A Meta-Analysis Reveals Opposite Effects of Biotic and Abiotic Stresses on Transcript Levels of Arabidopsis Intracellular Immune Receptor Genes

Leiyun Yang, Zhixue Wang and Jian Hua*

Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, United States

Plant intracellular immune receptor NLR (nucleotide-binding leucine-rich repeat) proteins sense the presence of pathogens and trigger strong and robust immune responses. NLR genes are known to be tightly controlled at the protein level, but little is known about their dynamics at the transcript level. In this study, we presented a meta-analysis of transcript dynamics of all 207 NLR genes in the Col-0 accession of Arabidopsis thaliana under various biotic and abiotic stresses based on 88 publicly available RNA sequencing datasets from 27 independent studies. We find that about two thirds of the NLR genes are generally induced by pathogens, immune elicitors, or salicylic acid (SA), suggesting that transcriptional induction of NLR genes might be an important mechanism in plant immunity regulation. By contrast, NLR genes induced by biotic stresses are often repressed by abscisic acid, high temperature and drought, suggesting that transcriptional regulation of NLR genes might be important for interaction between abiotic and biotic stress responses. In addition, pathogen-induced expression of some NLR genes are dependent on SA induction. Interestingly, a small group of NLR genes are repressed under certain biotic stress treatments, suggesting an unconventional function of this group of NLRs. This meta-analysis thus reveals the transcript dynamics of NLR genes under biotic and abiotic stress conditions and suggests a contribution of NLR transcript regulation to plant immunity as well as interactions between abiotic and biotic stress responses.

Keywords: NLR, biotic stress, abiotic stress, intracellular immune receptor, Arabidopsis

INTRODUCTION

Plants in nature are constantly challenged by a variety of environmental stresses including pathogen attacks. In order to fend off pathogens, plants utilize cell-surface receptors and intracellular immune receptors to sense the presence of microbes (Wang et al., 2020). The recognition of pathogens by immune receptors triggers a series of immune responses such as reactive oxygen species burst, Ca^{2+} influx, accumulation of salicylic acid (SA), and transcriptional reprogramming (Tsuda and Katagiri, 2010; Buscaill and Rivas, 2014). Transcriptional upregulation of defense genes and reduction of growth-related genes are critical for a successful inhibition or blocking of invasion and propagation of pathogens (Lewis et al., 2015). The plant hormone SA is often induced during defense responses, and key enzymes for SA biosynthesis are regulated by a few transcription factors. SAR DEFICIENT 1 (SARD1) and its close homolog CALMODULIN BINDING PROTEIN
High temperature (Yang and Mühlenbock et al., 2008; Cheng et al., 2013; Hua, 2004; Wang et al., 2009; Kim et al., 2010) and high humidity (Jambunathan et al., 2001; Panchal et al., 2016) are often associated with decreased disease resistance while low temperature (Huang et al., 2010; Yang et al., 2010; Kim et al., 2017) and high light (Mühlenbock et al., 2008) are often associated with high disease resistance. The intersection of abiotic factors with immunity could happen at multiple points, and NLR proteins likely occupy key intersection points. High temperature inhibits nuclear accumulation of NLR proteins SNC1 and RPP4, leading to the repression of their induced immune responses at elevated temperature (Zhu et al., 2010; Meng et al., 2012). However, whether or not abiotic stresses impact plant immunity through the regulation of NLR transcription is not thoroughly investigated.

Upregulation of NLR gene expression, in addition to their protein activity activation, has been linked to autoimmunity where immune responses are activated under normal non-pathogenic conditions often leading to spontaneous cell death and dwarfism (van Wersch et al., 2016; Wu et al., 2020). For instance, the immune response in the hos15-4 mutant defective in histone deacetylation is hyper-activated, which is partially dependent on a NLR gene SUPPRESSOR OF npr1, CONSTITUTIVE 1 (SNC1) (Yang et al., 2020). Additionally, about one third of total NLR genes are upregulated in the hos15-4 mutant (Yang et al., 2020), suggesting a contribution of NLR activation to autoimmunity. Autoimmune mutants often have increased SA accumulation and spontaneous cell death, which are also highly related to NLR function. For instance, the acd6-1 mutant is a gain-of-function mutant with increased disease resistance to Pseudomonas syringae, and the amount of SA in this mutant is positively correlated with the degree of disease resistance and defense gene expression (Lu et al., 2009). Similarly, the autoimmune mutant ssi2-1 defective in a stearyl-ACP desaturase accumulates a high level of SA under normal growth conditions (Shah et al., 2001). The bak1-4 serk4-1 double mutant defective in two receptor kinases coding BAK1 and SERK4 exhibited spontaneous cell death and other autoimmune phenotypes (de Oliveira et al., 2016). However, comprehensive analysis of NLR gene expression in these autoimmune mutants is still lacking.

In this meta-analysis, we systematically analyzed the transcript dynamics of all 207 NLR genes in the Col-0 accession of Arabidopsis thaliana under various biotic and abiotic stresses as well as in autoimmune mutants based on 88 RNA sequencing (RNAseq) datasets from 27 independent studies. We found that about 146 NLR genes were generally induced by pathogens and defense elicitors but repressed by abscisic acid, heat, and drought. The expression pattern of NLR genes in autoimmune mutants was
very similar to that in response to pathogen infection. Among the 131 NLR genes induced by pathogens, 87 NLR genes are induced and 44 NLR genes are not induced by SA or its analog BTH. Additionally, 26 NLR genes were repressed under biotic stress conditions. Therefore, this meta-analysis illustrates dynamics of transcript abundance of NLR genes under different conditions, which provides a foundation for further understanding of NLR gene regulation in plant immunity as well as interactions between biotic and biotic stress responses.

METHODS

Selection of RNAseq Data for the NLR Expression Study
In this meta-analysis, we utilized publicly available RNAseq data to investigate the transcript dynamics of all 207 NLR genes in the Col-0 accession of A. thaliana in response to biotic and abiotic stresses as well as autoimmune mutants. These NLR or NLR-like genes were listed in previous studies (Meyers et al., 2003; Yang et al., 2020). We also included in the analysis several key SA-related genes because SA signaling is closely linked with NLR activation (Shirano et al., 2002; Xiao et al., 2003; Yang and Hua, 2004). These SA-related genes include SA biosynthesis genes (ICS1, ICS2, PBS3, EPS1, PAL1, PAL2, PAL3, and PAL4), two master ICS1 transcription factors CBP60g and SARD1, and three EDSI family genes (EDS1, PAD4, and SAG101) that mediate SA and NLR signaling, as well as ED55 encoding a transporter for SA precursor. Also included are biosynthesis genes of another defense mediator N-Hydroxypipecolic Acid (NHP), ALDI, SARD4, and FMO1, which are coordinately regulated by the SA regulators SARD1 and CBP60g (Huang et al., 2020).

All the datasets in this meta-analysis were collected from peer-reviewed publications published before August 2020 (Supplementary Data 1). We searched literature via Google Scholar for RNAseq data of plants grown under various conditions using the keywords “transcriptome,” “RNA sequencing,” “biotic stress (pathogen infection; SA; flg22; chitin),” “abiotic stress (low temperature; cold; heat; high temperature; salt stress; ABA; drought),” and “autoimmunity.” The datasets used in the meta-analysis were selected based on the following criteria: datasets were performed on leaves or seedlings of Col-0 accessions; differentially expressed genes (DEGs) were listed in the publication or processed reads count for each gene (“reads count-only” datasets) were available in NCBI GEO database. We directly extracted the differentially expressed NLR genes, SA- and NHP-related genes from references if DEGs were listed in the references, and most of these studies used the same criteria (p or FDR < 0.05, FC ≥ 2). Otherwise, “reads count-only” datasets were first downloaded from NCBI GEO under the accession numbers provided in references and then DEGs were extracted by in-house pipeline in R with edgeR package. Genes with p value < 0.05 were defined as DEGs by the treatment. Because increase of the transcripts of NLR genes is often not very large (sometimes less than 0.5-fold change) after pathogen infection (Zou et al., 2014; Yang et al., 2020) and with or without fold change (FC) does not change the relative numbers of upregulated and downregulated DEGs, we used FDR criteria without an additional FC criterion to allow more sensitive capture of early induction of NLR genes.

RNAseq data with biotic and abiotic treatments as well as RNAseq data for autoimmune mutants were selected. For biotic stresses, we selected infection by representative strains of bacterial pathogen P. syringae pv. tomato (Pst) (Pst DC3000, Pst DC3000 AvrRps4, Pst DC3000 AvrRpm1, Pst DC3000 AvrRpt2, and Pst DC3000 cor-), fungal pathogen Botrytis cinerea, immunity elicitors flg22 and chitin, as well as SA and its functional analog BTH. For abiotic stresses, treatments by ABA, temperature, drought, and salt were included. When data were available, we included at least two independent datasets of the same treatment to increase data confidence. Also included are autoimmune mutants including host15-4, acl6-1, sis2-1, and bak1-1 serk4-1. In total, 88 RNAseq datasets from 27 independent studies were analyzed, including 57 datasets in response to biotic factors, 26 datasets to abiotic factors, and 5 datasets in autoimmune mutants (Table 1). The growth conditions and treatments used in the 27 independent studies are listed in Supplementary Data 1.

Procedures for Extracting DEGs From “Reads Count-Only” Datasets
Reads count of each treatment or mutant was downloaded from NCBI under GSE accessions provided in the references (Table 1). R/edgeR was used to call DEGs. The cpm (counts per million) values of each gene were calculated using “y <- DGEList(counts =x, group =group)”; “y <- calcNormFactors(y),” and genes with cpm value > 1 in at least two samples were used for DEG analysis using “keep <-rowSums(cpm(y)>1) ≥ 2”; “y <- y[keep].” Then, DEGs were called using “design<-model.matrix(~ 0+group)”; “y <- estimateGLMCommonDisp (y,design)”; “y <- estimateGLMTrendedDisp(y,design)”; “y <- estimateGLMTagwiseDisp(y,design)”; “fit <- glmFit(y,design)”; “lrt.2vs1 <- glmLRT(fit, contrast=(-1,1))”; “top2v1 <- topTags(lrt.2vs1, n=30000).” For more details, please see https://biohpc.cornell.edu/workshops.aspx.

Hierarchical Cluster Analysis of NLR Genes and SA- and NHP-Related Genes
The hierarchical cluster analysis was performed using “hclust” function in R program (https://www.r-project.org/). Because some NLR genes have been shown to have feedback regulation with SA, which is tightly connected with NHP, the SA- and NHP-related genes were also included in this analysis. To increase robustness of the analysis, we excluded 15 RNAseq datasets with fewer than 10 differentially expressed NLR genes. To reduce redundancy, one time point with the most drastic changes of NLR genes was selected for each of the time-course treatment. In total, 37 different samples were used for cluster analysis, with 16 biotic stress treatment, 16 abiotic stress treatment, and 5 autoimmune mutants. Briefly, the values of each gene with upregulation, downregulation, and no change were considered as 1, −1, and 0, respectively. The dissimilarity
| Treatments | Up | Down | Total DEGs | References | Source | Cutoffs |
|------------|----|------|------------|------------|--------|---------|
| Biotic stresses | | | | | | |
| DC3000, 1 hpi | 2 | 4 | 22 | Howard et al., 2013 | Directly from the article | p ≤ 0.1 |
| DC3000, 6 hpi | 9 | 3 | 646 | Howard et al., 2013 | Directly from the article | p ≤ 0.1 |
| DC3000, 12 hpi | 7 | 15 | 1,905 | Howard et al., 2013 | Directly from the article | p ≤ 0.1 |
| DC3000, 24 hpi | 20 | 20 | 7,254 | Yang et al., 2017 | Directly from the article | p ≤ 0.1 |
| DC3000, 1 hpi | 3 | 1 | 316 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000, 2 hpi | 0 | 0 | 107 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000, 3 hpi | 0 | 2 | 201 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000, 4 hpi | 3 | 0 | 335 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000, 6 hpi | 1 | 0 | 191 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000, 9 hpi | 26 | 0 | 1,022 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000, 12 hpi | 15 | 2 | 2,089 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000, 16 hpi | 40 | 7 | 5,624 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000, 20 hpi | 77 | 11 | 8,897 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000, 24 hpi | 81 | 12 | 9,388 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpm1, 1 hpi | 1 | 14 | 710 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpm1, 3 hpi | 15 | 2 | 1,638 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpm1, 4 hpi | 51 | 7 | 6,002 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpm1, 6 hpi | 50 | 16 | 8,857 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpm1, 9 hpi | 49 | 14 | 7,433 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpm1, 12 hpi | 61 | 13 | 7,977 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpm1, 16 hpi | 37 | 15 | 7,677 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpm1, 24 hpi | 71 | 4 | 6,881 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpm1, 3 day, 10h after dawn | 45 | 2 | 4,389 | Schwachtje et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpm1, 3 day, 15h after dawn | 25 | 0 | 1,942 | Schwachtje et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpm1, 4 day, 9h after dawn | 21 | 5 | 3,890 | Schwachtje et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpt2, 1 hpi | 8 | 2 | 333 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpt2, 2 hpi | 0 | 2 | 140 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpt2, 3 hpi | 8 | 0 | 312 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpt2, 4 hpi | 91 | 5 | 6,102 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpt2, 6 hpi | 79 | 17 | 11,305 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpt2, 9 hpi | 67 | 15 | 9,351 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpt2, 12 hpi | 67 | 15 | 9,579 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpt2, 16 hpi | 49 | 17 | 9,414 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpt2, 20 hpi | 77 | 10 | 10,430 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRpt2, 24 hpi | 61 | 4 | 7,177 | Mine et al., 2018 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRps4, 1 hpi | 0 | 0 | 901 | Howard et al., 2013 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRps4, 6 hpi | 47 | 6 | 2,581 | Howard et al., 2013 | Directly from the article | p ≤ 0.1 |
| DC3000 AvrRps4, 12 hpi | 33 | 1 | 2,501 | Howard et al., 2013 | Directly from the article | p ≤ 0.1 |
| DC3000 cor-, 24 hpi | 20 | 1 | 2,411 | Yang et al., 2017 | Directly from the article | p ≤ 0.1 |
| B. cinerea B05.10, 6 hpi | 0 | 0 | 1 | Coolen et al., 2016 | Directly from the article | p ≤ 0.1 |
| B. cinerea B05.10, 12 hpi | 0 | 0 | 67 | Coolen et al., 2016 | Directly from the article | p ≤ 0.1 |
| B. cinerea B05.10, 18 hpi | 12 | 0 | 780 | Coolen et al., 2016 | Directly from the article | p ≤ 0.1 |
| B. cinerea B05.10, 24 hpi | 13 | 0 | 1,974 | Coolen et al., 2016 | Directly from the article | p ≤ 0.1 |
| B. cinerea 2100, 14 hpi | 59 | 42 | 13,078 | Liu et al., 2015 | Directly from the article | p ≤ 0.1 |
| fig22, 30 min, 100 mM | 39 | 0 | 1,247 | Li et al., 2015 | Directly from the article | p ≤ 0.1 |
| fig22, 30 min, 1 µM | 65 | 0 | 2,253 | Bazin et al., 2020 | Directly from the article | p ≤ 0.1 |
| fig22, 1 h, 1 µM | 71 | 17 | 9,776 | Hillmer et al., 2017 | Directly from the article | p ≤ 0.1 |
| fig22, 2 h, 1 µM | 68 | 16 | 10,395 | Hillmer et al., 2017 | Directly from the article | p ≤ 0.1 |
| fig22, 3 h, 1 µM | 58 | 15 | 10,140 | Hillmer et al., 2017 | Directly from the article | p ≤ 0.1 |
| fig22, 5 h, 1 µM | 59 | 17 | 9,787 | Hillmer et al., 2017 | Directly from the article | p ≤ 0.1 |
TABLE 1 | Continued

| Treatments | Up | Down | Total DEGs | References | Source | Cutoffs |
|------------|----|------|------------|------------|--------|---------|
| fgl2, 9 h, 1 μM | 61 | 8 | 8,983 | Hillmer et al., 2017 | GSE78735 | p < 0.05 |
| fgl2, 18 h, 1 μM | 62 | 4 | 7,625 | Hillmer et al., 2017 | GSE78735 | p < 0.06 |
| chitin, 3h, 40 mM | 105 | 2 | 9,353 | Yamada et al., 2016 | GSE74955 | p < 0.05 |
| SA, 1 h, 50 μM | 38 | 0 | 3,367 | Ding et al., 2018 | Directly from the article | FDR < 0.05, FC ≥ 2 |
| BTH, 1 h, 300 μM | 59 | 0 | 1,577 | Yang et al., 2017 | GSE90077 | p < 0.05 |
| BTH, 5 h, 300 μM | 70 | 1 | 5,695 | Yang et al., 2017 | GSE90077 | p < 0.05 |
| BTH, 8 h, 300 μM | 58 | 1 | 4,017 | Yang et al., 2017 | GSE90077 | p < 0.05 |
| 44°C, 1 h | 18 | 55 | 8,851 | Suzuki et al., 2016 | GSE72806 | p < 0.05 |
| 37°C, 6 h | 16 | 92 | 13,700 | Pietzenuk et al., 2016 | GSE89077 | p < 0.05 |
| 37°C, 3 h | 7 | 78 | 8,615 | Zhang et al., 2017 | GSE94015 | p < 0.05 |
| 35°C, 4 h | 1 | 10 | 1,804 | Sewelam et al., 2020 | Directly from the article | p < 0.05, FC > 2 |
| 10°C, 1 h | 24 | 1 | 824 | Schlaen et al., 2015 | GSE63406 | p < 0.05 |
| 10°C, 24 h | 10 | 48 | 3,733 | Schlaen et al., 2015 | GSE63406 | p < 0.05 |
| 4°C, 3 h | 5 | 1 | 814 | Zhao et al., 2016 | Directly from the article | q < 0.05, FC ≥ 2 |
| 4°C, 24 h | 11 | 13 | 3,857 | Zhao et al., 2016 | Directly from the article | q < 0.05, FC ≥ 2 |
| 4°C, 24 h | 25 | 76 | 14,388 | Estève-Bruna et al., 2020 | GSE124812 | p < 0.05 |
| ABA, 3 h, 50 μM | 9 | 13 | 4,442 | Weng et al., 2016 | GSE65739 | p < 0.05 |
| ABA, 6 h, 100 μM | 36 | 27 | 11,771 | Zhan et al., 2015 | GSE66737 | p < 0.05 |
| ABA, 1 h, 10 μM | 1 | 1 | 491 | Song et al., 2016 | GSE80565 | p < 0.05 |
| ABA, 4 h, 10 μM | 4 | 8 | 2,318 | Song et al., 2016 | GSE80565 | p < 0.05 |
| ABA, 8 h, 10 μM | 2 | 10 | 2,090 | Song et al., 2016 | GSE80565 | p < 0.05 |
| ABA, 12 h, 10 μM | 3 | 12 | 2,287 | Song et al., 2016 | GSE80565 | p < 0.05 |
| ABA, 24 h, 10 μM | 3 | 11 | 1,960 | Song et al., 2016 | GSE80565 | p < 0.05 |
| ABA, 36 h, 10 μM | 4 | 16 | 2,498 | Song et al., 2016 | GSE80565 | p < 0.05 |
| ABA, 60 h, 10 μM | 2 | 18 | 2,334 | Song et al., 2016 | GSE80565 | p < 0.05 |
| ABA, 3 h, 50 μM | 24 | 38 | 8,527 | Zhu et al., 2017 | GSE99677 | p < 0.05 |
| drought, 5 days | 0 | 0 | 804 | Coolen et al., 2016 | Directly from the article | FDR < 0.05, FC > 2 |
| drought, 6 days | 4 | 33 | 2,488 | Coolen et al., 2016 | Directly from the article | FDR < 0.05, FC > 2 |
| drought, 7 days | 2 | 35 | 3,219 | Coolen et al., 2016 | Directly from the article | FDR < 0.05, FC > 2 |
| low water potential, 96 h | 5 | 31 | 2,856 | Wong et al., 2019 | Extracted from the article | p < 0.05 |
| NaCl, 1 h, 150 mM | 0 | 16 | 958 | Suzuki et al., 2016 | GSE72806 | p < 0.05 |
| NaCl, 7 h, 150 mM | 0 | 0 | 85 | Sewelam et al., 2020 | Directly from the article | p < 0.05, FC > 2 |
| NaCl, 24 h, 150 mM | 65 | 56 | 15,125 | GSE124812 | p < 0.05 |

| Autoimmune mutants | \(accd6-1\), 1 h after light onset | 100 | 2 | 8,595 | Zhang et al., 2019 | GSE115680 | p < 0.05 |
|---------------------|----------------------------------|----|----|-------------|------------|--------|---------|
| \(accd6-1\), 1 h after darkness onset | 75 | 1 | 5,548 | Zhang et al., 2019 | GSE115680 | p < 0.05 |
| \(bak1-4\) \(serk4-1\) | 43 | 2 | 3,637 | de Oliveira et al., 2016 | Directly from the article | FDR < 0.1, FC ≥ 2 |
| \(ssr2-1\) | 72 | 22 | 6,316 | Yang et al., 2016 | Directly from the article | FDR < 0.001, FC ≥ 2 |
| \(loe15-4\) \(smo1\) | 74 | 0 | 3,512 | Yang et al., 2020 | Directly from the article | FDR < 0.05 |

Shown are the selected RNAseq datasets including 57 biotic stresses, 26 abiotic stresses, and 5 autoimmune mutants. The differentially expressed genes (DEGs) are either directly from the publication or extracted from the deposited data on NCBI GEO website using in-house pipeline. The cutoff for in-house pipeline is \(p < 0.05\) to capture subtle changes of NLR genes. The cutoffs for DEGs from the references are either the same as in the publication or \(p < 0.05\) is used when the whole transcriptome changes are listed (for Wong et al., 2019).

"Up" indicates "induced NLRs" while "Down" indicates "repressed NLRs". "h," hour; "hpi," hour(s) post inoculation; "FDR," false discovery rate; "FC," fold change.

Values were calculated with the "dist" function and then used as input for "hclust." The Ward method was used for hierarchical clustering, because it identified the strongest clustering structure among the four methods (Average, 0.5804108; Single, 0.4357676; Complete, 0.7109366; Ward, 0.897766) analyzed. The clusters were identified with the "cutree" function, and the number of optimal clusters was determined with the Elbow method. Heatmap was generated in Excel. For visualization, NLR genes induced by the treatments or upregulated in the mutants were colored red while genes repressed were colored blue. Genes not expressed or the transcript level not altered were left blank. Among the 207 total NLR genes, 29 NLR genes had no altered
Kourelis and Kamoun (2020) contained. With DEG selected by

Figure 2 reduced expression after treatment (Table 1). More NLR genes had increased expression than reduced gene expression.

We further viewed the transcript dynamics in response to different treatments by plotting the number of increased and reduced NLR genes over time in the same treatment with a study. In general, the number of NLR genes with altered expression increased as the treatment progressed, and this was especially pronounced for the number of NLR genes with increased expression (Figure 1). The study of Mine et al. (2018) contained a set of treatment by various Pst DC3000 strains, which allowed us to compare over time and across different strains for DEGs selected by \( p < 0.05 \). After Pst DC3000 infection, fewer than 5 NLR genes had altered expression before 6 hpi (hours post-inoculation) while 40, 77, and 81 NLR genes were induced at 16, 20, and 24 hpi, respectively (Table 1; Figure 1A). By contrast, no more than 13 NLR genes were repressed during all the time points (Table 1; Figure 1A). A similar increase in number of induced NLRs over time was also observed under the infection by avirulent bacterial pathogens Pst DC3000 AvrRpm1 and Pst DC3000 AvrRpt2. Very few NLRs had altered gene expression before 3 hpi, but more NLR genes had increased expression after 4 hpi (Table 1; Figures 1B,C). A maximum of 71 and 91 NLR genes were induced by Pst DC3000 AvrRpm1 and Pst DC3000 AvrRpt2, respectively (Table 1; Figures 1B,C). The number of induced NLRs went up much faster in response to avirulent Pst DC3000 strains compared to the virulent Pst DC3000. No more than 26 NLR genes were induced in response to Pst DC3000 before 16 hpi whereas 51 and 91 NLR genes were induced at 4 hpi by Pst DC3000 AvrRpm1 and Pst DC3000 AvrRpt2, respectively (Table 1; Figures 1A–C). A similar pattern was also observed in the study of Howard et al. (2013). With DEG selected by \( p < 0.05 \), no more than 10 NLR genes were induced in response to Pst DC3000 at 1, 6, and 12 hpi while Pst DC3000 AvrRps4 induced 47 and 33 NLR genes at 6 and 12 hpi, respectively (Table 1; Figures 1D,E). This suggested a faster defense response in incompatible interaction than compatible interaction, as observed earlier in overall transcriptome responses (Tao et al., 2003).

The infection by fungal pathogens also induced the transcript level of some NLR genes. In response to fungal pathogen B. cinerea B05.10 strain, 12 and 13 NLR genes were upregulated at 18 and 24 hpi, respectively, and no NLR genes were repressed in the study of Coolen et al. (2016) (Table 1; Figure 1F). In another dataset of infection by the B. cinerea 2100 strain, with \( p < 0.05 \), 59 NLR genes were upregulated and 42 were downregulated at 14 hpi (Table 1). The latter dataset had an unusual high number of DEGs (comprising half of the genome), which might contribute

| Locus ID | Type | EV-NLR | Expression |
|----------|------|--------|------------|
| AT1G57670 | TX | Non-altered |
| AT1G63870 | TNL | Non-altered |
| AT2G31560 | TNL | Non-altered |
| AT3G31570 | TNL | Non-altered |
| AT4G27190 | CNL | Non-altered |
| AT5G17970 | TNL | Non-altered |
| AT5G42520 | TNL | Non-altered |
| AT1G57830 | TX | Non-altered |
| AT4G08450 | TNL | Non-altered |
| AT4G10780 | CNL | Non-altered |
| AT5G49140 | TNL | Non-altered |
| AT1G61105 | TX | Non-expressed |
| AT5G17950 | CN | Non-expressed |
| AT5G42520 | TNL | Non-expressed |
| AT1G60320 | TX | Non-expressed |
| AT2G03030 | TX | Non-expressed |
| AT2G03300 | ATTX12 | TX | Non-expressed |
| AT4G04110 | TN | Non-expressed |
| AT1G58842* | CNL | Non-expressed |
| AT3G25515* | TNL | Non-expressed |
| AT5G46480* | TN | Non-expressed |
| AT1G51280* | TX | Non-expressed |
| AT1G51485* | CNL | Non-expressed |
| AT2G0145* | TX | Non-expressed |
| AT4G11345* | TX | Non-expressed |
| AT4G19923* | TX | Non-expressed |
| AT4G19926* | TX | Non-expressed |
| AT4G23516* | TX | Non-expressed |

The locus, gene common name (ID), and NLR type of 29 NLR genes not included in the cluster analysis of Figure 2. These genes are either not expressed or their transcript levels are not altered in any of the treatment in the RNAseq datasets in Figure 2. "non-altered" indicates that the transcripts are neither increased nor decreased. "EV-NLR" means "experimentally validated NLRs" defined by Kourelis and Kamoun (2020). Genes with "*" are not valid genes on TAIR.

expression or were not expressed in response to any of the 37 conditions selected in this analysis (Table 2). None of these genes were experimentally validated and 13 of them did not contain NBS and LRR domains (Table 2). Additionally, 11 of them are no longer considered as valid genes on TAIR (Table 2). Therefore, we excluded these genes from the cluster analysis for clarity.

RESULTS

NLR Genes Are in General Induced Under Biotic Stresses

We tallied NLR genes that have increased or reduced expression respectively for each treatment (Table 1). In 47 out of 57 biotic stress treatments, more NLR genes had increased expression than reduced expression after treatment (Table 1). For instance, 81 NLR genes were induced while 12 were repressed at 24 h postinfiling with Pst DC3000 (Table 1). Also, chitin treatment at 40 mM for 3 h induced 105 NLRs while repressed only 2 NLRs (Table 1). The 10 treatments that did not show more NLR genes induced than repressed were either not reproduced in independent studies or were for the early time point after infection and became to have more induced NLR genes in later time points. Therefore, all biotic treatments analyzed, Pst DC3000 strains (virulent, avirulent, or non-virulent), B. cinerea, flg22, chitin, and SA, led to more NLR genes having increased gene expression than reduced gene expression.

The latter dataset had an unusual high number of DEGs (comprising half of the genome), which might contribute
NLR Genes Are in General Repressed in Response to Heat, ABA, and Drought

Abiotic stresses including high temperature and ABA are found to impact plant immunity through affecting NLR protein localization (Zhu et al., 2010; Mang et al., 2012). In addition, a previous study showed that the variation in NLR gene expression may be under natural selection to better adapt to the environment (MacQueen and Bergelson, 2016). Here, we analyzed NLR transcript change in response to abiotic stresses including heat, ABA, and drought and found that these abiotic stresses in general repressed the transcript level of NLR genes (Table 1).

Under three heat shock treatments, 44°C for 1 h, 37°C for 3 or 6 h, and 35°C for 4 h, much more NLR genes were repressed than induced (Table 1). Three-hour 37°C treatment repressed 78 NLR genes and induced 7 NLR genes among a total of 8,615 DEGs (Table 1). Similarly, 1-h 44°C treatment repressed 55 NLR genes and induced 18 NLR genes among 8,851 DEGs (Table 1). Therefore, more NLR genes had decreased transcripts than increased expression, which might contribute to high-temperature inhibition of disease resistance. By contrast, low temperature had a more complex effect on NLR transcript level depending on the duration of cold treatment. At the early period of low-temperature treatment (4°C for 3 h or 10°C for 1 h), no more than 25 NLR genes had altered expression with more genes induced than repressed by low temperature as observed in two...
Expression of Majority of NLR Genes Are Induced by Biotic Stresses and Repressed by Abiotic Stresses

To reveal potential gene network among all NLR genes, as well as SA- and NHP-related genes, we did a hierarchical cluster analysis of these genes based on their induction or repression under selected stress conditions and in autoimmune mutants (see Methods for details). Expression pattern was displayed for clustered NLR genes with grouped abiotic treatment, biotic treatment, and autoimmune mutants. Overall pattern from the cluster analysis revealed that abiotic and biotic stresses in general had opposite effects on expression of half of the NLR gene expression (Figure 2). Based on the expression pattern, NLR genes could be grouped into four modules A–D (Figure 2). Modules C and D comprised about half of the NLR genes and they were more similar to each other than to modules A and B. NLR genes in modules C and D were generally induced by biotic stresses and repressed by abiotic stresses, but they slightly differed in the extent of expressional changes.

Modules C and D contained 74 NLR genes, which were induced by most of the biotic stresses and repressed by most of the abiotic stresses (Figure 2). NLR genes in module D had more induction under biotic stresses while less repression under abiotic stresses as compared to NLR genes in module C. Fifty-five of them contained a TIR domain, suggesting that TNLs were more likely to respond to biotic and abiotic stresses than CNLs. Additionally, 31 out of the 51 experimentally validated NLR genes were in these two modules. For instance, RPM1 in module C was induced by both Pst DC3000 and Pst DC3000 AvrRpm1 at 24 hpi while it was repressed at 3 h or 6 h at 37°C. Notably, five major helper NLR genes ADR1, ADR1-L1, ADR1-L2, NRG1.1, and NRG1.2 were in these two modules (Figure 2). The drastic distinct expression changes of NLR genes under biotic and abiotic stresses suggested that transcriptional regulation of NLR genes might be an important mechanism for plants to cope with different environmental stresses.

Module B had 78 NLR genes including 13 experimentally validated NLRs, and 24 of them did not contain the LRR domain (Figure 2). NLR genes in this module were generally induced under a small number of biotic stress treatments and repressed under a small number of abiotic conditions, although some of them were induced under some abiotic conditions and repressed under certain biotic stresses (Figure 2). These NLR genes might have specificity in response to biotic and abiotic stresses. Alternatively, the expression level was too small to be detected in the RNAseq. Consequently, module B had a more heterogeneous pattern than modules C and D, which exhibited drastic expression changes of NLR genes under stress conditions.

A Small Number of NLR Genes Are Repressed by Biotic Stresses

Module A contained 26 NLR genes that were generally repressed under both biotic and abiotic stresses and in autoimmune mutants, with abiotic stresses more often repressing their expression than biotic stresses (Figure 2). Sixteen of them contained the CC domain, and 7 were experimentally validated
FIGURE 2 | Expression profile of 178 NLR genes as well as 17 SA- and NHP-related genes under stress conditions and in autoimmune mutants. Cluster display of 178 NLR or NLR-like genes along with 17 SA- and NHP-related genes by their transcript levels in 37 conditions. Gene induction is marked as a red box while gene (Continued)
NLR genes including **SUMM2** (SUPPRESSOR OF MKK1 MKK2 2) and **TAO1** (TARGET OF AVRB OPERATION 1). For instance, the transcript level of a CNL SUMM2 was decreased by 150 mM NaCl treatment at 24 h and 50 µM ABA treatment at 3 h. At the same time, it was repressed by biotic stresses including *B. cinerea*, *Pst* DC3000, and *Pst* DC3000 AvrRpt2. This might suggest a unique mode of action of CNLs under certain pathogenic conditions and further studies are needed to explore the biological relevance of this group of NLR genes in response to biotic and abiotic stresses.

**A Large Group of NLR Genes Have Similar Expression Pattern With SA- and NHP-Related Genes**

All the 17 SA- and NHP-related genes except for ICS2, EPS1, and PAL3 were in module C or D, and 11 of them were in module D, which had stronger induction of NLR genes under biotic stresses (Figure 2). Specifically, PAD4, SAG101, CBP60g, and SARD1 were in the same small subclade with ADR1-L1, AT5G41740, AT5G41750, AT1G66900, and AT1G72900. ED55 and SARD4 were in the same subclade with ADR1, ADR1-L2, ZAR1, AT4G14370, AT1G57630, and AT2G32140. The PAD4 subclade and the ED55 subclade were close to each other in the cluster. ICS1 and PBS3 were in another subclade, and so were ALD1 and FMO1. The SA biosynthesis genes PAL1 and PAL2 were in a subclade of module C. EDS1, a gene functionally related to PAD4, was not in the PAD4 subclade but was in the same subclade with two experimentally validated NLR genes AT1G17600 (SOC3) and AT1G17610 (CHS1). By contrast, EPS1 and PAL3 were in module A and ICS2 was in module B (Figure 2). In addition, the expression patterns of ADR1s (ADR1, ADR1-L1, and ADR1-L2) were more similar to SA-related genes as compared to that of NRG1s (NRG1.1, NRG1.2) (Figure 2). This was consistent with previous studies in which the ADR1 gene family regulates immunity through regulation of SA accumulation and subsequent activation of SA-dependent responses while the NRG1 gene family is not involved in SA regulation (Bonardi et al., 2011; Castel et al., 2019). Notably, 31 out of 38 SA-induced and 57 out of 78 BTH-induced NLR genes were also in modules C and D. These results suggest that SA and biotic stresses mostly activated a similar set of NLR genes.

**Assessment of the Contribution of SA to NLR Induction Under Biotic Stresses**

We further investigated the contribution of SA to NLR induction under biotic stresses. Datasets with time-course biotic stress treatments along with SA and BTH treatments were used for plotting the transcript dynamics of NLR genes. NLR genes were categorized into four major groups. Group I contained 90 NLR genes that are induced by SA or BTH, and 87 NLR genes among them were also induced by at least one of the biotic stresses (Figure 3). Most NLR genes are induced by pathogens at 4 hpi when SA-related genes, including PAD4, EDS1, SARD1, CBP60g, SAG101, ED55, PBS3, and ICS1, were induced (Figure 3). SA may potentially be involved in the induction of some NLR genes in this group. Group II consisted of 44 NLR genes that were induced by pathogens but not SA or BTH (Figure 3). All 131 NLR genes that were induced by pathogens or pathogen patterns were within Groups I and II, and 44 of them are not induced by SA or BTH, indicating a SA-independent induction by pathogens. Group III had 52 NLR genes including previously identified 29 non-expressed or “non-altered” NLR genes (Figure 3; Table 2). The transcripts of most NLR genes in this group were not altered in response to pathogen infection or SA and BTH treatment, except for two NLRs AT1G72840 and AT1G75850 showing inconsistent transcript changes in response to avirulent pathogens (Figure 3). Group IV contained 21 NLR genes repressed by pathogens, and 2 of them were repressed by BTH (Figure 3). These analyses indicate that biotic stresses had larger effects on NLR gene transcription and response to SA treatment is similar to responses to pathogens.

In order to determine whether or not SA accumulation is responsible for induction of some NLRs during pathogen infection, we analyzed RNAseq data of the *cbp60g sard1* double mutant where SA induction by pathogen was greatly compromised (Lu et al., 2018). As expected, the six genes related to biosynthesis of SA and NHP, PAD4, PBS3, ICS1, SARD4, ALD1, and FMO1 have reduced expression in the double mutant (Figure 3). Among the 61 NLR genes induced by *Pma* in wild type, 31 NLR genes were also induced in the *cbp60g sard1* double mutant (Figure 4A). Among the 61 NLR genes induced by *Pma* in wild type, 31 NLR genes were also induced in the *cbp60g sard1* double mutant.
FIGURE 3 | Expression profile of 207 NLR genes as well as 17 SA- and NHP-related genes under time-course biotic stress treatment. Expression levels of 207 NLR or NLR-like genes and 17 SA- and NHP-related genes during the time course of biotic stress treatments that are marked as triangles (light gray for Pst DC3000; dark (Continued)
Genes in the Same Gene Cluster More Likely Have Similar Expression Patterns

We analyzed the expression patterns of NLR genes residing in the same gene cluster to determine if they were co-regulated. The Col-0 accession of *A. thaliana* has a total of 42 NLR gene clusters with 2–11 of NLR genes in one cluster (Meyers et al., 2003). Co-expression was defined as genes residing in the same or adjacent subclade in the Cluster analysis. Fifteen NLR gene clusters had at least two genes in the cluster showing co-expression (Figure 2). For example, SOC3 (AT1G17600) and CHS1 (AT1G17610) are two well-known NLR genes with TIR domain in the same gene cluster, and they were next to each other in the expression cluster subclade, indicating a similar expression pattern (Figure 2). Likewise, SNC1, RECOGNITION OF PERONOSPORA PARASITICA 4 (RPP4), and SIDEKICK SNC1 2 (SIKIC2) resided in the RPP5 gene cluster, which contains eight NLR genes in the Col-0 background (Meyers et al., 2003), and they were clustered together in the expression cluster subclade (Figure 2). However, not all the genes in the same gene cluster shared a similar expression pattern. For instance, in the RPP5 gene cluster, SNC1, RPP4, and SIKIC2 were clustered together, while five other NLR genes in the RPP5 gene cluster were in other modules (Figure 2). Co-regulation of NLR genes in the RPP5 gene cluster has been implicated in previous studies (Yi and Richards, 2007; Zou et al., 2014, 2017). These results indicate that NLR genes in the same cluster are more likely to have a similar expression pattern,
which might enable plants to initiate a timely and effective immune response.

**Limitations of This Meta-Analysis**

Because this meta-analysis was from different datasets of separate studies, this raises an issue when comparing gene list across different studies. Biological differences and methodological differences could potentially make some cross comparisons impossible. These differences may come from (a) plant growth conditions (light quality and quantity; light cycle; humidity; growth medium); (b) developmental stage of plants; (c) tissues (leaf or whole plants) sampled for RNAseq analysis; (d) RNAseq method (library preparation; sequencing method and depth); and (e) RNAseq data analysis (methods and cutoffs for extracting DEGs). These factors can affect the DEG list extracted for analysis. The following measures have been used to minimize these differences: (1) Datasets from similar biological conditions were selected for this study. For instance, only leaves or young seedlings were selected (Supplementary Data 1); (2) When possible, datasets with processed reads count were selected so that the same in-house pipeline can be used to extract DEGs with the same selection criteria. In fact, DEGs from 66 out of the 88 RNAseq datasets were selected using the same criterion of \( p < 0.05 \). In addition, the selection criteria for the rest of the datasets (directly from the article) were mostly using the same selection criteria (\( p \) value, \( q \) value, or FDR < 0.05 with an additional FC \( \geq 2 \)). Characteristics that are not dependent on selection criteria or sample quality (such as more genes up than down) are used for cross-comparison between different studies. Characteristics that are dependent on selection methods are only compared within the same experimental set. Although we made efforts to minimize both biological and methodological differences, these factors need to be considered when it comes to cross-comparison of the number of NLR genes induced or repressed under stress conditions among different studies.

**DISCUSSION**

Plant NLR proteins are central intracellular immune receptors critical for pathogen recognition and immune response activation. They are known to be tightly regulated at the protein level for immunity/growth balance. However, their dynamics at the RNA transcript level was not extensively investigated before. This meta-analysis mined 88 RNAseq data and systematically revealed transcript dynamics of NLR genes during plant pathogen interaction and under abiotic stresses, which provides an extensive description of NLR expression under the changing environment.

**Transcriptional Induction of NLR Genes Is Prevalent in Plant Immune Response**

The first striking feature of the meta-analysis is that more than half of NLR genes are induced by pathogens or defense elicitors and much fewer NLR genes were repressed than induced by biotic stresses (Table 1; Figures 1, 2). Additionally, more NLR genes were induced at later stage after pathogen infection, and avirulent pathogens and defense elicitors triggered NLR gene activation much faster compared to virulent pathogens (Table 1; Figure 1). Also, the number of NLR genes induced or repressed fluctuated throughout the duration of pathogen or defense elicitor treatments (Figure 1), indicating that the transcription of NLR genes is very dynamic in immune responses. Indeed, NLR genes need to be induced upon pathogen infection to confer disease resistance while the transcription of NLR genes also needs to be tightly monitored to prevent overactivation. For instance, most NLR genes upregulated in the hos15-4 mutant are induced by pathogens while these NLRs are simultaneously repressed by HOS15 under pathogenic condition (Yang et al., 2020). These results suggest that regulation of NLR gene expression, in addition to the activation of NLR proteins, might be an important mechanism for plant to better fend off pathogen invasion.

**Transcriptional Repression of NLR Genes Might Be a Mechanism for Plant Adaption to Abiotic Stresses**

The second feature is that the same set of NLR genes that are induced under biotic stress conditions are repressed by heat, ABA, and drought (Figure 2). These abiotic factors have been shown to inhibit disease resistance in general. For instance, the ABA biosynthetic loss-of-function mutant aba3-1 is more resistant to 

\( Pst \) DC3000 and the ABA biosynthetic gain-of-function mutant 

\( cds2-1D \) exhibits susceptibility to various 

\( P. syringae \) strains as compared to wild type (Fan et al., 2009). The repression of plant defense response by ABA at least partially comes from its cross talk with SA (Mohr and Cahill, 2007; Yasuda et al., 2008; Fan et al., 2009). ABA treatment reduces SA concentration in plants and represses many genes involved in phenylpropanoid biosynthesis pathway, which is closely associated with SA biosynthesis (Mohr and Cahill, 2007). On the other hand, SA antagonizes ABA signaling through multiple mechanisms including inhibiting ABA-induced gene expression and acting against the role of ABA on protein degradation or stabilization (Manohar et al., 2017). Therefore, the opposite effects on NLR gene transcription by ABA and biotic stresses, which often induce SA accumulation, might be due to the antagonism between ABA and SA. In addition, plant drought response is largely through the regulation of ABA signaling pathways, which likely results in similar effects on NLR transcription by ABA and drought. Likewise, high temperature stimulates ABA biosynthesis (Toh et al., 2008), and it is possible that an ABA increase by heat stress contributes to the decrease of NLR transcript level at high temperature. Therefore, the induction of NLRs by biotic stresses and repression by abiotic stresses could result from antagonistic effects between SA and ABA. This study reveals that regulation of NLR gene expression might be an important node for balancing biotic and abiotic stress responses. It is not uncommon for a NLR gene to be functional in one natural accession but non-functional in another accession. Although this may result mainly from co-evolution between plants and pathogens, the balance between biotic and abiotic responses might also play a role. Indeed, functional and non-functional NLR gene ACQUIRED OSMOTOLERANCE (ACQOS) was maintained in Arabidopsis natural accessions.
due to trade-off between biotic and abiotic stress adaption (Ariga et al., 2017). The ACQOS gene was in module C, and it was generally induced under biotic stresses while repressed under abiotic stresses (Figure 2). Therefore, repression of NLR transcription might have evolved as a general mechanism for plant to survive under abiotic stresses.

**A Small Set of NLR Genes Are Repressed During Plant–Microbe Interaction**

Interestingly, a small set of NLR genes are repressed by biotic stresses in contrast to the majority of NLR genes (Figure 2). Most of these genes are not functionally characterized (Figure 2). Expression suppression of some NLR genes may occur simultaneously with expression increase of other NLR genes in order to prevent overactivation of immune responses and its consequent fitness costs. For example, an immunity regulator ENHANCED DOWNY MILDEW 2 (EDM2) positively regulates expression of RECOGNITION OF PERONOSPORA PARASITICA 7 (RPP7) and a small number of other NLR genes, whereas it represses the expression of a number of other NLR genes (Lai et al., 2020). Studying the molecular mechanism and biological relevance of NLR genes that are often repressed by pathogens in the context of plant–microbe interaction is expected to bring a new insight into NLR function.

**SA Contributes to NLR Induction Under Pathogenic Conditions**

Co-expression of SA-related genes with many NLRs under biotic stresses suggests a role of SA in defense signaling transduction and amplification. SA induction was reported for several NLR genes and was postulated to amplify their function (Shirano et al., 2002; Xiao et al., 2003; Yang and Hua, 2004). This study revealed that the majority of NLR genes (in modules C and D) that were induced by pathogens were also induced by SA and co-expressed with SA-related genes (Figure 2). Conversely, SA and BTH induced expression of about 90 NLR genes, and almost all of them were also induced by pathogens (Figure 3). Induction of some of these NLRs by pathogen invasion happened before measurable SA induction, suggesting that SA might amplify their induction during infection. Analysis of SA-deficient mutant cbp60g sard1 revealed that induction of 15 NLRs and full induction of the other 8 NLRs are dependent on CBP60g and SARD1 and therefore likely SA accumulation. Therefore, SA is an inducer and an amplifier of some NLR genes. Additionally, NLR or NLR-like genes with TIR domain were more likely to be induced by SA (Figure 2). This is consistent with the previous finding that SA induced the expression of several TNL genes including SS14, RPP1, and RPS4, but had little effect on the expression of two CNL genes RPM1 and RPS2 (Shirano et al., 2002). This might be due to a more prominent role of EDS1 and SA in TNL-mediated compared to CNL-mediated immune responses.

This analysis also identified 44 NLR genes whose expression are not significantly induced by SA or BTH. Their induction by pathogens is therefore likely SA independent. The transcriptional regulation of these NLR genes during pathogen infection awaits to be explored in the future. The systematic analyses of all NLR genes under various stress conditions in this study will be a useful resource for better understanding the correlation between SA and NLR induction during pathogen infection process.

**Co-regulation of NLR Genes in the Same Gene Cluster Is a Common Phenomenon**

This study also revealed that co-expression of NLR genes in the same gene cluster is quite common during plant pathogen interaction. More than one-third of NLR gene clusters have co-expressed genes under stress conditions (Figure 2). This co-expression might be efficient in co-ordinate genes with similar functions as genes in the same cluster tend to have high sequence similarity or are functionally related (such as gene pair). Mechanisms for co-expression have been studied for the RPP5 gene cluster. Chromatin-based gene regulation and RNA silencing have been postulated for achieving co-expression of multiple genes (Yi and Richards, 2007; Zou et al., 2014, 2017). An open or close chromatin structure initiated by transcriptional proteins influencing one NLR gene could cause chromatin structure changes in the gene cluster and thus affect the expression of neighboring genes. Alternatively, a common regulatory element (such as an enhancer) could be shared by NLR genes in the same gene cluster and thus enable co-expression. Co-regulation of NLR genes is expected to facilitate a timely and effective immune response upon pathogen infection.

**CONCLUSION**

In sum, NLR genes have dynamic transcript expression patterns in response to both biotic and abiotic stresses. The majority of NLR genes are induced by biotic stresses and are repressed by heat and drought. The opposite effects from biotic and abiotic stresses suggest an important role of NLR gene expression in plant adaptation to environmental stresses. Plant hormones SA and ABA, respectively induce and repress NLR expression in general, suggesting a contribution of these two hormones to NLR gene regulation by biotic and abiotic stresses. Strikingly, a small set of NLR genes are repressed by both biotic and abiotic stresses, and their function will warrant further investigation. This study revealed a broad picture of dynamics of NLR transcript level under environmental stresses, which will facilitate a molecular understanding of immunity regulation under diverse stress conditions.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author/s.

**AUTHOR CONTRIBUTIONS**

JH and LY designed the study. LY and ZW performed the data analyses and made the figures and tables. LY and JH wrote the manuscript with input from ZW. All authors contributed to the article and approved the submitted version.
This work was supported by National Science Foundation USA IOS-1353738 and IOS-1946174 to JH.

REFERENCES

Adachi, H., Derevina, L., and Kamoun, S. (2019). NLR singletons, pairs, and networks: evolution, assembly, and regulation of the intracellular immunoreceptor circuitry of plants. Curr. Opin. Plant Biol. 50, 121–131. doi: 10.1016/j.ppb.2019.04.007

Ariga, H., Katoki, T., Tsuchimatsu, T., Hirase, T., Tajima, Y., Parker, J. E., et al. (2017). NLR locus-mediated trade-off between abiotic and biotic stress adaptation in Arabidopsis. Nat. Plants 3, 1–8. doi: 10.1038/nplants.2017.72

Bazin, J., Mariappan, K., Jiang, Y., Blein, T., Voelz, R., Crespi, M., et al. (2020). Role of MPK4 in pathogen-associated molecular pattern-triggered alternative splicing in Arabidopsis. PLoS Pathog. 16:e1008401. doi: 10.1371/journal.ppat.1008401

Bonardi, V., Tang, S., Stallmann, A., Roberts, M., Cherkis, K., and Dangl, J. L. (2011). Expanded functions for a family of plant intracellular immune receptors beyond specific recognition of pathogen effectors. Proc. Natl. Acad. Sci. U.S.A. 108, 16463–16468. doi: 10.1073/pnas.1113726108

Buscaill, P., and Rivas, S. (2014). Transcriptional control of plant defence salicylic acid receptors NPR1 and NPR3/NPR4 in transcriptional regulation of low temperature and pathogen infection. Plant Cell. 20, 35–46. doi: 10.1016/j.pbi.2014.04.004

Castel, B., Ngou, P. M., Cevik, V., Redkar, A., Kim, D. S., Yang, Y., et al. (2019). Diverse NLR immune receptors activate defence via the RWP-8 NLR NRG1. New Phytol. 222, 966–980. doi: 10.1111/nph.15659

Chen, H., Xue, L., Chintamanani, S., Germain, H., Lin, H., Cui, H., et al. (2009). ETHYLENE INSENSITIVE3 and ETHYLENE INSENSITIVE-LIKE1 repress SALICYLIC ACID INDUCTION DEFICIENT2 expression to negatively regulate plant innate immunity in Arabidopsis. Plant Cell. 21, 2527–2540. doi: 10.1105/tpc.108.065193

Cheng, Y. T., Zhang, L., and He, S. Y. (2019). Plant-microbe interactions facing environmental challenge. Cell. Host Microbe. 26, 183–192. doi: 10.1016/j.chom.2019.07.020

Coelen, S., Proietti, S., Hickman, R., Davila Olivas, N. H., Huang, P. P., Van Verk, M. C., et al. (2016). Transcriptome dynamics of Arabidopsis during sequential plant immunity. Plant Cell. 27, 839–856. doi: 10.1105/tpc.114.134809

Ding, Y., Sun, T., Ao, K., Peng, Y., Zhang, L., Xu, Y., et al. (2018). Opposite roles of salicylic acid receptors NPR1 and NPR3/NPR4 in transcriptional regulation of plant immunity. Cell 173, 1454–1467. doi: 10.1016/j.cell.2018.03.044

Esteve-Bruna, D., Carrasco-López, C., Blanco-Touriñán, N., Iserte, J., Calleja-Cabrera, J., Pérez-Resa, C., et al. (2020). Prefoldsins contribute to maintaining the levels of the slycosome LSM2–8 complex through Hsp90 in Arabidopsis. Nucleic Acids Res. 48, 6280–6293. doi: 10.1093/nar/gkaa354

Fan, J., Hill, L., Crooks, C., Doerner, P., and Lamb, C. (2009). Absciscic acid has a key role in modulating diverse plant-pathogen interactions. Plant Physiol. 150, 1750–1761. doi: 10.1104/pp.109.173943

Gou, M., and Hua, J. (2012). Complex regulation of an R gene SNC1 involved in plant immunity by light, circadian rhythm, and temperature. Curr Opin Plant Biol. 15, 406–413. doi: 10.1016/j.ppb.2012.06.017

Howard, B. E., Hu, Q., Babaoglu, A. C., Chandra, M., Borghi, M., Tan, X., et al. (2013). High-throughput RNA sequencing of pseudomomas-infected Arabidopsis reveals hidden transcriptome complexity and novel splice variants. PLoS ONE 8:e74183. doi: 10.1371/journal.pone.0074183

Hua, J. (2013). Modulation of plant immunity by light, circadian rhythm, and temperature. Curr Opin Plant Biol. 16, 406–413. doi: 10.1016/j.ppb.2013.06.017

Jambunathan, N., Siani, J. M., and McNellis, T. W. (2001). A humidity-sensitive Arabidopsis copine mutant exhibits precocious cell death and increased disease resistance. Plant Cell. 13, 2225–2240. doi: 10.1016/j.plcel.2019.07.008

Kouris, J., and Kamoun, S. (2020). RePLANTNLR: a comprehensive collection of experimentally validated plant NLRs. bioRxiv [Preprint]. Available online at: https://www.biorxiv.org/content/10.1101/2020.07.08.139961v1 (accessed July 9, 2020).

Lai, Y., Xu, X., Daron, J., Pan, S., Wang, L., Renna, L., et al. (2017). CAMTA-mediated regulation of salicylic acid immunity pathway genes in Arabidopsis exposed to low temperature and pathogen infection. Plant. Cell. 29, 2465–2477. doi: 10.1105/tpc.16.008865

Koutris, J., and Kamoun, S. (2020). RePLANTNLR: a comprehensive collection of experimentally validated plant NLRs. bioRxiv [Preprint]. Available online at: https://www.biorxiv.org/content/10.1101/2020.07.08.139961v1 (accessed July 9, 2020).

Lim, S., Lapin, D., Liu, L., Sun, Y., Song, W., Zhang, X., et al. (2020). Direct regulation of ABA signaling by WRKY33 is critical for Arabidopsis immunity. Plant Cell. 32, 1038–1049. doi: 10.1105/tpc.119.187048

MacQueen, A., and Bergelson, J. (2016). Modulation of R-gene expression across environments. J. Exp. Bot. 67, 2093–2105. doi: 10.1093/jxb/erv530

SUPPLEMENTARY MATERIAL

This Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.625729/full#supplementary-material

FUNDING
Shah, J., Kachroo, P., Nandi, A., and Klessig, D. F. (2001). A recessive mutation in the Arabidopsis SSII2 gene confers SA-and NPR1-independent expression of PR genes and resistance against bacterial and oomycete pathogens. *Plant J.* 25, 563–574. doi: 10.1046/j.1365-313x.2001.00992x

Shirano, Y., Kachroo, P., Shah, J., and Klessig, D. F. (2002). A gain-of-function mutation in an Arabidopsis toll interleukin 1 receptor-nucleotide binding site–Leucine-rich repeat type R gene triggers defense responses and results in enhanced disease resistance. *Plant Cell* 14, 3149–3162. doi: 10.1105/tpc.0105348

Song, L., Huang, S. S., Wise, A., Castanon, R., Nery, J. R., Chen, H., et al. (2016). A transcription factor hierarchy defines an environmental stress response network. *Science* 354:598. doi: 10.1126/science.aag1550

Suzuki, N., Bassil, E., Hamilton, J. S., Inupakutika, M. A., Zandalinas, S. I., Tripathy, D., et al. (2016). ABA is required for plant acclimation to a combination of salt and heat stress. *PLoS ONE* 11:e0147625. doi: 10.1371/journal.pone.0147625

Tan, X., Meyers, B. C., Kozik, A., West, M. A., Morgante, M., St Clair, D. A., et al. (2007). Global expression analysis of nucleotide binding site-leucine rich repeat-encoding and related genes in Arabidopsis. *BMC Plant Biol.* 7:56. doi: 10.1186/1471-2229-7-56

Tao, Y., Xie, Z., Chen, W., Glazebrook, J., Chang, H. S., Han, B., et al. (2003). Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. *Plant Cell* 15, 317–330. doi: 10.1105/tpc.007591

Toh, S., Imamura, A., Watanabe, A., Nakabayashi, K., Okamoto, M., Ikumaru, Y., et al. (2008). High-temperature-induced abscisic acid biosynthesis and its role in the inhibition of gibberellin action in Arabidopsis seeds. *Plant Physiol.* 146, 1368–1385. doi: 10.1104/pp.110.117378

Tsuda, K., and Katagiri, F. (2010). Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity. *Curr. Opin. Plant Biol.* 13, 459–465. doi: 10.1016/j.pbi.2010.04.006

Van de Weyer, A. L., Monteiro, F., Furrer, O. J., Nishimura, M. T., Cevik, V., Witte, K., et al. (2019). A species-wide inventory of NLR genes and alleles in Arabidopsis thaliana. *Cell* 178, 1260–1272. doi: 10.1016/j.cell.2019.07.038

Van Wiersch, R., Li, X., and Zhang, Y. (2016). Mighty dwarfs: Arabidopsis autoimmune mutants and their usage in genetic dissection of plant immunity. *Front. Plant Sci.* 7:1717. doi: 10.3389/fpls.2016.01717

Wan, L., Essuman, K., Anderson, R. G., Sasaki, Y., Monteiro, F., Chung, E. H., et al. (2019). TIR domains of plant immune receptors are NAD+–cleaving enzymes that promote cell death. *Science* 365, 799–803. doi: 10.1126/science.aax1771

Wan, W. L., Kim, S. T., Castel, R., Chaoeinntit, N., and Chae, E. (2020). Genetics of autoimmunity in plants: an evolutionary genetics perspective. *New Phytol.* 229, 1215–1233. doi: 10.1111/nph.16947

Wang, L., Tsuda, K., Truman, W., Sato, M., Nguyen, L. V., Katagiri, F., et al. (2011). CBP60γ and SARD1 play partially redundant critical roles in salicylic acid signaling. *Plant J.* 67, 1029–1041. doi: 10.1111/j.1365-313X.2011.04655.x

Wang, W., Feng, B., Zhou, J. M., and Tang, D. (2020). Plant immune signaling: advancing on two frontiers. *J. Integr. Plant Biol.* 62, 2–24. doi: 10.1111/jipb.12898

Wang, X., Gao, J., Zhu, Z., Dong, X., Wang, X., Ren, G., et al. (2015). TCP transcription factors are critical for the coordinated regulation of *ISOCHORISMATE SYNTHASE* 1 expression in Arabidopsis thaliana. *Plant J.* 82, 151–162. doi: 10.1111/tjp.12803

Wang, Y., Bao, Z., Zhu, Y., and Hua, J. (2009). Analysis of temperature modulation of plant defense against biotrophic microbes. *Mol. Plant Microbe Interact.* 22, 498–506. doi: 10.1094/MPMI-22-5-0498

Weng, J. K., Ye, M., Li, B., and Noel, J. P. (2016). Co-evolution of hormone metabolism and signaling networks expands plant adaptive plasticity. *Cell* 166, 881–893. doi: 10.1016/j.cell.2016.06.027

Wong, M. M., Bhaskara, G. B., Chen, B. P., Chong, K. H., Mok, C. W., Toh, S., Imamura, A., Watanabe, A., Nakabayashi, K., Okamoto, M., Ikumaru, Y., et al. (2014). Phosphoproteomics of Arabidopsis highly adaptive accessions reveals the effects of temperature on alternative splicing and circadian rhythms. *Proc. Natl. Acad. Sci. U.S.A.* 112, 9382–9387. doi: 10.1073/pnas.1504511112

Xiao, S., Brown, S., Patrick, E., Brearley, C., and Turner, J. G. (2003). Enhanced transcription of the Arabidopsis disease resistance genes RPP8.1 and RPP8.2 via a salicylic acid–dependent amplification circuit is
required for hypersensitive cell death. Plant Cell 15, 33–45. doi: 10.1105/tpc.006940

Yang, H., Yamada, K., Yamaguchi, K., Shirakawa, T., Nakagami, H., Mine, A., Ishikawa, K., et al. (2016). The Arabidopsis CERK1-associated kinase PBL27 connects chitin perception to MAPK activation. EMBO J. 35, 2468–2483. doi: 10.15252/embj.201694248

Yang, H., Shi, Y., Liu, J., Guo, L., Zhang, X., and Yang, S. (2010). A mutant CHS3 protein with TIR-NB-LRR-LIM domains modulates growth, cell death and freezing tolerance in a temperature-dependent manner in Arabidopsis. Plant J 63, 283–296. doi: 10.1111/j.1365-313X.2010.04241.x

Yang, L., Chen, X., Wang, Z., Sun, Q., Hong, A., Zhang, A., et al. (2020). HOS15 and HDA9 negatively regulate immunity through histone deacetylation of intracellular immune receptor NLR genes in Arabidopsis. New Phytol. 226, 507–522. doi: 10.1111/nph.16380

Yang, L., Teixeira, P. J., Biswas, S., Finkel, O. M., He, Y., Salas-Gonzalez, L., et al. (2017). Pseudomonas syringae type III effector HopB1 promotes host transcriptional repressor degradation to regulate phytohormone responses and virulence. Cell Host Microbe 21, 156–168. doi: 10.1016/j.chom.2017.01.003

Yang, S., and Hua, J. (2004). A haplotype-specific resistance gene regulated by a plant-specific family of transcription factors. Proc. Natl. Acad. Sci. U.S.A. 101, 18220–18225. doi: 10.1073/pnas.1005225107

Yang, W., Dong, R., Liu, L., Hu, Z., Li, J., Wang, Y., et al. (2016). A novel BONZAI1 mediates temperature-dependent growth control in Arabidopsis. Plant Physiol. 171, 2744–2759 doi: 10.1104/pp.16.00533

Zhang, Y., Xu, S., Ding, P., Wang, D., Cheng, Y. T., He, J., et al. (2010). Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. Proc. Natl. Acad. Sci. U.S.A. 107, 18220–18225. doi: 10.1073/pnas.1005225107

Zhang, Y., Wang, B., Tang, K., Hsu, C. C., Xie, S., Du, H., et al. (2017). An Arabidopsis nucleoporin NUP85 modulates plant responses to ABA and salt stress. PLoS Genet. 13:e1007124. doi: 10.1371/journal.pgen.1007124

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Yang, Wang and Hua. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.