Plants tailor their metabolism to environmental conditions, in part through the recognition of a wide array of self and non-self molecules. In particular, the perception of microbial or plant-derived molecular patterns by cell-surface-localized pattern recognition receptors (PRRs) induces pattern-triggered immunity, which includes massive transcriptional reprogramming. An increasing number of plant PRRs and corresponding ligands are known, but whether plants tune their immune outputs to patterns of different biological origins or of different biochemical natures remains mostly unclear. Here, we performed a detailed transcriptomic analysis in an early time series focused to study rapid-signalling transcriptional outputs induced by well-characterized patterns in the model plant *Arabidopsis thaliana*. This revealed that the transcriptional responses to diverse patterns (independent of their origin, biochemical nature or type of PRR) are remarkably congruent. Many of the genes most rapidly and commonly upregulated by patterns are also induced by abiotic stresses, suggesting that the early transcriptional response to patterns is part of the plant general stress response (GSR). As such, plant cells’ response is in the first instance mostly to danger. Notably, the genetic impairment of the GSR reduces pattern-induced antibacterial immunity, confirming the biological relevance of this initial danger response. Importantly, the definition of a small subset of ‘core immunity response’ genes common and specific to pattern response revealed the function of previously uncharacterized GLUTAMATE RECEPTOR-LIKE (GLR) calcium-permeable channels in immunity. This study thus illustrates general and unique properties of early immune transcriptional reprogramming and uncovers important components of plant immunity.

Plants are challenged by a wide variety of potentially pathogenic organisms; their health relies on their ability to recognize and respond to this plethora of challenges. This recognition is partly accomplished through cell-surface-localized pattern recognition receptors (PRRs), which recognize pathogen-associated molecular patterns (PAMPs) or host-derived damage-associated molecular patterns (DAMPs), leading to pattern-triggered immunity (PTI). A wide variety of PRRs with an equivalent variety of cognate ligands have been identified in various plant species, but it is still unclear to what extent plants discriminate among patterns that are from different source organisms, of different chemical natures or recognized by different PRR classes. Notably, while a few studies have compared transcriptional responses (as proxies of a large, dynamic immune cellular output) triggered by two or three patterns together or used meta-analyses to compare responses, these studies were limited in scale or used different experimental conditions, which hinders meaningful comparisons.

To ascertain the timing and degree of discrimination among pattern-triggered transcriptional responses, we selected a panel of seven patterns with known PRRs, representing a variety of source organisms, chemical compositions and recognition mechanisms. This panel included bacterial flg22 (a 22-amino-acid epitope derived from bacterial flagellin), recognized by the leucine-rich repeat receptor kinase (LRR-RK) FLAGELLIN SENSING 2 (FLS2); elf18 (an 18-amino-acid epitope derived from bacterial elongation factor Tu), recognized by the LRR-RK EF-TU RECEPTOR (EFR); Pep1 (a 23-amino-acid peptide potentially released as a DAMP upon cellular damage), recognized by the LRR-RKs PEP1-RECEPTOR (PEPR1) and PEPR2; nlp20 (a 20-amino-acid peptide derived from bacterial, oomycete and fungal NECROSIS AND ETHYLENE-INDUCING PEPTIDE 1-LIKE PROTEINS), recognized by the LRR-receptor protein PROTEIN-RECEPTOR-LIKE PROTEIN 23 (RLP23); chitocttaoase (CO8, an octamer fragment of fungal cell walls), recognized by the LysM-RKs LYSM-CONTAINING RECEPTOR KINASE 4 (LYK4) and LYK5 (ref. 13); 3-OH-FA (a bacterial hydroxylated fatty acid), recognized by the S-lectin-RK LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE)15,16; and oligogalacturonides (OGs, derived from the plant cell wall), proposed to be recognized by the epidermal growth factor-like-RK WALL-ASSOCIATED KINASE 1 (WAK1). Both Pep1 and OGs are considered DAMPs, while the other patterns are PAMPs. Each pattern was applied in four replicate experiments to two-week-old *Arabidopsis thaliana* (hereafter *Arabidopsis*) seedlings grown in liquid culture, at concentrations either previously used in transcriptomics studies or shown to be saturating for upstream signalling responses. Each pattern was applied to Col-0 wild-type (WT) or cognate receptor mutant seedlings, and the seedlings were flash-frozen for RNA extraction at 0, 5, 10, 30, 90 and 180 min post-treatment (Fig. 1a).

Transcript abundance was assessed by RNA-seq, and differentially expressed genes (DEGs) were identified by comparison with time 0 (log$_2$(fold change, FC) > 1, $P_{	ext{adj}}$ < 0.05), resulting in a total of 10,730 DEGs throughout the experiment (5,718 upregulated and 5,012 downregulated), with the strongest treatment being flg22 (8,451 DEGs; 4,816 up and 3,635 down) and the weakest being 3-OH-FA (1,633 DEGs; 1,246 up and 387 down; Supplementary Tables 1 and 2 and Fig. 1b). One selection criterion for the treatments chosen here was the saturation of upstream signalling outputs (for example, reactive oxygen species production and Ca$^{2+}$ influx), but it cannot be ruled out that higher concentrations of ‘weaker’

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**The transcriptional landscape of Arabidopsis thaliana pattern-triggered immunity**

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patterns would match the responses observed here for ‘stronger’ patterns. The treatments in this study were also selected to match previously published transcriptomics experiments—indeed, the log₂(FC) expression values were similar to those published for single patterns⁴⁷, supporting the experimental and analysis setups used here (Extended Data Fig. 1a,b). A principal component analysis (PCA) of the DEGs revealed strong responses at 30, 90 and 180 min in WT plants that are absent in the receptor mutants and mock controls (Extended Data Fig. 1c). Any genes behaving similarly in the WT and the controls were removed from further analysis. Similar to the PCA, a correlation analysis implicated time post-treatment as the main factor determining transcriptome response; WT samples became highly correlated at later time points (Extended Data Fig. 1d; Pearson correlation at 5 min, 0.08; at 10 min, 0.49; at 30 min, 0.89; at 90 min, 0.80; at 180 min, 0.71).

We then collected the set of DEGs up- or downregulated by each pattern at each time point, and we subdivided these sets by the number of patterns similarly affecting each gene (Fig. 1b). This revealed a large set of DEGs induced by all tested patterns (n = 970; Supplementary Table 3 and Fig. 1b, darkest bar segment). Furthermore, with the exception of flg22, no pattern induced or repressed a large number of genes uniquely (Extended Data Fig. 2 and Supplementary Table 4). To ascertain whether there exist sets induced specifically by pattern subclasses (for example, by PRR type or pattern origin), we identified DEGs induced or repressed by all possible combinations of patterns (Extended Data Fig. 3) and determined the extent to which the relative sizes of these sets departed from that of a random assortment of genes among patterns (deviation[^1]). To avoid the potential effects of accelerated or delayed induction, we collected all DEGs induced by a pattern in this experiment into one representative set. As expected, this confirmed that the largest two sets were DEGs induced uniquely by flg22 (n = 1,041) or commonly by all tested patterns (Fig. 1c and Extended Data Fig. 3). Both of these sets were larger than would be expected by chance (deviation 0.16 for each). The next two largest sets comprised DEGs induced by at least five of the tested patterns—indeed, the treatments of CO8 and 3-OH-FA in this experiment were relatively weaker than other patterns (Fig. 1b), suggesting that DEGs in these sets may also be induced by all patterns under specific conditions. Remarkably, none of the pattern subsets we identified a priori induced unique sets of DEGs much larger or smaller than would be expected by chance (Extended Data Fig. 3). Taken together, these results suggest that gene induction within the first three hours mostly constitutes a general pattern-triggered response.

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[^1]: Calculated as the ratio of the observed sum of squares to the expected sum of squares.
(against ‘non-self’ or ‘damaged-self’), rather than being pattern-specific or pattern-subclass-specific.

To explore the set of ∼1,000 DEGs upregulated commonly by all treatments, we first hierarchically clustered these genes according to their log₂(FC) values for each pattern–time combination (Fig. 1d). This revealed four clusters with characteristic expression, described here as ‘Very rapid’, ‘Rapid transient’, ‘Rapid stable’, and ‘Late’ (Fig. 1e). Interestingly, though all tested patterns induced all DEGs and the overall expression kinetics were similar, some differences in the timing of gene induction could be observed. Among the ‘Very rapid’ and ‘Rapid’ sets, OGS, flg22, elf18 and Pep1 induced gene expression within 5 min, whereas expression was detectable in response to nlp20, 3-OH-FA and CO8 only after 10 min. The timing partially correlated with the total number of DEGs upregulated (Fig. 1b), suggesting a potential relationship between the amplitude and the rapidity of the transcriptional response, similar to that observed in some earlier steps of PTI signalling22. Note that differences in diffusion cannot be excluded as contributing to this observation.

A similar analysis of downregulated DEGs revealed no similar congruence in pattern response—indeed, most sets had similar sizes to those expected by chance (deviation, −0.03 to 0.11). There are approximately 100 DEGs downregulated by all tested patterns (Supplementary Table 5). Although this set was not significantly larger or smaller than expected by chance, we nevertheless clustered these genes to identify characteristic response kinetics, and we found differences similar to upregulated genes (Extended Data Fig. 4). Taken together, these results show that expression in response to pattern perception is dominated by a small number of pattern-specific responses and a large set of commonly induced genes.

To investigate transcriptional regulators controlling this response, we expanded this analysis from the genes upregulated by all patterns to the entire dataset. As timing was the dominant effect in pattern-induced transcriptome reprogramming (Fig. 1 and Extended Data Fig. 1), we grouped the upregulated DEGs by the time at which they first became induced, regardless of the inducing pattern, as previously done in response to other stimuli24. Gene Ontology (GO) term enrichment of these five gene sets supports progressive waves of transcriptional response (Fig. 2a). A cis-element enrichment analysis revealed the enrichment of binding sites for a large number of WRKY transcription factors (TFs) in the promoters of DEGs first induced at 10–30 min post-elicitation (Fig. 2b). This is in line with the established roles of many WRKY TFs in PTI25. In contrast, among genes first induced at 5 or 10 min post-elicitation, there is enrichment in the binding sites for CALMODULIN-BINDING TRANSCRIPTIONAL ACTIVATORS (CAMTAs; Fig. 2b). TFs of the CAMTA family bind the core element vCGGgb and are the major transcriptional regulators of the plant general stress response (GSR)—a rapid and transient induction of a core set of genes in response to a wide variety of stimuli26–28. Given the congruence of pattern-induced gene sets and the presence of CAMTA binding sites in the promoters of rapidly upregulated DEGs, we sought to ascertain the degree to which pattern-induced genes are also affected by varied abiotic stresses. To do this, we used the published AtGenExpress dataset of Arabidopsis seedling responses to cold, drought, genotoxic stress, heat, osmotic stress, salt, UVB irradiation and wounding29. We then classified each of the DEGs upregulated in this study according to (1) the time at which it is first induced, (2) the number of patterns that induce it throughout the experiment and (3) the number of abiotic stresses tested in the AtGenExpress experiment that induce it within three hours. Plotting each DEG according to these criteria, with the colour of the point determined by the maximum log₂(FC) observed in this study, revealed that rapidly induced genes tend to be strongly induced by all tested patterns and induced by most tested abiotic stresses (Fig. 2c). This analysis extended the observation of a common set of genes induced by all tested patterns to the conclusion that the rapid transcriptional response to pattern perception is dominated by the GSR. Our analysis of transcriptional responses therefore indicated that plant cells mostly respond to stress.

A similar analysis of downregulated DEGs revealed mostly later responses than for upregulated DEGs, with no downregulated DEGs identified at 5 min (P < 0.05). A comparison with gene repression under abiotic stress treatment did not reveal a trend like the GSR, although the most strongly affected genes do tend to be downregulated commonly by most or all tested patterns (Extended Data Fig. 5). Finally, while relatively few GO terms or TF binding sites were enriched in downregulated genes, many enriched GO terms were associated with growth hormones and response to light, consistent with previous reports that pattern treatment impedes photosynthesis30,31.

We next tested whether the GSR is required for PTI. CAMTA3 is the primary member of the CAMTA family in inducing the GSR29. The genetic analysis of the role of CAMTA3 in PTI is, however, confounded by the autoimmune phenotype of camta3 loss-of-function mutants, due at least in part to the activation of the two nucleotide-binding LRR-receptor proteins DOMINANT SUPPRESSOR OF CAMTA3 1 (DSC1) and DSC2 (ref. 32). We thus utilized the camta3dsc1dsc2 double mutant. While WT plants were able to mount an effective flg22-induced resistance to the bacterium Pseudomonas syringae pv. tomato DC3000 (Pto), this effect was almost completely lost in the GSR-deficient camta3dsc1dsc2 (P = 0.0007, Fig. 2d), consistent with similar results obtained with the dominant-negative camta3D allele33. Interestingly, basal susceptibility to Pto was also significantly reduced in camta3dsc1dsc2 compared with the WT (P = 0.0008; Supplementary Note 1), in contrast to camta3D but in line with studies showing a negative role for CAMTA3 in salicylic-acid-mediated immunity regardless of DSC1/2 (refs. 34–36).

Beyond highlighting the importance of the GSR in PTI, our comparison with AtGenExpress (extended to selected abiotic stress RNA-seq studies)37–39 further identified DEGs upregulated commonly by all tested patterns but not by abiotic stresses. Notably, among these 39 core immunity response (CIR) genes (Supplementary Table 6), the most strongly upregulated gene encodes GLUTAMATE RECEPTOR 2.9 (GLR2.9), and GLR2.7 is also among the CIR set. GLR2.7 and 2.9 are closely related and are present in a tandem repeat on the genome with GLR2.8 (ref. 40), which is similarly induced by all tested patterns (Supplementary Table 3). GLRs are Ca²⁺-permeable channels of which Arabidopsis GLR3 clade members, for example, are key for wound-responsive signalling41–44. In contrast, GLR2 clade members—to which GLR2.7, 2.8 and 2.9 belong—are poorly characterized. Previous pharmacological studies showed that GLRs contribute to pattern-induced Ca²⁺ influx in Arabidopsis45, but the identities of the relevant GLRs are still unknown. Given the high sequence similarity between GLR2.7, 2.8 and 2.9, as well as their chromosomal clustering, we generated a glr2.72.82.9 triple mutant using CRISPR–Cas9 in both the Col-0 WT and a genetically encoded YELLOW CHAMELEON 3.6 (YC3.6) indicator line. In both backgrounds, this resulted in a large deletion in the GLR2.7–2.9 genomic region (Extended Data Fig. 6). Interestingly, the increase in cytosolic calcium concentration ([Ca²⁺]cyt) triggered by flg22, elf18 and Pep1 was approximately 25% reduced in glr2.72.82.9 relative to the YC3.6 parental line in 12-day-old seedlings and leaf discs taken from 5- to 6-week-old plants (Fig. 3a and Extended Data Fig. 7a,b). In line with this reduced immune output, glr2.72.82.9 plants (in both WT Col-0 and YC3.6 backgrounds) were more susceptible to Pto infection by infiltration, to a similar degree as the immune-deficient bak1-5 mutant (Fig. 3c)46. Consistent with the specific regulation of GLR2.7 and 2.9 by pattern perception but not by abiotic stresses, glr2.72.82.9

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**Fig. 2** | Pattern-triggered transcriptional responses act in time-resolved waves, with the first wave constituting a GSR important for immune activation. 

a,b, GO term (a) and cis-element (b) enrichment analysis of induced genes, categorized according to the time point at which they first passed the induction threshold, regardless of which pattern caused the induction. The top three GO terms for each time point are indicated. ER, endoplasmic reticulum. c, Distribution of upregulated genes. Each gene induced in this study was plotted according to the time it is first induced (panels from top to bottom), the number of tested patterns that induce it (x axis) and the number of abiotic stresses in the AtGenExpress dataset that also induce it within the first three hours (y axis). The colour of each dot indicates the maximum log_{10}(FC) observed in this study. d, Box-and-bee-swarm plots of fig22-induced resistance to Pto infection. The box plots centre on the median, the boxes extend to the first and third quartiles, and the whiskers extend to the lesser value of the furthest point or 1.5x the interquartile range. The data were obtained from three independent experiments (indicated by the point shapes), n = 4 per genotype–treatment combination in each experiment. The data were analysed in R via a two-way analysis of variance (ANOVA) with experiment as a blocking factor, and the P value indicates the interaction between treatment and genotype.
We recently reported that Ca\(^{2+}\)-permeable channels from another family, OSCA1.3 and 1.7, contribute to pattern-induced stoma-
tal immunity\(^{46}\). In contrast, glr2.7/2.8/2.9 was not compromised in pattern-induced stomatal closure (Extended Data Fig. 7d), nor was this mutant more susceptible to Pto WT or a coronatine-defi-
cient mutant upon surface inoculation by spraying (Extended Data Fig. 7e,f). GLR2.7/2.8/2.9 are not strongly expressed before elic-
ation, and unlike OSCA1.3 and CNGC2/4 (calcium-permeable
channels previously shown to play roles in PTI), they do not show a
strong preference for or against stomatal expression (Extended Data
Fig. 8). Also, the previously reported role of CNGC2/4 is appar-
ent only under high external [Ca\(^{2+}\)] conditions\(^{49,50}\), indicating
that additional calcium-permeable channels must be involved in PTI
during normal conditions. These findings substantiate the emerg-
ning concept that multiple channels belonging to distinct Arabidopsis
families (for example, CNGCs, OSCAs and GLRs) contribute to
the overall pattern-induced calcium response observed at the
whole-plant level.

The CIR gene set includes several other genes associated with
immunity (Supplementary Table 6)\(^{51-53}\). We have here shown the
utility of this transcriptomic dataset in identifying signalling and
regulatory components of general stress and immune responses in
Arabidopsis. The future characterization of other CIR genes with
yet-uncharacterized functions or unknown roles in immunity
may thus reveal additional PTI players and improve our under-
standing of how the plant transitions from the rapid GSR to later
immunity-specific responses.

**Methods**

**Arabidopsis growth conditions.** Arabidopsis growth conditions followed
the standard protocols\(^{54}\). For in vitro culture, Arabidopsis seeds were surface-sterilized,
stratified three to five days at 4 °C and then plated on full-strength MS medium,
1% sucrose and 0.8% agar. The plates were placed at 22 °C, 16h/8h light/dark.
After four days, the germinated seedlings were transferred to liquid culture. For
RNA-seq, the seedlings were placed two per well in 24-well plates with 1 ml of MS
media lacking agar, and the plates were sealed with porous tape. For the seedling
Ca\(^{2+}\) measurements, the seedlings were transferred, 30–50 per plate, to sterile 9 cm
Petri dishes containing ~25 ml MS media lacking agar, and the plates were sealed
with porous tape.

For soil growth, Arabidopsis seeds were lightly surface-sterilized, stratified
three to five days and planted on soil. The plants were grown for four to six weeks at
20 °C, 60% humidity and 10 h/14 h light/dark before the assays were performed.
The lines used in this project include Col-0 (used as the WT control),
fls2c (SAIL_091_C04), cpr-1 (SALK_001152/SAIL_49_C05/FLAG014A11),
dsc2-1 (SALK_059281/SAIL_056564), rlp23-1 (SALK_034225),
ycd-5 (WiscDxLor297300_011C),
SALK_151911c, seeds obtained from G. Stacey\(^{55}\),
E06, seeds obtained from S. Ranf\(^{56}\),
cmock-5 (BAK1 C408Y)\(^{45}\),
ccms1_30/15 (refs. 4,55) (elicityl GAT114),
M CO8 (ref. 19) (IsoSep 57/12-001),
cm4p60/15 (refs. 47,48) (elicityl GAT114),
1 M Pep1 (ref. 54) (Scilight-Peptide), 1 M CO8 (ref. 47) (Scilight-Peptide),
1 M Pep1 (ref. 54) (Scilight-Peptide),
1 M flg22 (ref. 8) (Scilight-Peptide), 1 M flg22
M Pep1 (ref. 54) (Scilight-Peptide), 1 M flg22
M CO8 (ref. 19) (IsoSep 57/12-001),
cm4p60/15 (refs. 47,48) (elicityl GAT114),
1 M Pep1 (ref. 54) (Scilight-Peptide), 1 M CO8 (ref. 47) (Scilight-Peptide),
1 M flg22 (ref. 8) (Scilight-Peptide), 1 M flg22
M Pep1 (ref. 54) (Scilight-Peptide), 1 M CO8 (ref. 47) (Scilight-Peptide),
1 M flg22 (ref. 8) (Scilight-Peptide), 1 M flg22
M Pep1 (ref. 54) (Scilight-Peptide), 1 M CO8 (ref. 47) (Scilight-Peptide),
1 M flg22 (ref. 8) (Scilight-Peptide), 1 M flg22

**RNA-seq treatment.** Each plate contained an equal number of wells of Col-0
WT and PRR mutant control, except a single plate for the combined OG/mock
treatment. After nine days of growth in liquid MS medium, the sealing
taxe was removed from the plates, and the medium was replaced from the wells and
replaced with 0.6 ml of liquid MS per well. The following day, when the seedlings
were 14 days post-stratification, 400 µl of 2.5X pattern solution was added to each
well. Two wells, for a total of four seedlings, were harvested for each genotype–
treatment combination. For RNA-seq, the sample was collected
Tissue harvest, library preparation and sequencing. The samples were collected and the
libraries prepared using a combination of published high-throughput protocols\(^{59,60}\). Briefly, two wells per genotype–treatment–time combination were
pooled at each of 0, 5, 10, 30, 90 or 180 min following treatment. The seedlings
were blotted dry and flash-frozen in liquid nitrogen. The tissue was pulverized
while frozen via two 1 min pulses in a BioRad TissueLyser and divided in half for
library preparation. The divided powder was further disrupted for 1 min before
the addition of extraction buffer, and was then disrupted in the buffer for a further

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**Fig. 3** | A glr2.7/2.8/2.9 triple mutant is compromised in pattern-induced Ca\(^{2+}\) influx and bacterial disease resistance. **a, b,** Parent (darker shades) and
and glr2.7/2.8/2.9 (lighter shades) YC3.6 reporter lines were assayed for
responses to a variety of patterns (**a** and to salt (NaCl) or cold (4 °C)
treatment (**b**). The peak Ca\(^{2+}\) signal reported by YC3.6 within 25 min
(patterns), 1 min (NaCl) or 5 min (cold) is shown. Each point represents the
peak ratio (R) of yellow fluorescent protein (YFP) to cyan fluorescent
protein (CFP) (proportional to the Ca\(^{2+}\) concentration) for a single seedling,
normalized to the initial ratio (R\(_{0}\)). The different point shapes represent three
or four independent experiments, n=10–20 for each experiment-
treatment combination. **c,** Parent and glr2.7/2.8/2.9 mutants in Col-0
and YC3.6 backgrounds were assayed for bacterial susceptibility, alongside
the hypersusceptible bak1-5 mutant. Colony-forming units (c.f.u.)
were counted two days post-infiltration. Each point represents one infected
plant, and the different shapes represent three independent experiments,
n=5–7 for each experiment–treatment combination. The box plots
show the 25th and 75th percentiles, the median value, and the
whiskers extend to 1.5 times the interior quartile range. The statistical tests were performed in R via an ANOVA
with a blocking factor, on the square root of the peak-normalized Ca\(^{2+}\) response or log\(_2\)(c.f.u.). Post-hoc tests were performed
using the emmeans package in R. In **a** and **b,** glr2.7/2.8/2.9 was compared with the parent under each treatment, and in **c,** each genotype was
compared with Col-0 (dunnett test) (**left**), and YC3.6 glr2.7/2.8/2.9 was compared with YC3.6 (**right**).
two 1 min pulses. The samples were spun down, and the lysate was collected and incubated with biotin-oligo-dT and streptavidin magnetic beads. The full set of RNA washes and elution was performed twice, with DNase I treatment in between, to minimize endogenous RNA contamination. Complementary DNA synthesis was performed as described, except that only 2 µl of DNA Poly I was used. Serum-free double-stranded cDNA was quantified via SYBR-green-based plate assay and normalized to 2 ng/µl for quantification. Tagmentation was performed in 5 µl reactions containing 0.2 µM Tn-5 transposase, and the entire reaction was used as a template for PCR. PCR was performed using in-house primers to add 5' and 3' tags and the NEBnext 2x polymerase mix, amplifying for ten cycles. The libraries were again Seraph clean-up, SYBR quantified and normalized to 0.5 µM for pooling and sequencing. The pooled libraries were run on two or three flowcell lines of a NextSeq500, and pooling was adjusted after each run to maximize the overall read density per sample.

Read mapping and differential expression analysis. The read data were analysed using FastQC, trimmed using trimmomatic and mapped to the Arabidopsis TAIR10 genome via TopHat2 (refs. 29,30). The mapped reads were assigned to genes, and differential expression analysis was performed using DESeq2 (ref. 29). Before the differential expression analysis, 17,336 libraries were removed from later analysis, primarily for poor sequencing leading to few mapped reads. For each sample, differential expression was determined relative to the same genotype–treatment combination at time 0. To account for time and mechanical stress, for the WT samples, genes were removed if they were also differentially expressed in PRR mutant control as the basis of differential gene expression in the mock-treated WT. For data exploration (for example, PCA, correlation, GO term enrichment and cis-element enrichment), a relatively loose cut-off of (log2(FC)) > 1, FDR < 0.01 was used to obtain a broad landscape of DEGs. For analyses in which specific genes of interest would be analysed (for example, the CIR gene set), a more stringent cut-off of (log2(FC)) > 1, FDR < 0.01 was used. The data manipulation was done in R using functions from the tidyverse

Exploratory data analysis. PCA was performed using the prcomp function in R, and sample correlation was determined via the Pearson method, using the cor function in R. The visualization of genes induced by various combinations of patterns was done via user-modified adaptations of the UpSetR and SuperExactTest R packages29,30, and deviation was calculated as described in ref. 21. The expression of patterns was done via user-modified adaptations of the UpSetR and SuperExactTest R, and sample correlation was determined via the Pearson method, using the cor function. For each gene and each pattern, the fraction of total counts for that gene was calculated using the Fisher statistic74. The expression of patterns was done via user-modified adaptations of the UpSetR and SuperExactTest R, and sample correlation was determined via the Pearson method, using the cor function. For each gene and each pattern, the fraction of total counts for that gene was calculated using the Fisher statistic74.

The entire reaction set used as a template for PCR. PCR was performed using in-house primers to add 5' and 3' tags and the NEBnext 2x polymerase mix, amplifying for ten cycles. The libraries were again Seraph clean-up, SYBR quantified and normalized to 0.5 µM for pooling and sequencing. The pooled libraries were run on two or three flowcell lines of a NextSeq500, and pooling was adjusted after each run to maximize the overall read density per sample.

Measurement of intracellular Ca2+ concentration in seedlings. After six days of growth in liquid MS medium, the sealing tape was removed from the plates, and the seedlings were rinsed in sterile water and transferred one per well to black 96-well plates containing 150 µl of sterile water. The seedlings were gently pressed to ensure that the majority of the seedling was submerged, and the plates were incubated in the dark under bench conditions overnight. The following day, when the seedlings were 11 days post-stratification, the plates were imaged in a Tecan SPARK microplate reader under two conditions: excitation 440 nm and emission 480 nm (CFP), and excitation 440 nm and emission 530 nm (YPF). In the ratio metric Y3C.6 reporter, Ca2+ binding increases fluorescence resonance energy transfer from CFP to YFP, thus, the YFP/CFP ratio (R) is proportional to [Ca2+]. The initial ratios can vary from well to well; accordingly, the Y3C.6 ratios are normalized to the initial ratio (R0) and reported as (R – R0)/R0. Pattern treatment was performed through the addition of 38 µl of 5X solution injected after 5 min of visualization by the microplate reader. The focal plane for the fluorescence measurements was set to a single point in the centre of each well and was moved up 0.1 mm post-injection to accommodate the increased volume in the wells. Despite this adjustment, the overall fluorescence intensity and thus ratio was frequently altered post-injection, as the seedlings did not uniformly fill the wells. Due to this change and the generally slow pattern response, we normalized all subsequent fluorescence ratios to the first ratio measured post-injection (R0), as (R – R0)/R0. The wells were manually rejected if the pre-injection fluorescence was non-fluorescent or was vastly different from R0.

Salt (NaCl) treatment was performed similar to pattern treatment, with the following changes: to accommodate the faster response, the injection and imaging were performed on a well-by-well basis rather than across a subsection of the plate. Due to the faster response, the first measurement post-injection already reflects the beginning of the plant response. R0 was then used as the pretreatment fluorescence ratio, though this resulted in more noise in the final data. For cold treatment, the plate was first pre-imaged for baseline fluorescence; the plate was then removed from the plate reader, the overnight water was removed and 150 µl of fresh water at 22 or 4 °C (ice-water bath) was added. The plates were immediately placed back in the plate reader and imaged for 5 min. As with the salt response, the speed of the cold response necessitated defining R0 as the pretreatment fluorescence level, though this combined with the removal and addition of fresh water resulted in noise in the final peak levels.

As some silencing was observed both in parent Y3C.6 lines and in Y3C.6 glr2.7/2.8/2.9 lines, only seedlings with visible fluorescence at four days were transferred to liquid culture, and after treatment, only seedlings (wells) with pretreatment fluorescence in both wavelengths greater than 3× that of a non-fluorescent Col-0 control were considered. The total seedlings imaged were as follows: Y3C.6 mock, 56; Y3C.6 flg22, 54, Y3C.6 ehl18, 52; Y3C.6 Pep1, 55, Y3C.6 glr2.7/2.8/2.9 mock, 48; Y3C.6 glr2.7/2.8/2.9 flg22, 43; Y3C.6 glr2.7/2.8/2.9 ehl18, 45; Y3C.6 glr2.7/2.8/2.9 Pep1, 45; Y3C.6 mock (NaCl), 56; Y3C.6 NaCl, 51; Y3C.6 glr2.7/2.8/2.9 mock (NaCl), 38; Y3C.6 glr2.7/2.8/2.9 NaCl, 29; Y3C.6 22 °C water, 56; Y3C.6 2.2 °C water, 54; Y3C.6 glr2.7/2.8/2.9 22 °C water, 44; and Y3C.6 glr2.7/2.8/2.9 22 °C water, 42.

Measurement of intracellular Ca2+ concentration in leaf discs. Four leaf discs per plant were collected from five- to six-week-old soil-grown Arabidopsis plants and incubated overnight on 100 µl of sterile ultrapure water in black 96-well plates. As for the seedlings, the plates were imaged in a Tecan SPARK microplate reader under two conditions: excitation 440 nm and emission 480 nm (CFP), and excitation 440 nm and emission 530 nm (YPF). The flg22 treatment was performed through the addition of 25 µl of 5X solution injected after 5 min of visualization by the microplate reader. The focal plane for the fluorescence measurements was set to a single point in the centre of each well and was moved up 0.5 mm post-injection to accommodate the increased volume in the wells. The wells were manually rejected if the pre-injection fluorescence was not stable or was vastly different from R0.

For all infection assays, Arabidopsis plants were treated when four to five weeks old, and bacteria were grown overnight in Kings B medium and incubated at 37 °C for 16 h to reach high bacterial titers. Bacterial suspensions were made in 10 µl of PBS containing 1% Triton X-100 and 0.1% Tween 20, and 1 µl was injected into wild-type Arabidopsis plants. In order to allow more rapid infection and to exclude endogenous bacterial signals, plants were grown in soil in 15 cm pots on a growth chamber under a 16/8 h light/dark cycle with continuous illumination of 200 µE/m2/s prior to inoculation. After inoculation, plants were transferred to sterile distilled water and grown in a growth chamber under similar conditions. After 4 days post-inoculation, plants were transferred to sterile distilled water and grown in a growth chamber under similar conditions. After 4 days post-inoculation, plants were transferred to sterile distilled water and grown in a growth chamber under similar conditions.
medium liquid culture, refreshed via a 1–2 h subculture in the morning, spun down and resuspended in 10 mM MgCl₂. For induced resistance, three leaves from each plant were infiltrated with either 1 μM flg22 or water in the morning. The following morning, the selected leaves were re-infiltrated with Pto expressing luciferase at OD₆ₙ₅ = 0.0002 or ~1 × 10⁻⁶ c.f.u. ml⁻¹. The plants were covered, and the infection was allowed to proceed for two days. For the infiltration infection assays, infection was performed similarly with the following differences: WT Pto was used rather than the luciferase-expressing strain, the trays were incubated uncovered and there was no mock or pattern pretreatment. For spray infection, Pto was diluted to OD₆ₙ₅ = 0.2 or ~1 × 10⁻⁶ c.f.u. ml⁻¹ in MgCl₂, Silwet L-77 was added to 0.04% and the plants were sprayed to surface saturation (~4 ml per plant).

For all infection assays, after approximately 48 h, leaf discs were collected (for infiltration, from two each infiltrated leaf; for spray, six from each separate leaf) and ground in 10 mM MgCl₂, and serial dilutions from 1 × 10⁻¹ to 1 × 10⁻⁸ were plated to count c.f.u.

Tissue-specific expression datasets containing aerial (rosette) tissue were available in Supplementary Note 1. Source data are provided with this paper. Data availability

Received: 3 December 2020; Accepted: 8 February 2021; Published online: 15 March 2021

References

1. Yu, X., Feng, B., He, P. & Shan, L. From chaos to harmony: responses and signaling upon microbial pattern recognition. Annu. Rev. Phytopathol. 55, 1099–1137 (2017).
2. Albert, I., Hua, C., Nümburger, T., Pruitt, R. N. & Zhang, L. Surface sensor systems in plant immunity. Plant Physiol. 182, 1582–1596 (2020).
3. Sajo, Y., Loo, E. P.-I. & Yasuda, S. Pattern recognition receptors and signaling in plant–microbe interactions. Plant J. 93, 592–613 (2018).
4. Denoux, C. et al. Activation of defense response pathways by OGs and Flg22 elicitors in Arabidopsis seedlings. Mol. Plant 1, 423–445 (2008).
5. Wan, W.-L. et al. Comparing Arabidopsis receptor kinase and receptor protein-mediated immune signaling reveals BIK1-dependent differences. N. Phytol. 221, 2080–2095 (2018).
6. Stringlis, I. A. et al. Root transcriptional dynamics induced by beneficial rhizobacteria and microbial immune elicitors reveal signatures of adaptation to mutualists. Plant J. 93, 166–180 (2018).
7. Wan, J. et al. A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in Arabidopsis. Plant Cell 20, 471–481 (2008).
8. Zipfel, C. et al. Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. Cell 125, 749–760 (2006).

9. Gómez-Gómez, L. & Boller, T. FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Mol. Cell 5, 1093–1101 (2000).
10. Krol, E. et al. Perception of the Arabidopsis danger signal peptide 1 involves the pattern recognition receptor AtPEPR1 and its close homologue AtPEPR2. J. Biol. Chem. 285, 13471–13479 (2010).
11. Yamaguchi, Y., Hufnaker, A., Bryan, A. C., Tix, F. E. & Ryan, C. A. PEPR2 is a second receptor for the Pep1 and Pep2 peptides and contributes to defense responses in Arabidopsis. Plant Cell 22, 508–522 (2010).
12. Yamaguchi, Y., Pearce, G. & Ryan, C. A. The cell surface leucine-rich repeat receptor for AtPep1, an endogenous peptide elicitor in Arabidopsis, is functional in transgenic tobacco cells. Proc. Natl Acad. Sci. USA 103, 10104–10109 (2006).
13. Albert, I. et al. An RLP23–SOBIR1–BAK1 complex mediates NLP-triggered immunity. Nat. Plants 1, 1540 (2015).
14. Cao, Y. et al. The kinase LYK5 is a major chitin receptor in Arabidopsis and forms a chitin-induced complex with related kinase CERK1. eLife 3, e03766 (2014).
15. Kutschera, A. et al. Bacterial medium-chain 3-hydroxy fatty acid metabolites trigger immunity in Arabidopsis plants. Science 364, 178–181 (2019).
16. Ranf, S. et al. A lectin S-domain receptor kinase mediates lipopolysaccharide sensing in Arabidopsis thaliana. Nat. Immunol. 16, 426–433 (2015).
17. Brutsu, A., Sicilia, F., Macone, A., Cervone, F. & De Lorenzo, G. A domain swap approach reveals a role of the plant wall-associated kinase I (WAK1) as a receptor of oligogalacturonides. Proc. Natl Acad. Sci. USA 107, 9452–9457 (2010).
18. Navarro, L. et al. The transcriptional innate immune response to flg22: interplay and overlap with Avr gene-dependent defense responses and bacterial pathogenesis. Plant Physiol. 135, 1113–1128 (2004).
19. Libault, M., Wan, J., Czechowski, T., Udvardi, M. & Stacey, G. Identification of 118 Arabidopsis transcription factor and 30 ubiquitin-ligase genes responding to chitin, a plant-defense elicitor. Mol. Plant Microbe Interact. 20, 900–911 (2007).
20. Hu, X., Yeil, S. J., Cai, W. M. & Tang, Z. C. Induction of defence gene expression by oligogalacturonic acid requires increases in both cytosolic calcium and hydrogen peroxide in Arabidopsis thaliana. Cell Res. 14, 234–240 (2004).
21. Lex, A., Gehlenborg, N., Strohelt, H., Vuillemt, R. & Pfister, H. Upset: visualization of intersecting sets. IEEE Trans. Vis. Comput. Graph. 20, 1983–1992 (2014).
22. Jeworutzki, E. et al. Early signaling through the Arabidopsis pattern recognition receptors FLS2 and EFR involves Ca-associated opening of plasma membrane anion channels. Plant J. 62, 367–378 (2010).
23. Björnson, M., Dandekar, A. & Dehesh, K. Determinants of timing and amplitude in the plant general stress response. J. Integr. Plant Biol. 58, 119–126 (2016).
24. Varala, K. et al. Temporal transcriptional logic of dynamic regulatory networks underlying nitrogen signaling and use in plants. Proc. Natl Acad. Sci. USA 115, 6494–6499 (2018).
25. Birkenbihl, R. P. et al. Principles and characteristics of the Arabidopsis WRKY regulatory network during early MAMP-triggered immunity. Plant J. 96, 448–452 (2018).
26. Doherty, C. J., Van Buskirk, H. A., Myers, S. J. & Thomashow, M. F. Roles for Arabidopsis CAMTA transcription factors in cold-regulated gene expression and freezing tolerance. Plant Cell 21, 972–984 (2009).
27. Benn, G. et al. A key general stress response motif is regulated non-uniformly by CAMTA transcription factors. Plant J. 80, 82–92 (2014).
28. Walley, I. W. et al. Mechanical stress induces biotic and abiotic stress responses via a novel cis-element. PLoS Genet. 3, 1800–1812 (2007).
29. Kilian, J. et al. The AtGenExpress global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses. Plant J. 50, 347–363 (2007).
30. Bulgin, D. et al. Biotic and globally downregulates photosynthesis genes. Plant Cell Environ. 33, 1597–1613 (2010).
31. Göhr, V., Jones, A. M. E., Sklenář, J., Robatzeck, S. & Weber, A. P. Molecular crosstalk between PAMP-triggered immunity and photosynthesis. Mol. Plant Microbe Interact. 25, 1083–1092 (2012).
32. Lolle, S. et al. Matching NLR immune receptors to autoimmunity in cam3a mutants using antimicrobial NLR alleles. Cell Host Microbe 21, 518–529 (2017).
33. Jacob, F. et al. A dominant-interfering camt3a mutation compromises primary transcriptional outputs mediated by both cell surface and intracellular immune receptors in Arabidopsis thaliana. N. Phytol. 217, 1667–1680 (2018).
34. Yuan, P., Du, L. & Poovaiah, B. W. Ca⁺/calmodulin-dependent ATSR1/CAMTA3 plays critical roles in balancing plant growth and immunity. Int. J. Mol. Sci. 19, 1764 (2018).
35. Du, L. et al. Ca⁺/calmodulin regulates salicylic-acid-mediated plant immunity. Nature 457, 1154–1158 (2009).
65. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and p-value estimation for RNA-Seq data. Genome Biol. 15, 550 (2014).

66. R Core Team. R: a language and environment for statistical computing (R Foundation for Statistical Computing, 2020).

67. RStudio: integrated development environment for R (RStudio Team, 2020).

68. Wickham, H. et al. Welcome to the tidyverse. J. Open Source Softw. 4, 1686 (2019).

69. Conway, J. R., Lex, A. & Gehlenborg, N. UpSetR: an R package for the visualization of intersecting sets and their properties. Bioinformatics 33, 2938–2940 (2017).

70. Wang, M., Zhao, Y. & Zhang, B. Efficient test and visualization of multi-set intersections. Sci. Rep. 5, 16923 (2015).

71. Galili, T. dendextend: an R package for visualizing, adjusting and comparing trees of hierarchical clustering. Bioinformatics 31, 3718–3720 (2015).

72. Xiao, S.-J., Zhang, C., Zou, Q. & Ji, Z.-L. TisGed: a database for tissue-specific genes. Bioinformatics 36, 1273–1275 (2020).

73. Jiao, J. et al. Comparative transcriptional analysis reveals conserved transcriptional programs underpinning organogenesis and reproduction in land plants. Preprint at https://doi.org/10.1016/j.topo.2020.10.361501 (2020).

74. Alexa, A. & Rahnenfuehler, J. topGO: Enrichment analysis for gene ontology. R package version 2.4.2 (2020).

75. McLeay, R. C. & Bailey, T. L. Motif Enrichment Analysis: a unified framework and an evaluation on ChIP data. BMC Bioinform. 11, 160 (2010).

76. O’Malley, R. C. et al. Cistrome and epicistrome features shape the regulatory DNA landscape. Cell 165, 1280–1292 (2016).

77. Ritchie, M. E. et al. limma powers differential expression analyses for RNA-Seq, microarray and mass spectrometry studies. Nucleic Acids Res. 43, e47 (2015).

78. Fan, J., Crooks, C. & Lamb, C. High-throughput quantitative luminescence assay of the growth in planta of Pseudomonas syringae chromosomally tagged with Photorhabdus luminescens luxC/DABE. Plant J. 53, 393–399 (2008).

79. Lenth, R. emmeans: Estimated marginal means, aka least-squares means. R package version 1.5.4 (2020).

80. Mustroph, A. et al. Predictive translatomes of discrete cell populations resolves altered cellular priorities during hypoxia in Arabidopsis. Proc. Natl Acad. Sci. USA 106, 18843–18848 (2009).

81. Bates, G. W. et al. A comparative study of the Arabidopsis thaliana guard-cell transcriptome and its modulation by sucrose. PLoS ONE 7, e49641 (2012).

82. Ribeiro, D. M., Araújo, W. L., Fernie, A. R., Schippers, J. H. M. & Mueller-Roeber, B. Translome and metabolome effects triggered by gibberellins during rosette growth in Arabidopsis. J. Exp. Bot. 63, 2769–2786 (2012).

83. Yang, Y., Costa, A., Leonhardt, N., Siegel, R. S. & Schroeder, J. I. Isolation of a novel ankyrin protein, AAR5, is a regulator and an effector of salicylic acid signaling in the Arabidopsis defense response. Plant Cell 15, 2408–2420 (2003).

84. Liu, J. et al. Heterotrimeric G proteins serve as a converging point in plant defense signaling activated by multiple receptor-like kinases. Plant Physiol. 161, 2146–2158 (2013).

85. Zipfel, C. et al. Bacterial disease resistance in Arabidopsis through flagellin perception. Nature 428, 764–767 (2004).

86. Hufnaker, A., Pearce, G. & Ryan, C. A. An endogenous peptide signal in Arabidopsis activates components of the innate immune response. Proc. Natl Acad. Sci. USA 103, 10098–10103 (2006).

87. Ridley, B. L., O’Neill, M. A. & Mohnen, D. Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. Phytochemistry 57, 929–967 (2001).

88. Kumar, R. et al. A high-throughput method for Illumina RNA-seq library preparation. Front. Plant Sci. 3, 202 (2012).

89. Townsley, B. T., Covington, M. F., Ichihashi, Y., Zumstein, K. & Sinha, N. R. A RAD-seq breath adapter directional sequencing: a streamlined, ultra-simple and fast library preparation protocol for strand specific mRNA library construction. Front. Plant Sci. 6, 366 (2015).

90. Picelli, S. et al. RNA-seq transposase and tagmentation procedures for massively scaled sequencing projects. Genome Res. 24, 2033–2040 (2014).

91. Byrsonom, M., Kappa, J., Zipfel, C. & Dong, P. Low-cost and high-throughput RNA-seq library preparation for Illumina sequencing from plant tissue. Bio-protocol 10, e3799 (2020).

92. Rohland, N. & Reich, D. Cost-effective, high-throughput DNA sequencing for multiplexed target capture. Genome Res. 22, 939–946 (2012).

93. Leggatt, J., Allain, R., Isaac, L. & Blais, B. W. Microplate fluorescence assay for the quantification of double stranded DNA using SYBR Green I dye. Biotechnol. Lett. 28, 1587–1594 (2006).

94. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120 (2014).

95. Kim, D. et al. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 14, R36 (2013).

96. FastQC: a quality control tool for high throughput sequence data (Babraham Bioinformatics Institute, 2010); http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.
Extended Data Fig. 1 | Quality control and exploratory analysis of RNA-seq data. Expression changes in this study at a, 30 min and b, 3 h are plotted against previously published results for flg22, elf18/26, and chitoctoase (CO8). Linear correlation shown in red, with $R^2$ (linear regression) shown on each plot. c, PCA analysis of log$_2$(FC) of differentially expressed genes, showing (left) minimal changes in receptor-mutant treated plants, mostly corresponding with later time points, and rays of response (right) corresponding with plants at 30, 90, or 180 min post-treatment. d, Pearson correlation heatmap of DESeq2-calculated log$_2$(FC) showing clustering largely by time point, with the strongest correlations at 30 min.
Extended Data Fig. 2 | There is little specificity in pattern-induced genes. Among induced genes, for each pattern a specificity measure (expression in response to pattern/total expression in experiment) was calculated, and genes with at least one SPM > 0.33 (one pattern treatment responsible for approximately 1/3 total expression in study, n = 412) were gathered. flg22 is the only pattern treatment with a large number of pattern-selective genes expressed (flg22: 332, elf18: 8, Pep1: 33, nlp20: 31, OGs: 8, CO8 and 3-OH-FA: 0).
Extended Data Fig. 3 | Complete complement of set sizes among collapsed pattern-induced and pattern-repressed gene sets. Each circular ‘track’ represents one pattern treatment; when filled the pattern in question alters the expression of the gene set shown at the perimeter. Gene set size is shown via bar height of bars surrounding pattern tracks, and bar color shows deviation: indicating whether the set size is larger or smaller than would be expected by chance. Large diagrams show the overall set complement of genes induced or repressed by patterns taking all time points into account, whereas smaller diagrams to the left and right are specific for the complement of genes induced or repressed at the indicated time point. No genes were significantly repressed at five minutes post-treatment. Selected pattern subset of a priori interest are highlighted through open arrows on large combined plots; none has deviation far from 0.
Extended Data Fig. 4 | Pattern-responsive genes tend to be repressed by single patterns, though there does exist a core set of 93 genes repressed by all tested patterns. A single set of genes repressed \[\log_2(FC) < -1, p < 0.05\] by each pattern treatment was found through combining the lists genes repressed at each time. a, UpSet diagram showing the size of ‘collapsed’ gene sets repressed by each pattern (left) and the top 15 intersections (bottom right) by size (top right), colored by deviation from set size predicted by random mixing. b, Heat map of expression of the 93 genes repressed by all tested patterns. Genes are hierarchically clustered according to their behavior across all pattern/time combinations, and cut into three clusters. c, Visualization of average \(\log_2(FC)\) patterns of the three clusters identified in b, showing different patterns of expression. Error bars represent standard error of the mean.
Extended Data Fig. 5 | Pattern-triggered transcriptional repression acts in time-resolved waves. a, GO term and b, cis-element enrichment analysis of repressed genes, categorized according to the time point at which they first passed significance threshold, regardless of which pattern caused repression. The top three GO terms for each time point are indicated. c, Distribution of repressed genes. Each gene repressed in this study was plotted according to the time it is first repressed (panels from top to bottom), the number of tested patterns which repress it (x axis) and the number of abiotic stresses in the AtGenExpress dataset which also repress it within the first 3 h (y axis). The color of each dot indicates the most negative log2(FC) observed in this study.
Extended Data Fig. 6 | CRISPR deletes the majority of the GLR2.7/2.8/2.9 genomic region in assayed lines. Schematic of the GLR2.7/2.8/2.9 genomic region, with deletions in a Col-0, b and c YC3.6 background. In each case, a ‘fusion protein’ may be transcribed, consisting of approximately 90 (92, 92, 89) amino acids of GLR2.7, fused to approximately 12 (12, 13, 12) nonsense amino acids from the GLR2.9 genomic region. The potential fusion protein does not encode any transmembrane domains. GLR exons are represented by colored boxes, introns by grey boxes, and intergenic regions by black lines. Neighboring genes shown in black. Arrows represent direction of transcription.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Characterization of glr2.7/2.8/2.9 lines. a, b, c, Increase in intracellular Ca\(^{2+}\) concentration in response to treatment in seedlings (a, c) or leaf discs (b). Shown are mean corrected YFP/CFP ratio within 25 min (a, b), 1 min, or 5 min (c) post-treatment (timepoint 0) +/-1 standard error of the mean. Data were collected every 30 s (a, b), 5 s, or 12 s (c). For a and c corresponding peak values are shown in Fig. 3, for b peak values are shown to the right of response curve. In (b), each point represents peak ratio of YFP to CFP (proportional to Ca\(^{2+}\) concentration) for a single seedling, normalized to initial ratio. Different shapes represent 2 independent experiments, n=11-62 for each experiment/line/treatment combination. Statistical tests were performed in R, two-way ANOVA blocking by experiment. d, Stomatal aperture of WT, glr2.7/2.8/2.9, or flg22-hyporesponsive bak1-5 plants treated with water, 5 µM flg22, or 10 µM ABA. Each point represents one stoma, and plot represents stomata from a total of 12 plants assayed over 5 experiments (n=36-178 stomata per genotype/treatment/experiment). Statistical tests were performed in R, two-way ANOVA blocking by experiment. Post-hoc tests were performed using the emmeans package in R: within each genotype, stomatal aperture was compared with mock treatment with dunnettX multiple testing correction. In spray infection assays glr2.7/2.8/2.9 are not more susceptible to e WT Pto DC3000, or f Pto COR\(^{-}\), deficient in the stomata-opening toxin coronatine. Bacteria were harvested from leaf discs two days post-inoculation; each point represents one plant, and shapes represent three independent experiments (n=6 plants per genotype/treatment/experiment). Statistics were performed in R: one-way ANOVA blocking by experiment followed by dunnettX multiple comparison to Col-0 performed using the emmeans package. Box plots center on the median, with box extending to the first and third quartile, and whiskers extending to the lesser value of the furthest point or 1.5x the inter-quartile range.
Extended Data Fig. 8 | Leaf tissue expression patterns of genes encoding calcium-permeable channels implicated in PTI. Data collected from Genevestigator, and scaled by each experiment.
Extended Data Fig. 9 | AT3G32090 is likely miscalled as expressed in response to patterns. AT3G32090 is among the CIR set, (a), but all reads assigned to this gene map to a single exon (b, image from integrated genomics viewer), not the pattern expected from poly-A purification of mRNA. c, The top BLAST hit for each exon of AT3G032090 are shown, with strong similarity to WRKY40 (AT1G80840) in the ‘expressed’ exon of AT3G032090. d, WRKY40 is strongly expressed (note y axis) and strongly pattern-induced. A small fraction of mis-aligned reads likely account for the observed pattern AT3G032090.
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Last updated by author(s): Jan 28, 2021

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
- Fluorescence measurement: SparkControl (Tecan Group Ltd.)
- Stomatal aperture: ImageJ software (1.53c)
- Sanger sequencing: Benchling

Data analysis
- RNAseq: FastQC (0.11.8), trimmomatic (0.39), TopHat2 (v2.1.0)
- Data handling and statistical analysis: R (4.0.2), many packages including: DESeq2 (1.30.0), tidyverse (1.3.0), bioDict (1.60.0), cowplot (1.1.0), ggpubr (0.4.0), dendextend (1.14.0), viridis (0.5.1), scales (1.1.1), GO.db (3.11.4), topGO (2.40.0), Biobase (2.48.0), limma (3.44.3), readxl (1.3.1), emmeans (1.3.3), ggthemes (4.2.0), extraDistr (1.9.1), SuperExactTest (1.0.7), RColorBrewer (1.1.2), ggforce (1.1.0), gtools (3.8.2), gplots (3.8.1)
- cis element enrichment: AME (5.1.0)

Code for analysis with required packages in Supplementary Note 1.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The RNA-seq datasets generated and analyzed in the current study are available at the ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-9694.
Normalized abiotic stress microarray data (intensity) was obtained from http://jsp.weigelworld.org/AtGenExpress/resources/ in 2017 and analyzed using limma (NB: data are no longer hosted here, but CEL files can be downloaded through https://www.arabidopsis.org/portals/expressions/microarray/ATGenExpress.jsp).

Fold Change from abiotic stress RNAseq experiments was taken from supplementary information of cited articles (http://www.plantcell.org/content/28/6/1279, http://www.plantphysiol.org/content/172/2/668, https://pubmed.ncbi.nlm.nih.gov/24897929/).

Identifiers for publicly available Arabidopsis lines are given in the Material and Methods section.

Source data is available for Figures 2, 3 and Extended Data Figures 5 and 7.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

No sample size calculation was performed. Sample size was chosen as large as possible and in accordance with previous established protocols in the field, as follows:
- 4 replicates for RNAseq is more than the commonly used 3 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6367016/)
- 6-8 replicates in each of 3 experiments for infection assays (https://science.sciencemag.org/content/355/6322/287.abstract)
- >400 stomata for stomatal aperture (https://www.nature.com/articles/s41586-020-2702-1)
- 12 seedlings (leaf discs) in each of 3-4 experimental replicates for calcium measurements (https://www.nature.com/articles/s41586-020-2702-1)

Data exclusions

RNAseq: Several sequencing libraries were excluded from analysis due to low sequencing depth (<3 million reads) or contamination leading to poor mapping, 13 samples from Col-0 and 4 from PRR mutants, 17/336 prepared libraries.

YC 3.6 seedling Ca2+ measurement: Several fluorescent reporter line seedlings were excluded due to poor basal fluorescence (<3x background fluorescence of Col-0 control lacking YC3.6)

Replication

All findings were successfully reproduced in several replicates. Briefly, RNAseq was performed on 4 experimental replicates, infection assays performed in 2-3 independent experimental replicates, calcium reporter line experiments in 2-4 independent experimental replicates, and stomata aperture measurement across 2 independent experimental replicates.

Randomization

For mature plant assays, genotypes and treatments were randomly assigned position within trays for each experiment. For RNA-seq, elicitor treatment order was randomly determined, independently for each replicate.

Blinding

Experimenters set up, performed, and analysed experiments in succession. While some experiments were partially blinded (e.g. genotypes assigned numbers), experimenters did not fully forget the experimental setup while collecting data.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

- n/a Involved in the study
- Antibodies
- Eukaryotic cell lines
- Palaeontology
- Animals and other organisms
- Human research participants
- Clinical data

Methods

- n/a Involved in the study
- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging