Perception of pathogen-derived ligands by corresponding host receptors is a pivotal strategy in eukaryotic innate immunity. In plants, this is complemented by circadian anticipation of infection timing, promoting basal resistance even in the absence of pathogen threat. Here, we report that trichomes, hair-like structures on the epidermis, directly sense external mechanical forces, including raindrops, to anticipate pathogen infections in *Arabidopsis thaliana*. Exposure of leaf surfaces to mechanical stimuli initiates the concentric propagation of intercellular calcium waves away from trichomes to induce defence-related genes. Propagating calcium waves enable effective immunity against pathogenic microbes through the CALMODULIN-BINDING TRANSCRIPTION ACTIVATOR 3 (CAMTA3) and mitogen-activated protein kinases. We propose an early layer of plant immunity in which trichomes function as mechanosensory cells that detect potential risks.
innate immunity is an evolutionarily conserved front line of defense across the plant and animal kingdoms. In plants, pattern-recognition receptors (PRRs), such as leucine-rich repeat receptor-like kinases (LRR-RLKs) and LRR receptor proteins (LRR-RPs), specifically recognize microbe-associated molecular patterns (MAMPs) as non-self molecules, leading to the activation of pattern-triggered immunity (PTI) to limit pathogen proliferation. While adapted pathogens have evolved virulence effectors that can circumvent PTI, plants also deploy disease resistance (R) genes, primarily encoding nucleotide-binding LRR proteins, which mount effector-triggered immunity (ETI). ETI often culminates in a hypersensitive response as well as acute and localized cell death at the site of infection accompanied by profound transcriptional changes of defense-related genes to retard pathogen growth.

These ligand–receptor systems are largely dependent on a transient increase in intracellular calcium concentration ([Ca2+]i), followed by the initiation of phosphorylation-dependent signaling cascades, including mitogen-activated protein kinases (MAPKs) and calcium-dependent protein kinases, that orchestrate a complex transcriptional network and the activity of immune mediators.

In addition to PTI and ETI, plant immunity can be induced periodically in the absence of pathogen threat, a process controlled by the circadian clock and driven by daily oscillations in humidity as well as light–dark cycles. Such responses enable plants to prepare for the potential increased risk of infection at the time when microbes are anticipated to be most infectious. The anticipation of potentially pathogenic microorganisms through sensing climatological changes and their specific detection thus constitute two distinct layers of the plant immune system.

Among the climatological factors that affect the outcome of plant–microbe interactions, rain is a major cause of devastating plant diseases, as fungal spores and bacteria are spread through rain-dispersed aerosols or ballistic particles splashed from neighboring infected plants. Natural raindrops contain bacteria at a concentration of 1.06 × 10^4 (cm^3)^11, including plant pathogens such as *Pseudomonas syringae*, *Xanthomonas campestris*, and *Pantoea ananatis*. Likewise, raindrops contain fungi such as *Alternaria* sp., *Fusarium* sp., *Cladosporium* sp., *Phoma* sp., *Rhizopus* sp., and *Botrytis cinerea*. In addition, raindrops negatively regulate stomatal closure, which facilitates pathogen entry into leaf tissues. High humidity, which is usually associated with rain, enhances the effects of bacterial pathogen effectors, such as HopM1, and establishes an aqueous apoplast for aggressive host colonization. These findings suggest that it would be beneficial for plants to recognize rain as an early risk factor for infectious diseases.

How do plants respond to rain? Rain-simulating water spray induces the expression of mechanosensitive TOUCH (TCH) genes as bending stimulation, which induces plant growth retardation. Mechanostimulation affects a variety of plant physiological processes mediated by phytohormones such as auxin, ethylene, and gibberellin. *Arabidopsis thaliana* seedlings exposed to rain-simulating water spray accumulate the immune phytohormone jasmonic acid (JA) to promote the expression of JA-responsive genes. Thus, rain modulates both mechanotransduction and jasmonic acid (JA) to promote the expression of JA-responsive genes that can circumvent PTI, plants also deploy disease resistance (R) genes, primarily encoding nucleotide-binding LRR proteins, which mount effector-triggered immunity (ETI) to limit pathogen proliferation. While adapted pathogens have evolved virulence effectors that can circumvent PTI, plants also deploy disease resistance (R) genes, primarily encoding nucleotide-binding LRR proteins, which mount effector-triggered immunity (ETI).

To comprehensively identify mechanosensitive genes, we performed RNA-seq analysis of leaves brushed once 1–10 times along the main veins with a small paintbrush (Supplementary Fig. 1c; Methods) and analyzed the expression profile of the immune regulator WRKY33, which was responsive to raindrops. WRKY33 expression was maximally induced 15 min after brushing the leaves once to four times (Supplementary Fig. 1d). Next, we compared gene expression patterns between leaves that were brushed once and those that received 10 falling raindrops. Both raindrops and brushing strongly upregulated TCH2, TCH4, WRKY33, WRKY40, WRKY53, CBP60g, MYB51, ERF1, and jasmonate-ZIM-domain protein 1 (JAZ1) expression, suggesting that raindrops are likely recognized as a mechanical stimulus. This idea was supported by the result that natural rainfall-induced the expression of TCH4 and WRKY33 in Col-0 leaves (Supplementary Fig. 1e).

To validate this hypothesis, we mechanically stimulated rosette leaves by gently brushing them 1–10 times along the main veins with a small paintbrush (Supplementary Fig. 1c; Methods) and observed the expression profile of the immune regulator WRKY33, which was responsive to raindrops. WRKY33 expression was maximally induced 15 min after brushing the leaves one to four times (Supplementary Fig. 1d). Next, we compared gene expression patterns between leaves that were brushed once and those that received 10 falling raindrops. Both raindrops and brushing strongly upregulated TCH2, TCH4, WRKY33, WRKY40, WRKY53, CBP60g, MYB51, ERF1, and jasmonate-ZIM-domain protein 1 (JAZ1) expression, indicating that raindrops are likely recognized as a mechanical stimulus. This idea was supported by the result that natural rainfall-induced the expression of TCH4 and WRKY33 in Col-0 leaves (Supplementary Fig. 1e).

To comprehensively identify mechanosensitive genes, we performed RNA-seq analysis of leaves brushed once. We identified 1241 genes that were significantly induced 15 min after this treatment relative to control plants (3 biological replicates, log2 fold changes (log2FC) ≥ 1, likelihood ratio test; P < 0.05) (Supplementary Data 3). These mechanosensory stimuli (MS)-induced genes were primarily categorized as plant immune responses, such as response to chitin, defense response, and immune system response (Fig. 1d and Supplementary Data 4). We found that 87.3% of raindrop-induced genes and 73.9% of MS-induced genes overlapped (Fig. 1e): this set of 917 genes expressed upon both treatments were enriched for GO categories associated with stress responses (Supplementary Fig. 2). Furthermore, the expression levels of these 917 genes, including major immune regulators, were positively correlated between the two treatments (Pearson correlation coefficient r = 0.917) (Fig. 1f). In addition, we observed clear correlations between our profiles and previously published datasets obtained by water spray, bending, brushing, and cotton swabbing treatments (Supplementary Fig. 3 and Supplementary Data 11). Strong correlations observed in Fig. 1f are consistent with the fact that 10 falling droplets
applied the same level of the force intensity as brushing the leaf surface once (Supplementary Fig. 4). Taken together, these transcriptome analyses indicated that falling raindrops stimulate the expression of mechanosensitive genes involved in environmental stress responses, including plant immunity.

Rain and MS rapidly activate plant immune responses. To further characterize raindrop-induced genes, we conducted a comparative analysis with published transcriptome datasets. Many raindrop- and MS-induced genes were also expressed during major plant immune responses, such as those triggered by the immune phytohormones salicylic acid (SA), which is effective against biotrophic pathogens (21%; 193/917 genes), and JA, which mounts immune responses to necrotrophic pathogens (11.8%; 108/917 genes); the bacterial-derived peptide flg22, which activates PTI (37%; 339/917 genes); and the bacterial pathogen P. syringae pathovar maculicola ES4326 (Psm ES4326) (25.8%; 237/917 genes)1,2,29 (Fig. 2a, b). In total, 58.6% (537/917 genes) of raindrop- and MS-induced genes overlapped with those induced in response to different immune elicitors, suggesting that raindrops activate mechanosensitive immune responses.
Since stress-responsive gene expression is either positively or negatively regulated by phytohormones, we determined the changes in the accumulation levels of six phytohormones [SA, JA, JA-isoleucine (JA-Ile), abscisic acid (ABA), gibberellic acid 4 (GA4), and indole-3-acetic acid (IAA)] in leaves treated with 10 falling droplets and in those brushed once. No significant changes in the levels of the phytohormones were observed 5 min and 15 min after treatment (Fig. 2c and Supplementary Fig. 5). However, the slight increase in JA and JA-Ile could explain the observation that 11.8% of raindrop- and MS-induced genes are JA-responsive (Fig. 2a) as previously confirmed by Van Moerkercke et al.23. Although 21% of raindrop- and MS-induced
Fig. 2 Raindrop- and MS-induced mechanosensation triggers defense responses. a, b Venn diagram (a) and the upset plot (b) between 917 raindrop- and MS-induced genes and transcriptome datasets obtained from salicylic acid (SA), jasmonic acid (JA), and flg22 (PAMP) treatment and Pseudomonas syringae pv. maculicola ES4326 infection (P < 0.05). Overlap with raindrop- and MS-induced genes: SA, 21%, 193/917 genes; JA, 11.8%, 108/917 genes; flg22, 37%, 339/917 genes; Psms ES4326, 25.8%, 237/917 genes; any of the four factors, 58.6%, 537/917 genes. c Accumulation of plant hormones (ng/g fresh weight) SA, JA, JA-isoleucine (JA-Ile), abscisic acid (ABA), and indole-3-acetic acid (IAA) 5 min after treatment with 10 falling droplets (raindrop), 1 brushing (MS), or cutting (wounding). Data are presented as mean ± SD. n = 6 plants examined over three independent experiments. Each dot indicates a biological replicate. Different letters above bars indicate significant differences (one-sided Tukey’s multiple comparison test; P < 0.05). d, e Raindrop (4 droplets)- and MS (4 brushings)-induced (e) MAPK activation in Col-0. Total proteins were extracted from 4-week-old plants treated with raindrops and detected by immunoblot analysis with anti-phospho-p44/42 MAPK antibodies. Relative phosphorylation levels are shown below each blot. Similar results were obtained in four independent experiments. f MS-induced MAPK activation in Col-0, fls2, and bak1-3. Total proteins were extracted from 4-week-old plants after 5 min of MS treatment (1 brushing) and detected by immunoblot analysis with anti-phospho-p44/42 MAPK antibodies. Relative phosphorylation levels are shown below each blot. Similar results were obtained in three independent experiments. g, h Disease progression of Alternaria brassicicola in Col-0 leaves 3 days after inoculation with (+) or without (−) raindrop (10 falling droplets) pretreatment (g) or with (+) or without (−) MS (4 brushings) pretreatment (h). Error bars represent SE. Asterisks indicate significant difference (two-sided Tukey’s t test; **P < 0.0001). I Growth of Psms ES4326 in Col-0 leaves 2 days after inoculation with (+) or without (−) MS (4 brushings) pretreatment. Error bars represent SE. Asterisks indicate significant difference (two-sided Tukey’s t test; ****P < 0.0001). Cfu colony-forming units. n = 29 (g), (−): n = 13, (+: n = 12 (h), and n = 8 (i) samples examined over three independent experiments. Each dot indicates a biological replicate.

Rain and MS confer resistance to both biotrophic and necrotrophic pathogens. We then investigated whether raindrops and MS confer resistance to pathogenic microbes. Raindrops containing the spores of the necrotrophic pathogen Alternaria brassicicola Ryo-1 were placed on fully expanded leaves after pretreatment with raindrops or MS for 3 h at an interval of 15 min. Both stimuli significantly suppressed lesion development compared to control plants without pretreatment (Fig. 2g, h). Pretreatment of leaves with MS for 3 h also efficiently protected plants from infection with the biotrophic pathogen Psm ES4326 (Fig. 2i). These results confirmed that mechanostimulation induces a PTI-like response to confer a broad spectrum of resistance to both biotrophic and necrotrophic pathogens, as MS activates immune MAPKs and upregulates a large subset of flg22-induced genes. In support of this argument, exposure to the fungal cell wall, chitin, also upregulated 42.1% (386/917 genes) of raindrop-induced genes (Supplementary Fig. 7c).

Mechanosensitive genes are regulated by calmodulin-binding transcription activator 3. To dissect rain-induced mechanotransduction, we searched for a conserved cis-regulatory element in the promoter sequences of mechanosensitive genes. From an unbiased promoter analysis of the top 300 genes among 917 differentially expressed genes, we obtained the highest enrichment for the GCCG box (CCCGTG or CGTGTC), which is recognized by CAMTA3s that are conserved from plants to mammals38–42 (Fig. 3a). A similar motif analysis that detects the CAMTA-binding sites among the brushing-induced gene promoters was reported27. The Arabidopsis transcription factor CAMTA3s that were involved in immune signal transduction are CAMTA3s, which are negative regulators of plant immunity; camta3 null mutants exhibit constitutive expression of defense-related genes and enhanced resistance to virulent P. syringae infection33,44. CAMTA transcription

- **Fig. 2**: Raindrop- and MS-induced mechanosensation triggers defense responses. This figure shows the overlap between the raindrop- and MS-induced gene sets with transcriptomics data from various treatments, including salicylic acid (SA), jasmonic acid (JA), and flg22 (PAMP) treatment and Pseudomonas syringae pv. maculicola ES4326 infection. The Venn diagram and upset plot illustrate the overlap in gene expression between these treatments, with annotations indicating the significance levels for each overlap.

- **Fig. 3a**: Arabidopsis transcription factor CAMTA3 binding sites. This figure shows the enrichment of the GCCG box (CCCGTG or CGTGTC) in the promoter regions of brushing-induced genes, indicating the role of CAMTA3 in mechanosensation-induced defense responses.

The text discusses the role of MAPK activation in mechanosensation, with specific examples of phosphorylation levels in response to raindrops and MS. It also explores the role of mechanosensitive genes regulated by the calmodulin-binding transcription activator 3 (CAMTA3) in conferring resistance to both biotrophic and necrotrophic pathogens. The figure data support the hypothesis that mechanostimulation induces a PTI-like response, which is critical in mechanotransduction.
Fig. 3 MS-induced genes are regulated by CAMTA3. a Promoter analysis of the top 300 (among 917 genes) raindrop- and MS-induced genes in terms of expression levels revealed that the CAMTA-binding CGCG box [CGCG/TGT] was overrepresented among these genes. **c Transcript levels of WRKY33 and CBP60g in 4-week-old camta2 camta3 CAMTA3pro:CAMTA3-GFP (CAMTA3) and camta2 camta3 CAMTA3pro:CAMTA3A855V-GFP (CAMTA3) plants 15 min after the plants were treated with 1 falling droplet (b) or brushed 4 times (c), determined by RT-qPCR and normalized to UBQ5 transcript levels in Col-0 Mock. Data are presented as mean ± SD. Asterisks indicate significant difference (one-sided Tukey’s multiple comparison test; ***P < 0.001, ****P < 0.0001). n = 6 plants examined over three independent experiments. Each dot represents a technical replicate.

b Venn diagram depicting the overlap between genes with CAMTA3-binding sites in their promoters, as determined by ChIP-seq, and raindrop- and MS-induced genes as determined by RNA-seq. A total of 314 genes, shown in red, were identified as CAMTA3-target genes. The CGCG box was identified as an overrepresented motif among the sequence peaks of 314 genes by MEME-ChIP. Localization of CAMTA3 on the promoters of the MS-induced genes TCH2, TCH4, CAM2, CBP60g, CML23, and WRKY40, as representative of the 314 genes shown in (d). Blue and red lines indicate CGCGG and CGTGTG, respectively. g Growth of Psm ES4326 in camta2 camta3 CAMTA3pro:CAMTA3-GFP (CAMTA3) and camta2 camta3 CAMTA3pro:CAMTA3A855V-GFP (CAMTA3A855V) plants 2 days after inoculation with (+) or without (−) MS (4 brushing) pretreatment. Error bars represent SE. Asterisks indicate a significant difference (one-sided Tukey’s multiple comparison test and two-way ANOVA; ***P < 0.0001). Cfu colony-forming units, NS not significant. CAMTA3 (−, +), CAMTA3A855V (−): n = 8, CAMTA3A855V (+): n = 6 samples examined over three independent experiments. Each dot indicates a biological replicate.
factors possess a CaM-binding domain and an IQ domain to which CaM binds in a calcium-dependent manner to negate their function (Supplementary Fig. 8a). CAMTA3^A835V transgenic plants, which possess a mutation in the IQ domain, suppress the constitutive expression of defense-related genes seen in the *camta2 camta3* double mutant and are no longer regulated by calcium-mediated responses. In agreement with our promoter analysis, 28.7% of constitutively upregulated genes (309/1075 genes) in the *camta1 camta2 camta3* triple mutant overlapped with raindrop- and MS-induced genes detected in wild-type plants (Supplementary Fig. 8b and Supplementary Data 7). Upon application of raindrops and MS, WRKY33 and CBP60g transcript levels were significantly reduced in plants expressing the CAMTA3^A835V variant compared to a CAMTA3-GFP transgenic line expressing a transgene that complemented the phenotype of the *camta2 camta3* mutant (Fig. 3b, c), suggesting that CAMTA3 is involved in mechanotransduction.

To confirm whether CAMTA3 directly targets mechanosensitive genes, we investigated the genome-wide distribution of CAMTA3 binding sites by chromatin immunoprecipitation followed by deep sequencing (ChiP-seq) using CAMTA3^A835V-GFP plants, as the mutant protein stably represses the transcription of CAMTA3-regulated genes. With the aid of model-based analysis of ChiP-seq (MACS2) software, we identified 2641 and 2728 CAMTA3-binding genes, respectively, in two replicates (binomial distribution; *P* < 0.05); about 40% of these peaks are located in the promoter regions and another 30% in gene bodies (Supplementary Fig. 8c and Supplementary Data 8). The overlap between the two replicates highlighted 2011 CAMTA3-targeted genes that included 272 raindrop- and 297 MS-induced genes such as *TCH2*, *TCH4*, and *CBP60g* (Fig. 3d), consistent with our hypothesis that CAMTA3 regulates the transcription of mechanosensitive genes.

To validate the results from the promoter analysis of mechanosensitive genes, we next investigated specific DNA sequences to which CAMTA3 selectively binds by analyzing CAMTA3-binding peaks by Multiple EM for Motif Elicitation (MEME)-ChiP (Methods). We again identified the CGCG box (CCGGT or CGTGT) as the motif with the highest enrichment.

MS initiates intercellular calcium waves concentrically away from trichomes. To visualize changes in cytosolic Ca$^{2+}$ concentrations ([Ca$^{2+}$]$_{cyt}$) induced by MS on the leaf surface, we used transgenic *Arabidopsis* expressing the GFP-based [Ca$^{2+}$]$_{cyt}$ indicator GCaMP3. Leaf brushing induced a marked increase of [Ca$^{2+}$]$_{cyt}$ in the surrounding leaf area of trichomes 1 min after stimulation (Fig. 4d and Supplementary Movie 1). Flicking a single trichome with a silver chloride wire triggered an intercellular calcium wave that propagated concentrically away from the trichome and surrounding skirt cells at a speed of 1.0 μm/s (Fig. 4e, f and Supplementary Movie 2). This pattern showed striking consistency with the area of induction observed with the WRKY33pro:EFYP-NLS and CBP60gpro:EFYP-NLS reporters (Fig. 4a, b and Supplementary Fig. 9a, b). The base of trichomes exhibited a rapid and transient increase in [Ca$^{2+}$]$_{cyt}$ before the concentric propagation of calcium waves was initiated (Fig. 4g and Supplementary Movie 3).

Trichomes are mechanosensory cells activating plant immunity. To investigate the possible involvement of trichomes in mechanosensation in *Arabidopsis* leaves and activation of the immune response, we observed calcium waves using the knockout mutant of *glabrous 1* (*GL1*) mutants, which lacks trichomes. The *gl1* mutants exhibit effective basal resistance comparable to that of wild-type Col-0 plants, and its local resistance to *Psm ES4326 and A. brassicicola RyO-1* is similar to that of Col-0 plants (Supplementary Fig. 11). However, the trichome-defective *gl1 gl3* (?brous 3), ttg1 (transparent testa glabra 1) mutants lost cuticle and systemic acquired resistance (SAR), a secondary immune response in uninfected distal tissues after primary infection in a local site, while basal levels of immunity were not dramatically affected (Fig. 5 and Supplementary Fig. 11). The lack of SAR in these trichome mutants is associated with impairment of cuticle formation but not of trichomes. *cer1-1* (enceriferum 1) and *cer3-1* (WAX 2) mutants, which have defects in synthesizing cuticular wax but form normal
Fig. 4 Trichomes initiate intercellular calcium waves. a–c YFP fluorescence in a whole leaf from WRKY33pro:EYFP-NLS (Col-0) with (MS, bottom half) or without brushing (untreated, top half) (a), along with zoomed-in views of brushed (b), and untreated (c) areas. Arrowheads indicate trichomes (b, c). Scale bars, 0.5 mm (a), 0.3 mm (b, c). Similar results were obtained in three independent experiments. d Ca\(^{2+}\) imaging using 35Spro:GCaMP3 (Col-0). The leaf surface of a 4-week-old plant was treated with MS by brushing. MS-induced intercellular calcium waves propagated concentrically from trichomes. Scale bar, 1.0 mm. See also Supplementary Movie 1. e Ca\(^{2+}\) imaging using 35Spro:GCaMP3 (Col-0). A single trichome from a 2-week-old seedling was flicked with a silver chloride wire. MS-induced intercellular calcium waves propagated concentrically from the trichome (dashed outline). Scale bar, 0.2 mm. See also Supplementary Movie 2. Similar results were obtained in three independent experiments. f \([\text{Ca}^{2+}]_\text{cyt}\) changes at sites indicated by numbers in (e). Similar results were obtained in three independent experiments. g Side view of a trichome whose neck was flicked with a silver chloride wire. MS-induced intercellular Ca\(^{2+}\) influx was transiently observed in the trichome base (arrowheads) followed by the formation of circular waves. Scale bar, 0.1 mm. See also Supplementary Movie 3. Similar results were obtained in three independent experiments.
trichomes, fail to induce SAR\textsuperscript{56}. Therefore, the immunodeficient phenotype of \textit{gl} and \textit{ttg} mutants is due to disordered cuticle formation. To investigate whether trichome-mediated immunity is compromised in \textit{cer1} and \textit{cer3} mutants as in \textit{gl1}, we performed the pathogen test using \textit{A. brassicicola} Ryo-1 after mechanical stimulation of these mutants (Supplementary Fig. 12). Pretreatment with brushing significantly suppressed lesion development. These data strongly indicate that the lack of trichome, but not cuticle, in \textit{gl} mutants is responsible for failed MS-induced immunity. The mechanostimulation-induced propagation of concentric calcium waves was compromised in the \textit{gl1} mutant (Fig. 5a, b and Supplementary Fig. 13), confirming that trichomes are true MS sensors and initiate calcium waves (Supplementary Movies 4 and 5). Furthermore, approximately 70.5\% of mechanosensitive genes were expressed in a trichome-dependent manner (3 biological replicates, log\textsubscript{2}FC $\geq$ 1, likelihood ratio test; $P$ < 0.05) (Fig. 5c, Supplementary Fig. 14a and Supplementary Data 9), and transcript levels of 18 representative mechanosensitive immune genes were markedly lower at all time points in the \textit{gl1} mutant than they were in the wild type in RNA-seq.
Fig. 5 Trichomes are mechanosensory cells. a Ca\(^{2+}\) imaging using 35Spro:GCaMP3 (Col-0) and 35Spro:GCaMP3 (gl1). Leaf surfaces were exposed to MS by brushing. MS-induced calcium waves were compromised in the gl1 mutant. See also Supplementary Movies 4 and 5. Scale bars, 0.5 mm. b [Ca\(^{2+}\)\(_{\text{cyt}}\)] signature of (a). c Venn diagram of transcriptome datasets for MS-induced genes in Col-0 and gl1 (likelihood ratio test; P < 0.05). NS, not significant. Lower, fold change (FC) (gl1)/FC (Col-0) < 0.5. High, MS (gl1)/Mock (gl1), log\(_2\)FC ≥ 1 in gl1 (likelihood ratio test; P < 0.05). d Heatmap of differentially expressed defense-related genes obtained from transcriptome datasets from Col-0 and gl1 plants treated with MS (4 brushings). e Transcript levels of WRKY33, WRKY40, and CBP60g in 4-week-old Col-0 and gl1 plants 15 min after treatment with brushing 4 times, determined using RT-qPCR and normalized to UBQ5. Data are presented as mean ± SD. Asterisks indicate significant difference (one-sided Tukey’s multiple comparison test; ****P < 0.0001, ****P < 0.0001). n = 6 plants examined over three independent experiments. Each dot indicates a technical replicate. f MS-induced MAPK activation in Col-0 and gl1. Total proteins were extracted from 4-week-old leaves 5 min after MS (4 brushings) treatment and detected by immunoblot analysis with anti-phospho-p44/42 MAPK antibodies. Relative phosphorylation levels are shown below each blot. Similar results were obtained in three independent experiments. Each dot indicates a biological replicate. g Disease progression of Psm ES4326 in Col-0 and gl1 leaves 2 days after inoculation with (+) or without (−) MS (4 brushings) pretreatment. Error bars represent SE. Asterisks indicate a significant difference (one-side Tukey’s t test and two-way ANOVA; ****P < 0.0001). Clu colony-forming units, NS not significant. n = 8 samples examined over three independent experiments. Each dot indicates a biological replicate. h Disease progression of Alternaria brassicicola in Col-0 and gl1 leaves 3 days after inoculation with (+) or without (−) MS (4 brushings) pretreatment. Error bars represent SE. Asterisks indicate a significant difference (one-side Tukey’s t test and two-way ANOVA; ****P < 0.0001). NS not significant. Col-0: n = 15, gl1: n = 14 samples examined over three independent experiments. Each dot indicates a biological replicate.

Fig. 6 Mechanosensory trichomes evoke an immune response. Model showing how trichomes directly sense the mechanical impact of raindrops as an emergency signal in anticipation of possible infections. Mechanosensory trichome cells initiate intercellular calcium waves in response to MS. [Ca\(^{2+}\)\(_{\text{cyt}}\), initiates the de-repression of Ca\(^{2+}\)/CaM-dependent CAMTA3 and activates the phosphorylation of MPK3 and MPK6, thereby inducing WRKY-dependent transcription.
Mimosa pudica and Arabidopsis pollination, tentacles of bees are a common intercellular network of cell signaling pathways. Sensory hairs of the short- and long-term responses. Two successive stimulations of Ca2+ are thought to play a pivotal role in mechanosensation (Supplementary Fig. 16). Our work demonstrated that raindrops and MS only partially activate the JA signal but rather strongly induce a PTI-like response via the Ca2+ and CAMTA3-dependent pathway, which is effectively observed between both mechanotransduction and biotrophic signaling (Fig. 2g–i). Because rain disseminates diverse pathogens with different parasitic strategies, including fungi, bacteria, and viruses, it is reasonable that plants perceive raindrops as a risk factor and activate broad-spectrum resistance.

Plants possess mechanosensory cells with a variety of functions, such as flower anthers of Catanese species for pollination, tentacles of Drosera rotundifolia for insect trapping, root hairs of Arabidopsis for water tracking, and red cells of Mimosa pudica for evading herbivores. The carnivorous Venus flytrap (Dionaea muscipula) captures insects by sensing mechanostimulation via sensory hairs on leaf lobes. To monitor diverse MS applied to plants, several sensing mechanisms have been proposed that include detection of cell wall components, distortion of the plasma membrane, and displacement of the plasma membrane against the cell wall. In all these systems, a transient increase in [Ca2+]cyt is thought to play a pivotal role in short- and long-term responses. Two successive stimulations of sensory hairs of the flytrap are required to meet the threshold of [Ca2+]cyt for rapid closure of the leaf blade. As the trichome on the leaf surface is widely found in many land plants, there may be a common intercellular network of cell-cell communication that initiates calcium waves for activating immune responses.

**Methods**

**Plants.** A. thaliana accession Columbia-0 (Col-0) was the background for all plants used in this study. WRKY33pro:EEFP-NLS (Col-0) and CBP60gpro:EEFP-NLS (Col-0) were generated as described in the subsection “Promoter-reporter imaging”. 35Spro:GCaMP3 (Col-0)35, camt2a camt3 CAMTA3pro:CAMTA3-GFP, and camt2a camt3 CAMTA3pro:CAMTA3GFP were provided from Michael F. Thomashow (Michigan State University)64.35S:Goll(Glu)1 was obtained from Lehle Seeds (TX, USA) and was previously designated as g1 mutant. The Arabidopsis mutants fis2 (SALK_093905) and bak1-3 (SALK_034523) were obtained from the Arabidopsis Biological Resource Center (ABRC). 35Spro:GCaMP3 was introduced into the g1 mutant background by crossing. The selection of homozygous lines was performed by genotyping using primers listed in the Supplementary Data 11. Plants were grown on soil (peat moss; Super Mix A and vermiculite mixed 1:1) at 22 °C under diurnal conditions (16-h-light/8-h-dark) and 70% relative humidity. The peak weight applied to the leaf surface was obtained as the force. The force per unit area (N/m2) is converted from the peak weight (kg) and the contact area of the brush tip (5.6 × 10−3 m2) or raindrop (9.73 × 10−6 m2).

**RNA-seq library construction.** Total RNA was extracted from 80 to 100 mg frozen samples using Sepasol-RNA I Super G (Nacala Tesque, Kyoto, Japan) and the TURBO DNase free kit (Thermo Fisher Scientific, IL, USA) according to the manufacturer’s protocols. Total RNA was further purified with the NRSaRNA Isolation Kit (IQGEN, Halden, Germany) and assessed for quality and quantity with the NanoDrop Spectrophotometer (Thermo Fischer Scientific). Total RNA was used at 1 μg total RNA for mRNA purification with NERBNext Oligo d(T)20 (NEBNext poly(A) mRNA Magnetic Isolation Module; New England Biolabs, MA, USA), followed by first-strand cDNA synthesis with the NEBNext Ultra II RNA Library Prep Kit for Illumina (New England Biolabs) and NBDNext Multiplex Oligo for Illumina (New England Biolabs) according to the manufacturer’s protocols. For the analysis of the RNA-seq and MS-induced gene expression, the amount of cDNA was determined on an Agilent 4150 TapeStation System (Agilent, CA, USA). cDNA libraries were sequenced as single-end reads for 36 nucleotides on an Illumina NextSeq 550 (Illumina, CA, USA). The reads were mapped to the Arabidopsis thaliana reference genome (TAIR10, http://www.arabidopsis.org) on the web (BaseSpace, Illumina, https://basespace.illumina.com/). Pairwise comparisons between samples were performed with the EdgeR package on the web (Degust, https://degest.erc.monash.edu/).

For the comparative analysis of differentially expressed genes between leaves in the g1 mutant and Col-0, the amount of cDNA was determined by the QuantFluor dsDNA System (Promega, WI, USA). cDNA libraries were sequenced as single-end reads for 36 nucleotides on an Illumina NextSeq 550 (Illumina). The reads were mapped to the A. thaliana reference genome (TAIR10) by Bowtie2 with the options “--best” and “--strata”. Pairwise comparisons between samples were performed with the EdgeR package in the R program and enrichment analysis for biological categories was performed using BiNGO (http://www.psb.ugent.be/cdb/papers/BiNGO/Home.html) (one-sided hypergeometric test; P < 0.05). Pearson correlation coefficient of expression levels between two transcriptome profiles was calculated with Excel function (PEARSON (r = 0.2–0.4: weak correlation, r = 0.4–0.7: slightly correlated, r = 0.7–1.0: strongly correlated).

**Quantiﬁcation of the force density.** The abaxial side of leaves from 4-week-old plants was physically attached to the measuring pan of the electronic balance QUINTIX224-1S (Sartorius Lab Instruments GmbH & Co., Göttingen, Germany) with surgical tape (3 M Company, MN, USA). The adaxial side of the leaf was treated with a falling droplet or brushed once (shown in the above subsections). The peak weight applied to the leaf surface was obtained as the force. The force per unit area (N/m2) is converted from the peak weight (kg) and the contact area of the brush tip (5.6 × 10−3 m2) or raindrop (9.73 × 10−6 m2).

**Re-analysis of immune-related transcriptome datasets.** We used the following public transcriptome datasets for comparative analysis with the RNA-seq data obtained in this study: 10-day-old Arabidopsis seedlings treated with 1 μg flg22 for 30 min (Array Express; E-NSAC-76)26, 8-day-old Arabidopsis seedlings treated with 40 μM chitin for 1 h (Gene Expression Omnibus; GSE76955), leaves from 4-week-old Arabidopsis plants inoculated with Pseudomonas syringae pv. maculicola (Psm) ES4326 (24 h post inoculation) (GSE18978), 2-week-old Arabidopsis seedlings treated with 10 μM JA for 24 h (DNA Data Bank of Japan; DRA003119)33, 2-week-old Arabidopsis seedlings grown with MS medium and treated with water spray for 10 min or 25 min (E-MTAB-8021)27, leaves from 4-week-old Arabidopsis plants treated with brushing for 30 min (NCBI BioProject: PRJNA3782)22, 2-week-old Arabidopsis seedlings grown with MS medium and treated with cotton swabbing for 30 min (accession is now not available)31, leaves from 4-week-old Arabidopsis plants treated with brushing for 30 min (NCBI BioProject: PRJNA537102)22, 12-day-old Arabidopsis
RT-qPCR. Total RNA was extracted from 30~40 mg leaf tissue with Sepasol-RNA I Super G and the TURBO DNase free kit (Thermo Fisher Scientific) according to the manufacturer’s protocols, followed by reverse transcription with the PrimeScript RT reagent kit (Takara Bio, Shiga, Japan) using oligo dT primers. RT-qPCR was performed on the first-strand cDNAs diluted 20-fold in water using KAPA SYBR FAST qPCR Master Mix (2×) kit (Roche, Basel, Switzerland) and gene-specific primers in a LightCycler 96 (Roche). Primer sequences are listed in Supplemental Data 10.

ChIP-Seq library construction. ChIP-seq libraries for the input and two biological replicates were constructed from 2 ng purified DNA samples with the NEB Ultra II DNA Library Prep Kit for Illumina (New England Biolabs) according to the manufacturer’s instructions. The amount of DNA was determined on an Agilent 4150 TapeStation System (Agilent). All ChIP-seq libraries were sequenced as 12-nucleotide single-end reads using an Illumina NextSeq 500 system.

Analysis of ChIP-seq. Reads were mapped to the Arabidopsis thaliana genome (TAIR10, http://www.arabidopsis.org/) using Bowtie2 with default parameters74. The Sequence Alignment/Map (SAM) file generated by Bowtie2 was converted to a Binary Alignment/Map (BAM) format file by SAMtools45. To visualize mapped reads, Tiled Data Files file were generated from each BAM file using the igvtools package in the IGV48. ChIP-seq peaks were called by comparing the IP with the input using MACS2 with the “–p 0.05 -g 1.196” option (binomial distribution; P<0.05)75. The peaks were annotated using the nearest gene using the Bioconductor and the ChIPPeaksAnno packages in the R program, from which we identified 2111 genes detected in both biological replicates. Enrichment of GO categories of the set of 314 genes overlapping between raindrop- and MS-induced genes for biological processes was determined using BINGO (http://www.psb.ugent.be/cdbs/papers/BiNGO/Home.html)76. Sequences of the peaks were extracted from the Arabidopsis thaliana genome as FASTA files with BEDtools46. To identify the candidates of CAMTA3-binding motifs, the FASTA files were subjected to MEME-ChIP with the default parameters (meme-mw 6-meme-maxw 10)77, and a density plot of the distribution of the motifs were generated.

Immuno blot analysis for detection of MPK3 and MPK6 phosphorylation. The adaxial side of leaves from 4-week-old plants was brushed four times or treated with four raindrops, and samples (0.1~0.15 g) were snap-frozen in liquid nitrogen. Total proteins were extracted in protein extraction buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 2 mM EDTA, 0.1% (v/v) Triton X-100, 0.5% (w/v) Nonidet P-40, 50 mM β-glycerophosphate, and proteinase inhibitor cocktail) and centrifuged once at 6000 g, 4 °C, for 20 min and then at 17,000 g, 4 °C for 10 min. The supernatant was mixed with SDS sample buffer (50 mM Tris-HCl pH 6.8, 2% (w/v) SDS, 5% (w/v) glycerol, 0.02% (w/v) bromophenol blue, and 200 mM DTT) and heated at 70 °C for 20 min. The protein samples were subjected to SDS-PAGE, electrophoresis and transferred onto a nitrocellulose membrane (GE Healthcare, IL, USA). The membrane was incubated with an anti-phospho-p44/42 MAPK polyclonal antibody (Cat#9101; Cell Signaling Technology, MA, USA) (1:1000 dilution) and goat anti-rabbit IgG(H + L)-HRP secondary antibody (Cat#70745-551; BIO-RAD, USA) (1:5000 dilution) for 1 h. The bands for MPK3 and MPK6 were visualized using chemiluminescence solution mixed 5:1 with ImmunoStar Zeta (FUJIFILM Wako Chemicals, Osaka, Japan) and SuperSignal West Dura Extended Duration Substrate (Thermo Fisher Scientific). The Rubisco bands were stained with Ponceau S (Merck Sharp & Dohme Corp., NJ, USA) as a loading control. The phosphorylation levels of MPK3 and MPK6 were quantified with the blot analysis plug-in in ImageJ (https://imagej.nih.gov/ij/).

Treatment with the calcium ionophore A23187. Twelve-day-old Col-0 seedlings were treated with 50 μM calcium ionophore A23187 (Sigma-Aldrich Co, MO, USA) for 15, 30, and 60 min. Samples were processed for the phosphoimmunohistochemistry of MPK3 and MPK6 as described in the “Immuno blot analysis for detection of MPK3 and MPK6 phosphorylation” section. The leaf tissue was stored at −80 °C until use.

Promoter-reporter imaging. The 3.0-kbp promoters for WRKY33 and CBP60g, both of which covered the previously analyzed respective regulatory sequences, were amplified from Col-0 genomic DNA by PCR and cloned into the pENTR/D-TOPO vector (Invitrogen). The promoter regions were recombined using Gateway technology into the binary vector pbyGVN. The resulting pBGYN-pWRYK33-EYFP-NLS and pBGYN-pCBP60g-EYFP-NLS vectors were introduced into A. tumefaciens GV3101 (mpP90) and then into Arabidopsis Col-0 plants using the floral dip method. A representative homozygous line was selected for each construct for further detailed analyses.

Promoter-reporter imaging was performed using an MA205A automated stage microscope (Nikon, Japan) equipped with a 40× objective (Leica Wetzlar, Germany) and DFC356FX CCD camera (Leica Microsystems) in 12-bit mode. Chlorophyll autofluorescence and YFP fluorescence were detected through Texas Red (TXR) (excitation 560/40 nm, emission 610 nm) and YFP (excitation 510/20 nm, emission 560/40 nm) filters (Leica Microsystems). To image fluorescence emanating from the WRKY33pro:EYFP-NLS (Col-0) and CBP60gpro:EYFP-NLS (Col-0) plants47, the leaves of 3-week-old Arabidopsis plants were brushed 10 times at an interval of 15 min for 2 h or left untreated.
Promoter analysis. The statistical analysis for overrepresented transcriptional regulatory elements across transcriptome datasets described above was calculated using the Bioinformatics program. The P-values were calculated using the Statistical Motif Analysis in Promoter or Upstream Sequences (https://www.arabidopsis.org/tools/bulk/motiffinder/index.jsp). Figures of promoter motif sequences are generated with WebLogo (https://weblogo.berkeley.edu/logo.cgi).

Real-time [Ca2+]cyt imaging. We used 4-week-old and 3-week-old plants expressing the GFP-based cytosolic Ca2+ concentration ([Ca2+]cyt) indicator GCaMP32,35, To image the fluorescence from the GCaMP3 reporter (in Col-0 and gl1) in whole leaves, the axilial sides of leaves from 4-week-old plants were blunted. To monitor the calcium waves propagating from trichomes, a single trichome from a 2-week-old seedling was flanked with a silver chloride wire. Samples were imaged with a motorized fluorescence microscope (SMZ–25; Nikon, Tokyo, Japan) equipped with a 1x objective lens (NA = 0.156, P2-ShIR PLAN APO; Nikon) and an sCMOS camera (ORCA-Flash 4.0 V2; Hamamatsu Photonics, Shizuoka, Japan)33.

To detect the accumulation levels of GCaMP3 protein in 35Spro:GCaMP3:Col-0 and 35Spro:GCaMP3:gl1, leaves from 4-week-old plants (0.1–0.15 g) were snap-frozen in liquid nitrogen. Total proteins were extracted in protein extraction buffer [50 mM Tris–HCl pH 7.5, 150 mM NaCl, 2 mM DTT, 0.5% (w/v) Nonidet P-40, and proteinase inhibitor cocktail] and centrifuged once at 6000 g, 4 °C, for 20 min and twice at 17,000 g, 4 °C for 10 min. The supernatant was mixed with SDS sample buffer (50 mM Tris–HCl pH 6.8, 2% (w/v) SDS, 5% (w/v) glycerol, 0.02% (w/v) bromophenol blue, and 200 mM DTT) and heated at 70 °C for 20 min. The protein samples were subjected to SDS-PAGE electrophoresis and transferred onto a nitrocellulose membrane (GE Healthcare). The membrane was incubated with an anti-GFP antibody (CatFab290; Abcam) (1:4000 dilution) and a goat anti-rabbit IgG(H+L) HRP secondary antibody (CatFab165–651; Biorad) (1:2000 dilution). GCaMP3 proteins were visualized using chemiluminescence solution mixed 5:1 with ImmunoStar Zeta (FUJIFILM Wako Chemicals) and SuperSignal West Dura Extended Duration Substrate (Thermo Fisher Scientific). Rubisco proteins were stained with Ponceau S (Merck Sharp & Dohme Corp.) as the loading control. The protein levels of GCaMP3 proteins were quantified with the blot analysis plug-in in ImageJ.

Propidium iodide staining. A stock solution of 10 mM propidium iodide (PI) was prepared with phosphate-buffered saline pH7.5. Rosette leaves of 4-week-old Col-0 plants were cut into 5 mm squares, floated in a glass petri dish with 20 μl PI solution, and incubated for 1 h at room temperature. Stained tissues were observed under the all-in-one fluorescence microscope (BX-800; Keyence Corporation, Osaka, Japan) equipped with a 2× objective lens (CFI S Plan Fluor LWD ADM 20Xc, Nikon) and TRITC dichroic mirror (excitation 545/25 nm, extinction 605/70 nm) (KEYENCE).

Bacterial infection. MS was applied to the adaxial leaf surface of 4-week-old plants by brushing 4 times at an interval of 15 min for 3 h. Sample leaves were then inoculated by infiltration, using a plastic syringe (Terumo Tuberculin Syringe 1 mL; ES4326 (OD600 0.001) resuspended in 10 mM MgCl2. Bacterial growth was measured 2 days after inoculation as described previously37.

Fungal infection. Alternaria brassicicola strain Ryo-1 was cultured on 3.9% (w/v) potato dextrose agar plates (PDA; Becton, Dickinson and Company, NJ, USA) for 4–20 days at 28 °C in the dark. After incubation of the agar plates for 3–7 days under ultraviolet C light, a conidial suspension of A. brassicicola was obtained by mixing with RO water26. The adaxial side of leaves from 4-week-old plants was treated with 10 droplets or MS by brushing 4 times at an interval of 15 min for 3 h, followed by spotting with 5 μl conidia suspension (2 ×106 per ml) of A. brassicicola on the adaxial side of leaves. Inoculated plants were placed at 22 °C under diurnal conditions (16-h-light/8-h-dark cycles) with 100% relative humidity. The lesion size of fungal infection was measured with ImageJ 3 days after infection.

Statistics and reproducibility. GraphPad Prism 9 (GraphPad Software, CA, USA) was used for all statistical analyses. One-sided or two-sided Tukey’s multiple comparison test, one-sided Sidak’s multiple comparison test, or two-way analysis of variance (two-way ANOVA) were used for multiple comparisons. In all graphs, asterisks indicate statistical significance tested by one-sided or two-sided Tukey’s multiple comparison test, one-sided Sidak’s multiple comparison test, or two-way ANOVA (multiple groups).

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

Date availability

The authors declare that all data supporting the findings of this study are available within this article and its Supplementary Information files. RNA-seq and ChIP-seq data have been deposited in the DDBJ Sequence Read Archive at the DNA Data Bank (http://www.ddbj.nig.ac.jp/) with the accession numbers DRA0101970, DRA009248, and DRA011123. Source data are provided with this paper.

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Author contributions

M.M., M.N., S.H.S., and Y.T. designed the research. M.M. and T.I. established the artificial rain device. M.M. optimized the protocols for artificial rainfall and brush treatment. M.M. and M.N. conducted the illumina sequencing libraries for RNA-seq and ChIP-seq. M.N. performed RNA-seq and analysis. M.N. performed the ChIP and analysis of CAMTA3 T.M. and I.C.M. performed the quantitative of phytomolecules. M.M., Y.H., and T.K. performed the detection of MPK3 and MPK6 phosphorylation.

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M.M., T.M., and Y.Y.Y. performed the promoter analysis. Y.A. and M.T. generated 35Spro:GCaMP3 (gl1) plants, and M.N., M.T., and Y.A. visualized real-time [Ca\textsuperscript{2+}]\text{cyt.} M.I. and S.B. generated the transgenic lines WRKY33pro:EYFP-NLS (Col-0) and CBP60gpro:EYFP-NLS (Col-0). M.M., M.I., and S.B. performed promoter-reporter imaging. M.N. and M.M. performed the rest of the experiments. M.M., M.N., M.J.S., S.H.S., and Y.T. wrote the paper with input from all authors.

**Competing interests**
The authors declare no competing interests.

**Additional information**

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