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SHORT COMMUNICATION

Expression profiling of H3K27me3 demethylase genes during plant development and in response to environmental stress in *Arabidopsis*

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ABSTRACT

Histone modification influences gene expression. Among histone modifications, H3K27me3 is associated with downregulation of nearby genes via chromatin compaction. In *Arabidopsis thaliana*, a subset of JUMONJI C DOMAIN-CONTAINING PROTEIN (JMJ) proteins play a critical role in removal of H3K27me3 during plant development or in response to environmental cues. However, the regulation of H3K27me3 demethylase gene expression is not yet fully characterized. In this study, we computationally characterized the expression patterns of JMJ H3K27me3 demethylase genes using public transcriptome datasets created across plant development and after various environmental cues. Consistent with the available transcriptome datasets, GUS staining validated that *JMJ30* was highly expressed in the L1 layer of the shoot apical meristem. Furthermore, expression data for panel of five H3K27me3 demethylase genes revealed *JMJ30* to be the most highly affected by abiotic and biotic stress. In addition, *JMJ30* expression was variable between *Arabidopsis thaliana* accessions. Finally, the expression of a *JMJ30* orthologue from the related species *Arabidopsis halleri, AhgJMJ30*, fluctuated under field conditions. Taken together, our results suggest that transcriptional changes of H3K27me3 demethylase genes may play key roles in development and environmental responses.

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Arabidopsis thaliana; development; environmental response; H3K27me3; Jumonji

One aspect of robust and flexible gene expression is epigenetic regulation,[1, 2] which provides regulatory information to a genome without changing the nucleotide sequence.[3,4] Epigenetic regulation is mediated by heritable epigenetic marks, such as DNA methylation, histone modification, and chromatin remodeling. Whereas methylation of DNA creates long-lasting marks, histone modification, and chromatin remodeling are fast-changing marks.

In plants, histone modifications are often used to control developmental transitions and environmental responses.[5,6] This modification is particularly important for plants, because a plant has to produce new organs throughout its life cycle and cannot move away from unfavorable conditions. Histones can be modified via methylation, phosphorylation, acetylation, and/or ubiquitylation. The histone H3 trimethyl Lys 27 (H3K27me3) is particularly important for silencing nearby genes.[5,6] In *Arabidopsis thaliana*, a subset of JUMONJI C DOMAIN-CONTAINING PROTEIN (JMJ) proteins play critical roles in removal of H3K27me3 modifications;[7,8] these include JMJ11/EARLY FLOWERING 6 (ELF6), JMJ12/RELATIVE OF EARLY FLOWERING (REF6), JMJ13, JMJ30, and JMJ32.[9–13] REF6 binds directly to DNA in a sequence-specific manner via C2H2-type Zn-finger (ZnF) domains.[12] Like JMJ30 and JMJ32, JMJ31 belongs to JmjC contain-only group and might have a similar function.[7,8] Although JMJ13 belongs to the same family as REF6, JMJ13 recognizes the H3K27me3 peptide rather than binding directly to DNA.[14] How other three demethylases recognize target genes remains unknown.

Molecular, genetic, and biochemical analyses of these five demethylase genes have revealed their critical roles during plant development and environmental responses. Based on whole-mount GUS expression analysis, these H3K27 demethylases accumulate in various tissues.[11,14,15] In seedlings, REF6-GUS, ELF6-GUS, JMJ13-GUS, and JMJ32-GUS accumulate in rapidly dividing cells of leaves. Additionally, JMJ30-GUS is expressed at high levels throughout the entire leaf. However, our understanding of JMJ accumulation is largely limited to the organ level. In this study, we used transcriptome data from publicly available databases to increase our understanding of demethylase functions in *Arabidopsis* during development and in response to environmental stimuli.

To understand the expression patterns of H3K27 demethylase genes, we utilized data from TRAnscriptome Variation Analysis (TraVA). TraVA is a database of spatiotemporal gene expression profiles that were captured by RNA-seq at various stages of *Arabidopsis thaliana* development.[16–18] When we clustered *REF6, ELF6, JMJ13, JMJ30, JMJ31*, and *JMJ32* expression data from TraVA, we identified three clades, consisting of *JMJ13* and *ELF6, JMJ30* and *JMJ31*, and *REF6* and *JMJ32* (Figure 1a). *JMJ13* and *ELF6* were weakly expressed throughout development, and, consistent with their antagonistic roles during fertility regulation,[22] both were highly expressed in mature flowers and floral organs (Figure 1a: Opened anthers, Anthers of the mature flower before opening). *JMJ30* was expressed throughout plant development, and, in agreement with the known expression of JMJ30-GUS in

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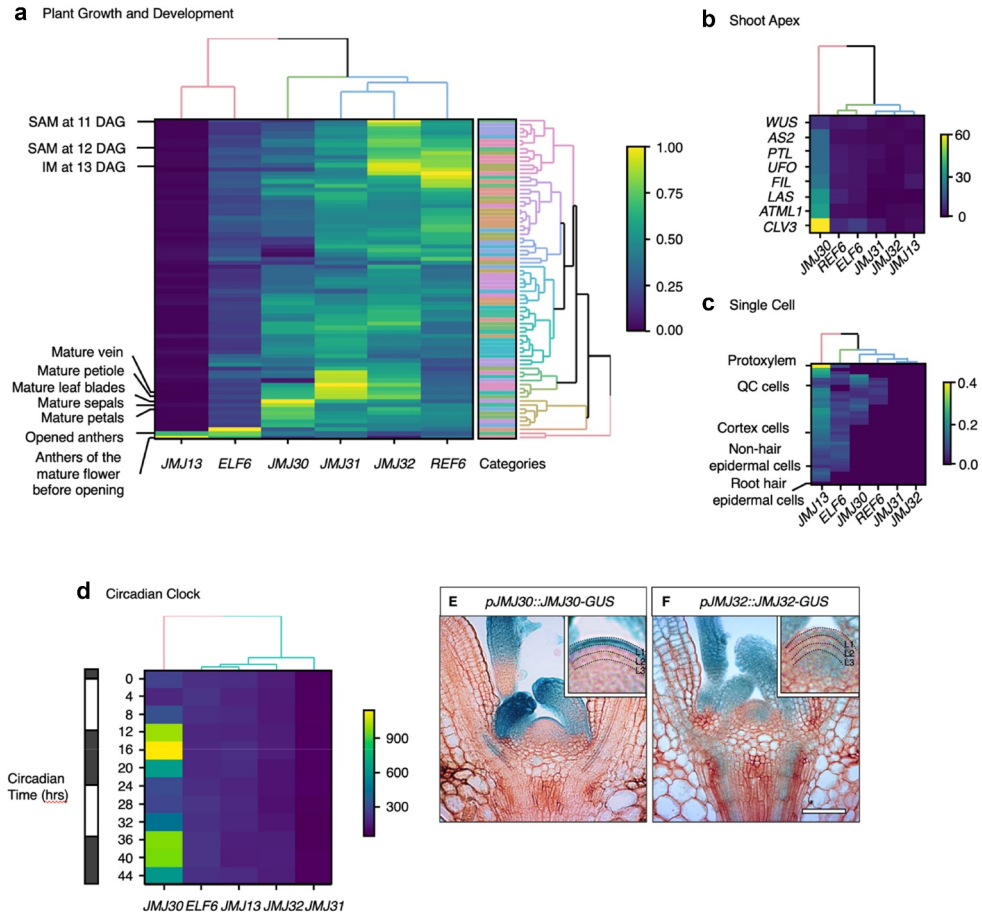

Figure 1. Expression of *JUMONJI* (*JMJ*) genes during plant development in *A. thaliana*. (a) Heat map of the expression of six *JMJ* genes in the *A. thaliana* developmental transcriptome based on RNA-seq profiling.[16] The data were obtained from the TraVA database (http://travadb.org). (b) Heat map of the expression of six *JMJ* genes in the *A. thaliana* shoot apex transcriptome based on RNA-seq profiling.[19] The data were obtained from the Arabidopsis eFP browser database (http://bar.utoronto.ca/efp_arabidopsis/cgi-bin/efpWeb.cgi). (c) Heat map of the expression of six *JMJ* genes in the *A. thaliana* single-cell transcriptome in roots based on scRNA-seq profiling.[20] The data were obtained from the Arabidopsis eFP browser database. (d) Heat map of five circadian oscillation patterns of *JMJ* genes from the *A. thaliana* transcriptome, based on ATH1 GeneChip microarray profiling.[21] The data were obtained from the supplemental data from their paper. All heat maps were generated using the heatmaply package in R. (e, f) *pJMJ30::JMJ30-GUS* (e) and *pJMJ32::JMJ32-GUS* (f) expression in shoot apical meristem. Insets indicate close-up views of shoot apical meristem. Dash lines indicate L1, L2, and L3 layer of shoot apical meristem. Bar = 50 µm.

mature flowers, the highest *JMJ30* transcript levels were seen in mature floral organs[11] (Figure 1a: Mature sepals, Mature petals). During plant growth and development, *REF6* and *JMJ32* transcripts showed an expression pattern similar to that observed in the JMJ30-GUS expression analysis:[11,15] they were highly expressed in young leaves near the shoot apical meristem (Figure 1a: SAM at 11 DAG, SAM at 12 DAG, SAM at 13 DAG). Potential interactions between *REF6* and *JMJ32* should therefore be investigated in the future.

We next assessed the spatial expression patterns of the six *Arabidopsis* H3K27me3 demethylase genes using shoot-apex-specific RNA-seq and cell-type-specific scRNA-seq data available from the Arabidopsis eFP browser.[19,20,23] Based on the shoot-apex-specific transcriptome, *JMJ30* was highly expressed in the shoot apical meristem (Figure 1b). The other *JMJ* genes were weakly expressed (Figure 1b). *CLAVATA3* (*CLV3*) and *ARABIDOPSIS THALIANA MERISTEM LAYER 1* (*ATML1*) are specifically expressed in stem cells and L1 layer, respectively.[24,25] Out of the eight different domains within the shoot apical meristem, the *CLV3* and *ATML1* expression domains contained the highest *JMJ30* expression (Figure 1b). In the root, *JMJ13, ELF6, JMJ30*, and *REF6* were expressed in a cell-type-specific manner (Figure 1c: Protoxylem). The high *JMJ13* expression in the protoxylem suggests that JMJ13 may have a specific function in this tissue (Figure 1c). *ELF6* transcripts were present in root cells, contrary to what might be expected based on the GUS expression data; however, the regulation of *ELF6* mRNA and protein might differ. These high-resolution differential expression patterns suggest that histone demethylation is tissue or cell-type specific.

We also assessed the circadian regulation of the five *Arabidopsis* H3K27me3 demethylase genes based on previous publication.[21] As reported previously, *JMJ30* expression pattern was rhythmic with a broad peak around subjective dusk and into the night (Figure 1d). On the other hand, clear circadian regulation of *ELF6, JMJ13, JMJ32*, and *JMJ31*was not observed.

To validate the shot-apex-specific transcriptome results, we made use of previously established *pJMJ30::JMJ30-GUS* and *pJMJ32::JMJ32-GUS* lines.[11] Consistent with the transcriptome data, JMJ30-GUS expression was generally higher than JMJ32-GUS expression (Figure 1e, f). JMJ30-GUS accumulated at high

levels in the L1 layer of the shoot apical meristem. In the stem, although the JMJ30-GUS accumulation was higher in the L1 layer, it was not as high in the L2 and L3 layers, a surprise given the high *CLV3* domain expression that was observed in the shoot-apex-specific transcriptome dataset (Figure 1b, e). Thus, high accumulation of *JMJ30* in the *CLV3* expression domain is likely due to higher levels of *JMJ30* in the L1 layer of the shoot apical meristem. Future work could study whether JMJ30 removes H3K27me3 in a cell-type specific manner.

Epigenetic regulation is thought to be important for responses to environmental stimuli, and there have been reports of relationships between environmental stresses and the induction of *JMJ* genes. *JMJ30* is induced by the stress hormone abscisic acid and by salt stress, drought stress, and heat stress.[26–31] The expression of *JMJ13* is affected by photoperiod and temperature, according to the GUS expression data.[14] *REF6* expression is induced by long-term heat exposure through HEAT SHOCK FACTOR A2 (HSFA2).[32] To date, no effects of environmental stress on the regulation of *ELF6* and *JMJ32* expression have been reported. Although *REF6* is not present in the Genechip Arabidopsis Genome ATH1 array, the other published results are largely supported by microarray data from the Arabidopsis eFP browser (Figure 2a). Various abiotic stresses, such as heat, drought, salt, and osmotic stress, induced *JMJ30* expression in shoot tissues (Figure 2a: Heat shoot 12 h, Drought shoot 12 h, Osmotic Shoot 12 h). Furthermore, rapid *JMJ13* induction was observed after exposure to salt stress (Figure 2b: Salt root 15 min). Biotic stress also triggered *JMJ30* and *JMJ13* expression (Figure 2b: HRP 1 h, FLG22 1 h, NPP 1 h, Virulent half, Avirulent half). However, almost nothing is known about the relationship between H3K27me3 demethylases and biotic stress in *Arabidopsis thaliana*, a topic that will require more detailed characterization in the future.

To understand the change in spatial distribution of JMJ30 after heat stress, *pJMJ30::JMJ30-GUS* was examined with and without heat stress. At 22°C, JMJ30-GUS accumulated at the edge of cotyledons to a limited extent. Consistent with the transcriptome data, JMJ30-GUS levels increased after 43.5°C treatment. We observed the highest expression levels at the edge of cotyledons when we grew plants at 43.5°C. Under heat stress, JMJ30-GUS accumulation was observed throughout cotyledons except for the petiole. Increased JMJ30-GUS accumulation could be due to the stabilization of *JMJ30* mRNA and JMJ30 protein that was described previously.[11]

Numerous accessions of *Arabidopsis. thaliana* are available from across the species geographic range, which covers different climate zones and habitats, and analysis of their differences can provide ecological and evolutionary insights.[33] We compared the expression patterns of *JMJ* genes using microarray data of different accessions grown under continuous light[33] (Figure 3a). The expression of *JMJ30* varied greatly between accessions, whereas that of other *JMJ* genes was extremely similar across accessions. One notable difference between *Arabidopsis thaliana* accessions is the basal heat tolerance capacity of seeds.[35] Col and C24 are more resistant to heat stress, whereas Cvi and Ler were more sensitive to heat stress. This phenotype did not correlate well with the expression levels

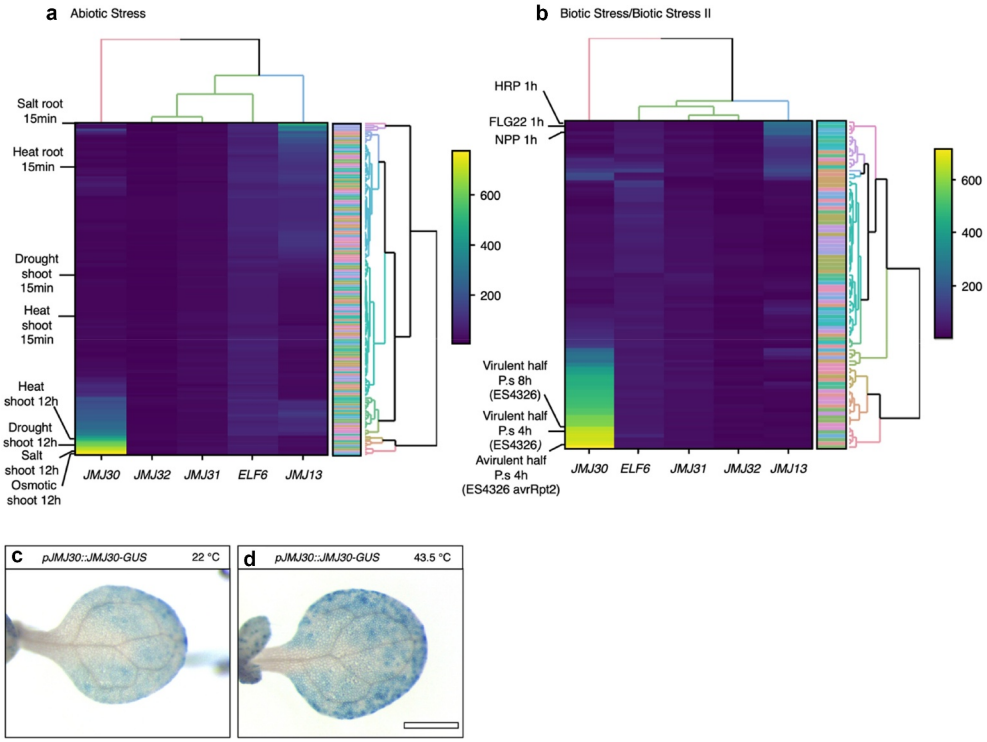

Figure 2. Expression of *JUMONJI* (*JMJ*) genes in response to stress in *A. thaliana*. (a) Heat map of five *JMJ* genes from the *A. thaliana* transcriptome in response to abiotic stress, based on ATH1 GeneChip microarray profiling.[23] The data were obtained from the Arabidopsis eFP browser database (http://bar.utoronto.ca/efp_arabidopsis/cgi-bin/efpWeb.cgi). (b) Heat map of the expression of five *JMJ* genes in the *A. thaliana* transcriptome in response to biotic stress, based on ATH1 GeneChip microarray profiling.[23] The data were obtained from the Arabidopsis eFP browser database. Note that *REF6* is not in the ATH1 source data. All heat maps were generated using the heatmaply package in R. (c, d) *pJMJ30::JMJ30-GUS* without (c) and with heat stress (d). Bar = 0.5 mm.

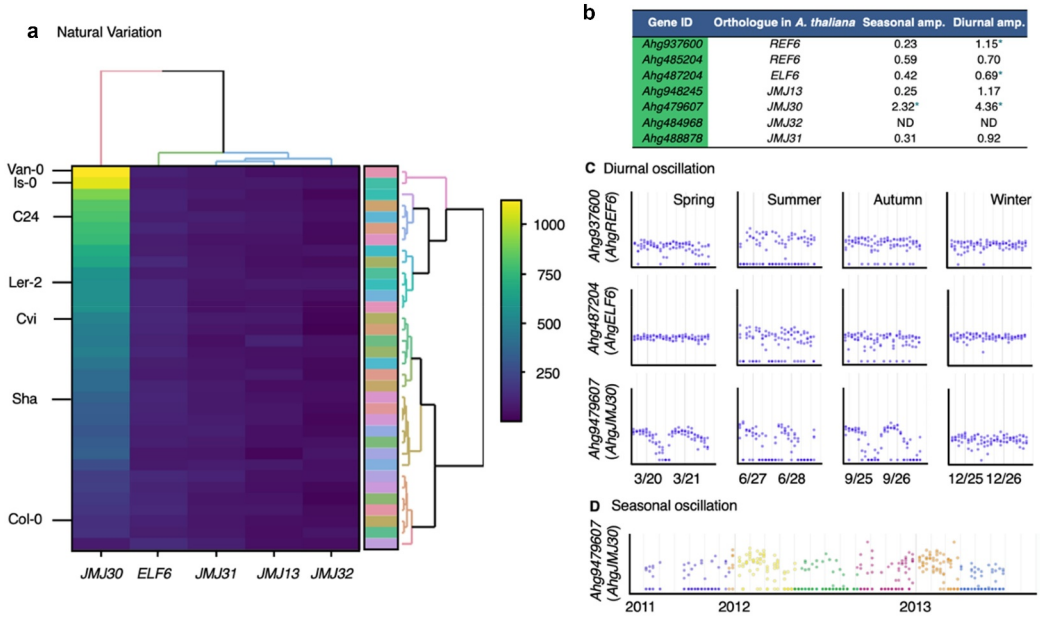

Figure 3. Expression of *JUMONJI* (*JMJ*) genes in *A. thaliana* and *A. halleri*. (a) Heat map of the expression of five *JMJ* genes from the *A. thaliana* natural variation transcriptome based on ATH1 GeneChip microarray profiling.[33] The data were obtained from the Arabidopsis eFP browser database (http://bar.utoronto.ca/efp_arabidopsis/cgi-bin/efpWeb.cgi). The heat map was generated using the heatmaply package in R. (b) Amplitude (amp.) of seasonal and diurnal oscillation in the expression of *JMJ* orthologues in *A. halleri*. The data were obtained from the database of seasonal transcriptome of *A. halleri* in the natural environment[34] (http://sohi.ecology.kyoto-u.ac.jp/AhgRNAseq/). (c) Diurnal oscillation in the expression of *AhgREF6, AhgELF6,* and *AhgJMJ30* (above) and (d) seasonal oscillation in the expression of *AhgJMJ30*. The data were obtained from the database of the seasonal transcriptome of *A. halleri* in the natural environment[34] (http://sohi.ecology.kyoto-u.ac.jp/AhgRNAseq/).

of *JMJ30*. A genome-wide association study (GWAS) may be useful to find genetic variations associated with heat stress.

Arabidopsis halleri has recently been established as a perennial model system in which to study local adaptation under natural conditions and seasonal and diurnal changes in gene transcription.[34] An orthologue of *JMJ30, AhgJMJ30* (*Ahg479607*), was identified as a seasonally and diurnally oscillating gene in the flowering pathway (Figure 3b–d). The seasonal and diurnal amplitude of *AhgJMJ30* expression was the highest of all *AhgJMJ* genes. *AhgREF6* (*Ahg485204*) and *AhgELF6* (*Ahg487204*) also showed seasonal and diurnal changes in expression, but with lower amplitudes; *AhgJMJ13* showed a relatively high diurnal amplitude. From these patterns, we conclude that *AhgJMJ30* likely plays a more critical role in environmental responses than do other genes from the same family. The expression of *AhgREF6* and *AhgJMJ13* is also potentially affected by temperature and day length under natural conditions. Although nothing is known about the relationship between *ELF6* expression and environmental stimuli in *A. thaliana*, a link was recently identified by transcriptome analysis of *A. halleri* under natural conditions,[34] and multiple stimuli might be important for *ELF6* regulation. Evolution of JMJ family needs to be addressed in more details.

Materials and methods

Plant materials and growth conditions

Arabidopsis thaliana plants were grown under continuous light condition at 22°C or 43.5°C on half-strength of Murashige and Skoog salt and 0.8% agar at pH 5.6. *pJMJ30::JMJ30-GUS* and *pJMJ32::JMJ32-GUS* were described previously.[11]

Transcriptome data analysis

The transcriptome data were obtained from the TraVA database[16] (http://travadb.org), the Arabidopsis eFP browser database[19,20,33,36] (http://bar.utoronto.ca/efp_arabidopsis/cgi-bin/efpWeb.cgi), the Plant Molecular Phenology database[34] (http://sohi.ecology.kyoto-u.ac.jp/AhgRNAseq/), or the supplemental data of published paper.[21] For Figure 1a (Plant Growth and Development), data from TraVa is used. Read counts normalized by method applied in median-of-raito method and divided by maximum value of expression level as described previously.[37] For Figure 1b (Shoot Apex), gene expression levels were calculated by Cuffdiff2 (version 2.1.1) and normalized by edgeR.[19] For Figure 1c, raw reads were demultiplexed by 10X Genomics Cell Ranger pipeline (version 2.1.1). A gene with expression in more than 3 cells was considered as expressed. Each dataset was log-normalized, scaled and corrected for dataset-specific batch effects using ComBat (version 3.26.00). For Figure 1d (Circadian Clock), expressed genes were identified by MAS 5.0 Software (Affymetrix) for at least four of the 12 time-points.[21] For Figures 2a,b, and 3a, data using the ATH1 GeneChip were normalized by GCOS normalization.[33,36] All heat maps were generated using the heatmaply package in R (Version 3.5.2).

GUS staining

For GUS staining, the aboveground parts of 10-day-old seedlings of *pJMJ30::JMJ30-GUS* and *pJMJ32::JMJ32-GUS*[11] were used. GUS lines were fixed in 90% acetone and kept for 20 min at room temperature. Tissues were rinsed with GUS staining buffer (100 mM Sodium phosphate, 5 mM Potassium ferricyanide, 5 mM Potassium ferrocyanide, 1% Triton X) without

5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-Gluc) three times and put into GUS staining buffer with X-Gluc. They were placed under vacuum until the tissues sank, and then left overnight at 37°C. After removing chlorophyll by 70% EtOH, tissues were observed under an AXIO Zoom. V16 (Zeiss) microscope.

Sectioning

For GUS sectioning, GUS stained tissued were transferred through a gradient series of ethanol solutions in water (80% EtOH, 90%, 95%, 100%) for 15 min each and then a gradient series of Technovit H1 in EtOH (25% Technovit H1, 50%, 75%, 100% × 2) for 30 min each. The resulting tissues were put into Technovit H1 and H2 solution and kept overnight at room temperature. The block was sliced by a RM2255 microtome (Leica). After background staining with neutral red, the sections were observed under an Axio Scope A1 microscope (Zeiss) and analyzed using ZEN2 software.

Heat treatment

Heat treatment was conducted as described previously.[30] MS plates containing *A. thaliana* were placed into resealable zipper storage bags and submerged into a PERSONAL-11 water bath shaker (TAITEC). Heat shock treatment (43.5°C for 20 min) was performed on 7-day-old plants. After the heat treatment, plants were returned into the 22°C growth chamber and allow to recover for further experiments.

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