock and PHYB work together to regulate plant growth through the modulation of auxin signaling[15]. Therefore, we speculate that the functions of auxin, and possibly other plant hormones, may be modulated by amino acid conjugation, contributing to growth acceleration by microwave irradiation. Interestingly, the role of the circadian clock in regulating the accumulation of organic acids and amino acids, as well as starch synthesis has been revealed in previous studies[51,52]. Dawn components positively regulate the accumulation of amino acids, whereas the evening components likely activate starch accumulation but represses sucrose recycling[52]. These findings together with our results of transcriptome and metabolome analyses suggest that microwave-induced alterations in the patterns of circadian clock could lead to the activation of plant hormone signaling and amino acid synthesis, resulting in accelerated cell growth via increasing extensibility of cell wall and facilitating the synthesis of proteins and compound required for growth (Fig. 9).

Conclusions

This research demonstrated the beneficial impact of microwave irradiation on plant growth and its underlying mechanisms. Notably, just one time exposure of microwave irradiation showed long-lasting effects on young Arabidopsis plants, even accelerated reproductive development as well as growth of rosette leaves during vegetative growth stage with non-thermal effects of microwave. The most effective microwave irradiation condition for Arabidopsis growth is pulse microwave irradiation oscillating approximately 90 times. This specific condition found in this study may be the magic that can solve the problems of irreproducibility of microwave effects on plants.

The microwave irradiation neither raised the plants' temperature nor induced heat responsive gene expression such as heat shock protein. Furthermore, overall transcriptome profile in microwave irradiation treated plants were significantly different from heat treated plants, suggesting that growth promotion might be attributed to non-thermal effects of microwave. However, it is still challenging to distinguish and analyze the effects of microwave-induced material heating from other factors. Microwave is thought to create hotspots, which are microscopic high-temperature regions generated by the electric field, as well as exert as-yet-unknown effects of the magnetic field on magnetic substances within plants, such as oxygen and iron ions. These phenomena suggest that specific parts of the plant body may be selectively affected within the microwave field, leading to behaviors distinct from those caused by heat energy transmitted externally. This study aims to serve as a starting

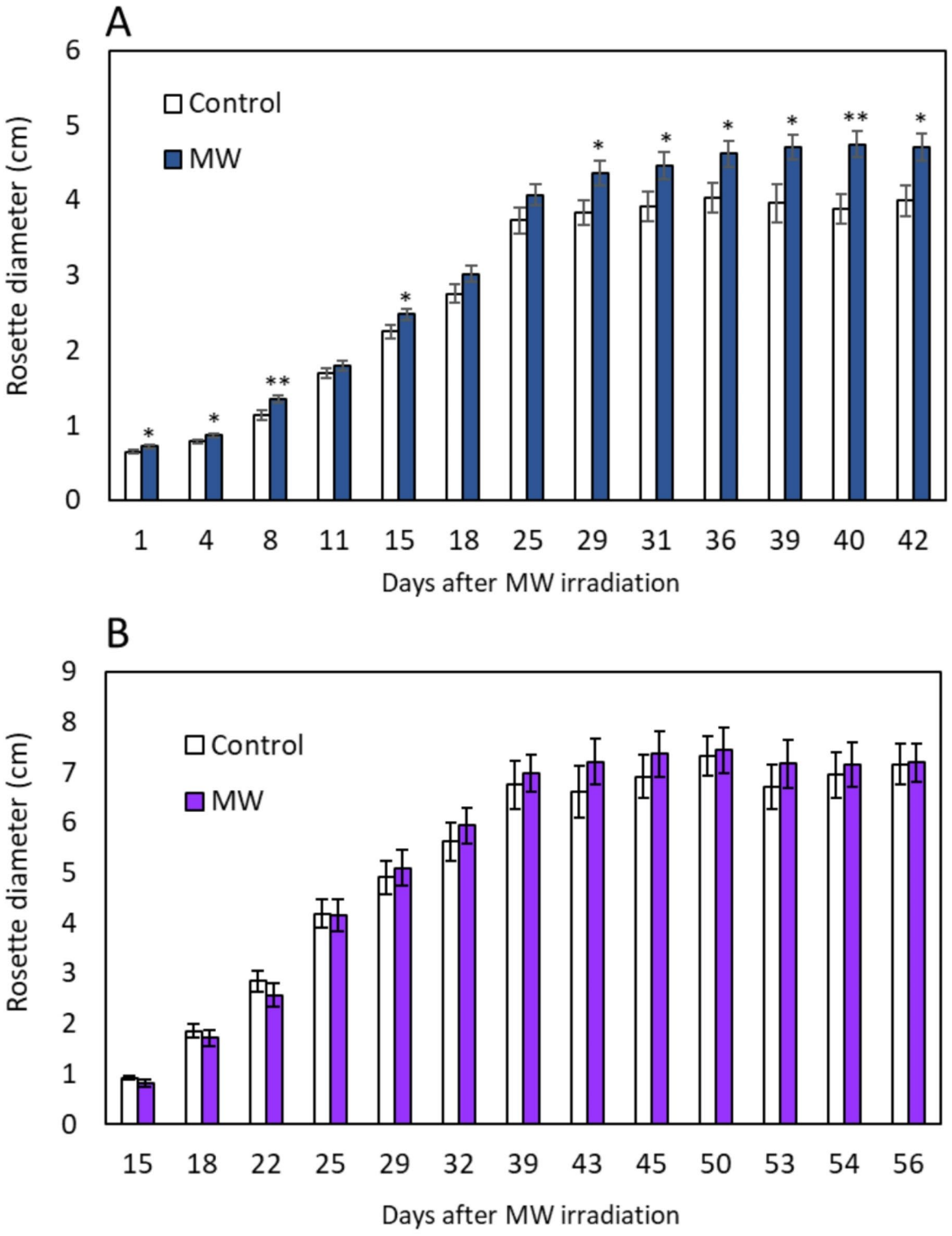


Fig. 7. Effects of microwave (MW) irradiation on growth of in Arabidopsis. Graphs indicate effects of MW-irradiation on rosette diameter of WT (**A**) and *phyB-9* (**B**) plants. Bars indicate standard error ($n = 14$). * and **: Student's *t*-test significant at 5 and 1% compared to control, respectively. "n" indicates the number of plants analyzed in the experiments.

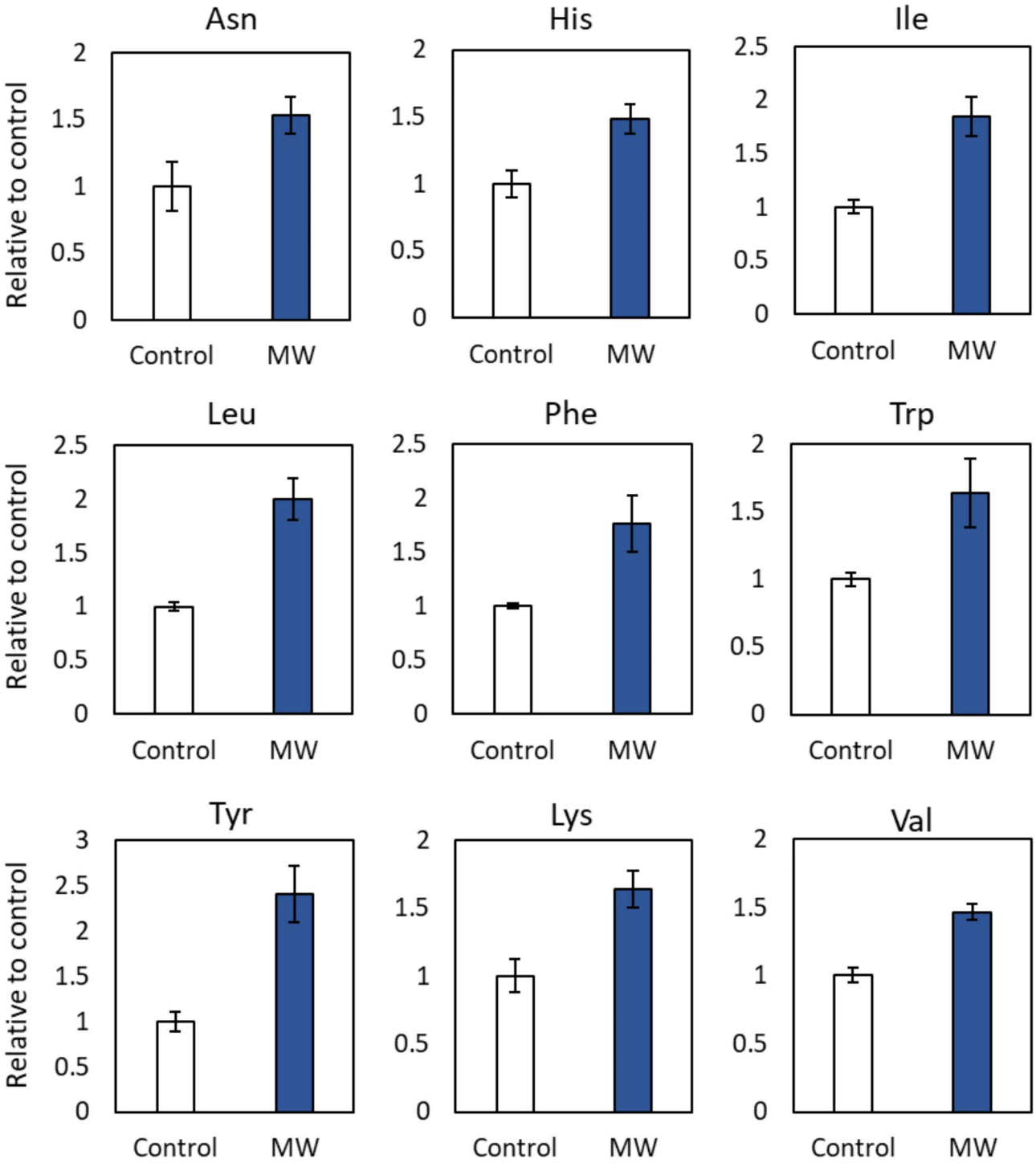


Fig. 8. Changes of amino acids accumulation in plants exposed to microwave (MW) irradiation. Level of amino acid is indicated as relative values compared to control. All metabolites detected in the metabolome analyses are listed in Supplemental Table 14.

point for uncovering the unknown phenomena of microwave effects on biological systems. it is anticipated that these mechanisms will be elucidated in the future.

While the detailed mechanisms underlying microwave effects require further investigation, our findings provide new insights into the molecular and physiological processes influenced by microwave irradiation. Transcriptome and metabolome analysis indicated that microwave irradiation altered circadian clock as well as hormonal response especially in auxin and gibberellin, which promoted plant growth by inducing amino acid biosynthesis and stress tolerance, and reducing cell wall thickness. Further studies are required to elucidate the interplay among multiple mechanisms that contribute to positive effects of microwave irradiation on plant

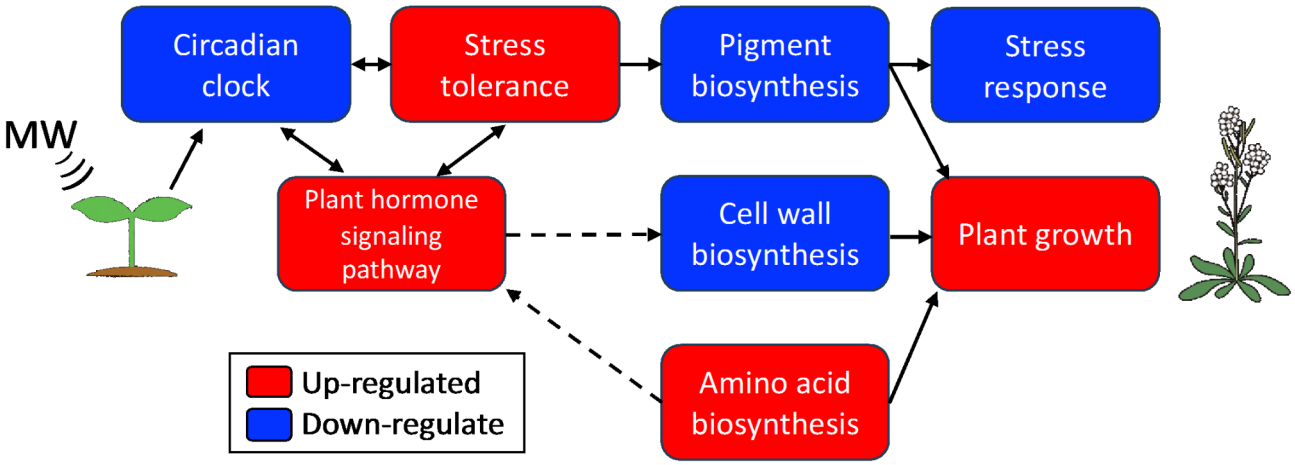


Fig. 9. Putative model of the mechanisms underlying enhanced growth and stress tolerance induced by microwave irradiation.

growth by analyzing response of the mutants deficient in the key regulators of these mechanisms, such as TOC1, CCA1, LHY1 or PIF4, to microwave irradiation.

Uncovering the optimal conditions for microwave irradiation in this study might pave the way for a breakthrough in implementing the microwave-based strategies to promote plant growth, leading to increase in crop yield. Additionally, this study serves as a starting point for advancing research in this field. Further investigation into the underlying mechanisms may lead to the development of more detailed strategies for implementing these techniques in agricultural practices.

Data availability

Law data of the microarray analysis was deposited to Gene Expression Omnibus with accession number: GSE241899.

Received: 22 October 2024; Accepted: 17 February 2025
Published online: 22 April 2025

References

1. Maxwell, J. C. A dynamical theory of the electromagnetic field. *Philos. Trans. R. Soc. Lond.* **155**, 459–512 (1865).
2. International Telecommunication Union Radiocommunications Sector (ITU-R). Nomenclature of the frequency and wavelength bands used in telecommunications, V.431-8, 08. https://www.itu.int/dms_pubrec/itu-r/rec/v/R-REC-V.431-8-201508-I!!PDF-E.pdf (2015).
3. Horikoshi, S., Schiffmann, R. F., Fukushima, J. & Serpone, N. *Microwave Chemical and Materials Processing; A Tutorial* (Springer Singapore, 2018).
4. Stavinoha, W. B., Pepelko, B. & Smith, P. W. Microwave radiation to inactivate cholinesterase in the rat brain prior to analysis for acetylcholine. *Pharmacologist* **12**, 257 (1970).
5. Stavinoha, W. B., Weintraub, S. T. & Modak, A. T. The use of microwave heating to inactivate cholinesterase in the rat brain prior to analysis for acetylcholine. *J. Neurochem.* **20**, 361–371 (1973).
6. Soran, M. L., Stan, M., Niinemets, Ü. & Copolovici, L. Influence of microwave frequency electromagnetic radiation on terpene emission and content in aromatic plants. *J. Plant Phys.* **171**, 1436–1443 (2014).
7. Tafforeau, M. et al. Plant sensitivity to low intensity 105 ghz electromagnetic radiation. *Bioelectromagnetics* **25**, 403–407 (2004).
8. Saitou, H. et al. Effects of 2.45GHz microwave on the plant growth rate - Promotion of germination, root elongation, and synthesis of the Chlorophyl. *Tech. Rep. IECE*, 16 SPS2006 (Japanese) (2007).
9. Qiu, Z. B., Li, J. T., Zhang, Y. J., Bi, Z. Z. & Wei, H. F. Microwave pretreatment can enhance tolerance of wheat seedlings to $CdCl_2$ stress, ecotoxicology environ. *Safety* **74**, 820–825 (2011).
10. Miler, N. & Kulus, D. Microwave treatment can induce chrysanthemum phenotypic and genetic changes. *Sci. Hort.* **227**, 223–233 (2018).
11. Verma, S., Sharma, V. & Kumari, N. Microwave pretreatment of tomato seeds and fruit to enhance plant photosynthesis, nutritive quality and shelf life of fruit. *Postharvest Biol. Technol.* **159**, 111015 (2020).
12. Majda, M. & Robert, S. The role of auxin in cell wall expansion. *Int. J. Mol. Sci.* **19**, 951 (2018).
13. Ritonga, F. N. et al. The roles of gibberellins in regulating leaf development. *Plants (Basel).* **12**, 1243 (2023).
14. Zenglin, L., Xiaofeng, L., Lei Wang, W. & Kai, S. ABSCISIC ACID INSENSITIVE 5 mediates light-ABA/gibberellin crosstalk networks during seed germination. *J. Exp. Bot.* **73**, 4674–4682 (2022).
15. Choi, H. & Oh, E. PIF4 integrates multiple environmental and hormonal signals for plant growth regulation in Arabidopsis. *Mol. Cells* **39**, 587–593 (2016).
16. Covington, M. F. & Harmer, S. L. The circadian clock regulates auxin signaling and responses in Arabidopsis. *PLoS Biol.* **5**, e222 (2007).
17. Balodis, V. et al. Does the Skrunda radio location station diminish the radial growth of pine trees? *Sci. Total Environ.* **180**, 57–64 (1996).
18. Horikoshi, S., Hasegawa, Y. & Suzuki, N. Growth stimulation system of plants using microwave irradiation and elucidation of its molecular mechanisms, *Proceedings of IMPI's 50th Annual Microwave Power Symposium*, June 21–23 (The Caribe Royale All-Suite Hotel & Convention Center, 2016).

19. Horikoshi, S., Suzuki, N. & Serpone, N. In *Agritech: Innovative agriculture using microwaves and plasmas: Thermal and non-thermal processing*, Chap. 5 (eds. Horikoshi, S. et al.) (Springer Nature Asia, 2021).
20. Horikoshi, S. & Serpone, N. On the influence of the microwaves' thermal and non-thermal effects in Titania photoassisted reactions. *Catal. Today*. **224**, 225–235 (2014).
21. Fichman, Y. et al. Phytochrome B regulates reactive oxygen signaling during abiotic and biotic stress in plants. *New. Phytol*. **237**, 1711–1727 (2023).
22. Katano, K., Kataoka, R., Fujii, M. & Suzuki, N. Differences between seedlings and flowers in anti-ROS based heat responses of Arabidopsis plants deficient in Cyclic nucleotide gated channel 2. *Plant. Physiol. Biochem.* **123**, 288–296 (2018).
23. Cronin, N. J. *Microwave and optical waveguides*, 27–40 (Institute of Physics Publishing, 1995).
24. Hsu, P. Y., Devisetty, U. K. & Harmer, S. L. Accurate timekeeping is controlled by a cycling activator in Arabidopsis. *Elife* **2**, e00473 (2013).
25. Suzuki, N., Bajad, S., Shuman, J., Shulaev, V. & Mittler, R. The transcriptional co-activator MBF1c is a key regulator of thermotolerance in Arabidopsis thaliana. *J. Biol. Chem.* **283**, 9269–9275 (2008).
26. Suzuki, N. et al. Ultra-fast alterations in mRNA levels uncover multiple players in light stress acclimation in plants. *Plant. J.* **84**, 760–772 (2015).
27. Suzuki, N., Miller, G., Sejima, H., Harper, J. & Mittler, R. Enhanced seed production under prolonged heat stress conditions in Arabidopsis thaliana plants deficient in cytosolic ascorbate peroxidase 2. *J. Exp. Bot.* **64**, 253–263 (2013).
28. Kumazaki, A. & Suzuki, N. Enhanced tolerance to a combination of heat stress and drought in Arabidopsis plants deficient in ICS1 is associated with modulation of photosynthetic reaction center proteins. *Physiol. Plant*. **165**, 232–246 (2019).
29. Nakamura, Y. et al. Arabidopsis florigen FT binds to diurnally oscillating phospholipids that accelerate flowering. *Nat. Commun.* **5**, 3553 (2014).
30. Inoue, K., Araki, T. & Endo, M. Circadian clock during plant development. *J. Plant. Res*. **131**, 59–66 (2018).
31. Endo, M., Araki, T. & Nagatani, A. Tissue-specific regulation of flowering by photoreceptors. *Cell. Mol. Life Sci.* **73**, 829–839 (2016).
32. Shim, J. S., Kubota, A. & Imaizumi, T. Circadian clock and photoperiodic flowering in arabidopsis: CONSTANS is a hub for signal integration. *Plant. Physiol.* **173**, 5–15 (2017).
33. Tiwari, S. B. et al. The flowering time regulator CONSTANS is recruited to the FLOWERING LOCUS T promoter via a unique cis-element. *New. Phytol*. **187**, 57–66 (2010).
34. Suzuki, N., Sejima, H., Tam, R., Schlauch, K. & Mittler, R. Identification of the MBF1 heat-response Regulon of *Arabidopsis thaliana*. *Plant. J.* **66**, 844–551 (2011).
35. Suzuki, N. Fine tuning of ROS, redox and energy regulatory systems associated with the functions of chloroplasts and mitochondria in plants under heat stress. *Int. J. Mol. Sci.* **24**, 1356 (2023).
36. Sprunck, S. et al. Egg cell-secreted EC1 triggers sperm cell activation during double fertilization. *Science* **338**, 1093–1097 (2012).
37. Wang, W. et al. EGG CELL 1 contributes to egg-cell-dependent Preferential fertilization in Arabidopsis. *Nat. Plants*. **10**, 268–282 (2024).
38. Matsukura, C., Itoh, S., Nemoto, K., Tanimoto, E. & Yamaguchi, J. Promotion of leaf sheath growth by gibberellic acid in a Dwarf mutant of rice. *Planta* **205**, 145–152 (1998).
39. Liu, X-D. et al. Diverse functional interactions between ABA and ethylene in plant development and responses to stress. *Physiol. Plant.* **176**, e70000–e70017 (2024).
40. Walker, C. H. & Bennett, T. Cytokinin and reproductive shoot architecture: bigger and better? *Biochem. Soc. Trans.* **52**, 1885–1893 (2024).
41. Aardening, Z., Khandal, H., Erlichman, O. A. & Savaldi-Goldstein, S. The whole and its parts: cell-specific functions of brassinosteroids. *Trends Plant. Sci.* **18**, S1360–1385 (2024).
42. Timofeeva, A. M., Galyamova, M. R. & Sedykh, S. E. How do plant Growth-Promoting Bacteria use plant hormones to regulate stress reactions?? *Plants* **13** (17), 2371–2395 (2024).
43. Katano, K., Honda, K. & Suzuki, N. Integration between ROS regulatory systems and other signals in the regulation of various types of heat responses in plants. *Int. J. Mol. Sci.* **19**, 3370 (2018).
44. Soran, M-L., Stan, M., Niinemets, Ü. & Copolovici, L. Influence of microwave frequency electromagnetic radiation on terpene emission and content in aromatic plants. *J. Plant. Physiol.* **171**, 1436–1443 (2014).
45. Devlin, P. F. Signs of the time: environmental input to the circadian clock. *J. Exp. Bot.* **53**, 1535–1550 (2002).
46. Hildebrandt, T. M., Nesi, A. N. & Araújo, W. L. Braun, amino acid catabolism in plants. *Mol. Plant.* **8**, 1563–1579 (2015).
47. Ohyama, T., Matsumoto, K., Goto, H., Saito, A. & Higuchi, K. Nitrogen metabolism in Non-Nodulated and nodulated soybean plants related to Ureide synthesis. *Nitrogen* **4**, 209–222 (2023).
48. Hafez, R. M., Abdel-Rahman, T. M. & Naguib, R. M. Uric acid in plants and microorganisms: biological applications and genetics - A review. *J. Adv. Res.* **8**, 475–486 (2017).
49. Tegeder, M. Transporters for amino acids in plant cells: some functions and many unknowns. *Curr. Opin. Plant. Biol.* **15**, 315–321 (2012).
50. Westfall, C. S., Herrmann, J., Chen, Q., Wang, S. & Jez, J. M. Modulating plant hormones by enzyme action. *Plant. Signal. Behav.* **5**, 1607–1612 (2010).
51. Flis, A. et al. Multiple circadian clock outputs regulate diel turnover of carbon and nitrogen reserves. *Plant. Cell. Environ.* **42**, 549–573 (2019).
52. Cervela-Cardona, L., Alary, B. & Mas, P. The Arabidopsis circadian clock and metabolic energy: A question of time. *Front. Plant. Sci.* **12**, 804468 (2021).

Acknowledgements

We are grateful to the Japan Society for the Promotion of Science (JSPS) for financial support through a Grant-in-aid for Scientific Research (No. 19K22316).We would like to thank Prof. Ron Mittler for providing seeds of *phyB-9* mutant.

Author contributions

N.S. and S.H. conceived the study, designed the experiments and wrote the first draft. Y.H., K.K. K.Y. and M.S. performed the experiments. A.A. discussed and examined the data's interpretation with N.S. and S.H.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-90859-7.

Correspondence and requests for materials should be addressed to S.H.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

© The Author(s) 2025