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Ribosomal protein QM/RPL10 positively regulates defence and protein translation mechanisms during nonhost disease resistance

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Abstract

Ribosomes play an integral part in plant growth, development, and defence responses. We report here the role of ribosomal protein large (RPL) subunit QM/RPL10 in nonhost disease resistance. The *RPL10*-silenced *Nicotiana benthamiana* plants showed compromised disease resistance against nonhost pathogen *Pseudomonas syringae* pv. *tomato* T1. The RNA-sequencing analysis revealed that many genes involved in defence and protein translation mechanisms were differentially affected due to silencing of *NbRPL10*. *Arabidopsis AtRPL10* RNAi and *rpl10* mutant lines showed compromised nonhost disease resistance to *P. syringae* pv. *tomato* T1 and *P. syringae* pv. *tabaci*. Overexpression of *AtRPL10A* in *Arabidopsis* resulted in reduced susceptibility against host pathogen *P. syringae* pv. *tomato* DC3000. RPL10 interacts with the RNA recognition motif protein and ribosomal proteins RPL30, RPL23, and RPS30 in the yeast two-hybrid assay. Silencing or mutants of genes encoding these RPL10-interacting proteins in *N. benthamiana* or *Arabidopsis*, respectively, also showed compromised disease resistance to nonhost pathogens. These results suggest that QM/RPL10 positively regulates the defence and translation-associated genes during nonhost pathogen infection.

KEYWORDS

disease resistance, plant immunity, ribosome, RNA sequencing, translation regulation

1 | INTRODUCTION

Ribosomal proteins are an integral part of ribosomes and are involved in their biogenesis and assembly, thus regulating protein synthesis. It is difficult to attribute an individual function of translational activity to a single ribosomal protein. This difficulty is rooted in the highly cooperative nature of the interactions between ribosomal ribonucleic acid (rRNA) and ribosomal proteins. Ribosomal proteins regulate their own synthesis by controlling the expression of their transcripts in association with transcription factors. The ribosome consists of large and small subunits. In eukaryotes, 47 ribosomal protein large (RPL) subunits and 32 ribosomal protein small (RPS) subunits form a ribosome complex with rRNAs (Ben-Shem *et al.*, 2011). Interestingly, extraribosomal functions of many ribosomal proteins have been reported (Wool, 1996; Freed *et al.*, 2010). Several ribosomal protein-encoding genes are shown to be differentially

expressed under environmental stress conditions (Saha *et al*., 2017; Vemanna *et al*., 2019).

The role of ribosomal proteins in plant immunity has not been well studied. Plants have a sophisticated and layered defence mechanism against invading pathogens. Plants can perceive pathogen-associated molecular patterns (PAMPs) through their pattern recognition receptors and induce a general defence response, termed PAMP-triggered immunity (PTI; Jones and Dangl, 2006). A few successful pathogens can overcome or suppress PTI by delivering virulence proteins or metabolites termed as effectors. Certain plants have the ability to detect such effectors and trigger a strong defence response called effector-triggered immunity (ETI; Feng and Zhou, 2012). Another type of plant defence response, operating under less understood mechanisms, provides resistance against pathogens throughout all members of a species and is referred to as nonhost disease resistance (Heath, 2000; Mysore and Ryu, 2004; Senthil-Kumar and Mysore, 2013; Ayliffe and Sorensen, 2019). A pathogen that cannot cause disease in a nonhost plant is referred to as a nonhost pathogen. Nonhost resistance can be used to confer broad and durable disease resistance in crop plants (Gill *et al*., 2015; Fonseca and Mysore, 2019). Although nonhost resistance mechanisms are not fully understood, a variety of preformed and inducible responses are implicated (Mysore and Ryu, 2004; Senthil-Kumar and Mysore, 2013).

Ribosomal proteins are involved in basic cellular machinery and any abnormalities in these mechanisms may lead to compromised disease resistance. Multiple rare genetic diseases have been attributed to defects in ribosome function in humans and other mammals (Freed *et al*., 2010). Silencing of *RPL12* and *RPL19* in *Nicotiana benthamiana* and *Arabidopsisthaliana* showed compromised nonhost disease resistance against multiple bacterial pathogens (Nagaraj *et al*., 2016). RPL12 interacting receptor for activated C-kinase 1 (RACK1) was identified in *Arabidopsis* (Kundu *et al*., 2013) and plays a key role in plant innate immunity (Nakashima *et al*., 2008; Wang *et al*., 2014). Differential expression of genes encoding ribosomal proteins has been reported in vanilla in response to infection by *Fusarium oxysporum* f. sp. *vanillae* (Solano-De la Cruz *et al*., 2019). Similarly, induction of *RPS10* in soybean in response to *Phytophthora sojae* (Zhang *et al*., 2013) and induction of *RPS6*, *RPL19*, *RPL13*, *RPL7*, and *RPS2* associated with the plant response to turnip mosaic virus (TuMV), tobacco mosaic virus (TMV), and tomato bushy stunt virus (TBSV) have been reported (Yang *et al*., 2009). In a rice genotype resistant to *Xanthomonas* bacterial infection, 50 genes encoding ribosomal proteins were up-regulated (Narsai *et al*., 2013). The differential responses of all the ribosomal protein-encoding genes, including small and large subunit encoding genes, have been studied using genome-wide studies in rice in response to multiple stress conditions (Moin *et al*., 2016; Saha *et al*., 2017). Even though many stress-induced ribosomal protein-encoding genes have been reported, the precise function of each ribosomal protein during stress is not known (Vemanna *et al*., 2019).

Ribosomal proteins also possess extraribosomal functions such as regulation of transcription, chaperone activity, and protein phosphorylation in addition to their role in protein synthesis (Kubo and Arimura, 2010). A tomato QM)-like protein (chromosome 11 [Q26, 23]) has been shown to be involved in regulating oxidative stress (Chen *et al*., 2006). QM was able to rescue the *Proline dehydrogenase (PUT1)* mutant of *Saccharomyces cerevisiae* from oxidative stress (Chen *et al*., 2006). *QM10* was initially reported as a Wilm's tumour suppressor gene in humans and has homology with RPL10 (Rivera-Madrid *et al*., 1993; Shi *et al*., 2004; Dong *et al*., 2007; Chen *et al*., 2011). QM/RPL10 interacts with c-Jun transcription factor in humans and has been shown to be involved in regulating reactive oxygen species (ROS) levels in prostate cancer patients (Yang *et al*., 2018). RPL10 is a 60S large subunit protein mainly involved in ribosome biogenesis and assembly of both large and small subunits (Eisinger *et al*., 1997; Loftus *et al*., 1997). RPL10 proteins are present at the stalk of ribosomes and are involved in the rotation of ribosomes to recognize the mRNA strand for protein synthesis (Sulima *et al*., 2014). *Arabidopsis* has three QM/RPL10 protein-encoding gene family members that are differentially regulated by various stress stimuli. A specific set of genes is expressed in different individual *rpl10 Arabidopsis* mutants, signifying their role in the regulation of specific mechanisms (Falcone Ferreyra *et al*., 2013). Nuclear shuttle protein-interacting kinase 1 (NIK1) phosphorylates RPL10 during viral disease signalling and translocates to the nucleus to associate with MYB transcription factor (Carvalho *et al*., 2008). The *Arabidopsis rpl10a* mutant shows reduced plant protein synthesis and reduced viral protein synthesis, resulting in improved tolerance to viral infection (Rocha *et al*., 2008; Zorzatto *et al*., 2015). The NIK1 protein is homologous to a well-characterized plant defence protein called *Botrytis*-induced kinase1 (BRI1)-associated receptor kinase 1 (BAK1). *Arabidopsis nik1/bik1* mutants exhibited resistance against *Pseudomonas syringae* pv. *tomato* DC3000 (Lal *et al*., 2018; Li *et al*., 2019).

Here we report the role of QM/RPL10 in nonhost disease resistance against bacterial pathogens. *NbRPL10*-silenced *N. benthamiana* plants and *Arabidopsis rpl10* mutants showed higher nonhost bacterial multiplication. The transcriptome profiling of *NbRPL10*-silenced plants identified differential expression of several plant defence-related genes and protein translation-associated genes. Furthermore, we identified and characterized QM/RPL10 interacting proteins and determined their role in nonhost disease resistance. This study demonstrates that RPL10 acts as a central regulator of many ribosomal proteins and plant defence responses.

2 | RESULTS

2.1 | Silencing of *QM10/RPL10* in *N. benthamiana* compromises nonhost disease resistance

We performed a virus-induced gene silencing (VIGS)-based forward genetics screen in *N. benthamiana* to identify genes involved in nonhost disease resistance (Rojas *et al*., 2012; Wang *et al*., 2012; Senthil-Kumar *et al*., 2013; Kaundal *et al*., 2017). One of the cDNA clones

NbME23C12 (Senthil-Kumar *et al*., 2018; https://vigs.noble.org/line2.php?id=NbME23C12) compromised nonhost disease resistance when silenced. The *NbME23C12* sequence has homology with *Arabidopsis QM/RPL10* family genes, and the domain architecture clearly shows that it belongs to the ribosomal L16_L10e superfamily of genes (Figure S1a). In *Arabidopsis*, *RPL10* has three gene family members, A, B, and C, whereas we could find only two of them in *N. benthamiana* and named them *NbRPL10A* and *NbRPL10B*. To test whether silencing of a specific *NbRPL10* gene in *N. benthamiana* compromises nonhost resistance, the VIGS constructs with different regions specifically targeting *NbRPL10A* or *NbRPL10B* or both were designed using the *PssRNAit* webserver tool (Ahmed *et al*., 2020) with minimum off-target genes, PCR amplified, and cloned into TRV2 VIGS vector (Senthil-Kumar and Mysore, 2014; Figure S1b). Silencing of the *NbRPL10A* clone showed stunted plants with variegated, mottled, and crinkled leaves (Figure 1a). The silenced plants also showed cell death starting at the petiole and midrib. Interestingly, silencing of *NbRPL10B* did not produce a variegated leaf phenotype when compared to *NbRPL10A* or *NbRPL10A* + *NbRPL10B* (*NbRPL10s*)-silenced plants (Figure 1a). All silenced plants showed more than 50% down-regulation of target transcripts (Figure S1c). To test the response of *NbRPL10*-silenced plants to pathogens, silenced plants and control (TRV::*GFP* inoculated; the green fluorescent protein gene, *GFP*, has no sequence similarity to plant genomic DNA and thus will not cause gene silencing) were vacuum infiltrated with *GFPuv* (Wang *et al*., 2007)-expressing nonhost pathogen *P. syringae* pv. *tomato* T1 and host pathogen *P. syringae* pv. *tabaci* at 10^4 cfu/ml concentration. All the silenced plants had a higher accumulation of nonhost bacteria *P. syringae* pv. *tomato* T1 than the control plants (Figures 1b,c and S1d,e). In contrast, the host pathogen *P. syringae* pv. *tabaci* multiplied to the same extent in both control and silenced plants. These results suggest that *NbRPL10* silencing in *N. benthamiana* compromises nonhost resistance but not basal resistance.

2.2 | *NbQM/NbRPL10*-silenced *N. benthamiana* plants have fewer differentially expressed genes after nonhost pathogen treatment

To understand the molecular mechanisms involved in nonhost disease resistance by *NbQM/NbRPL10* silencing in *N. benthamiana*, we generated a transcriptome profile of *NbRPL10A*-silenced and control (TRV::*GFP*) plants by RNA-Seq after inoculation with the nonhost pathogen *P. syringae* pv. *tomato* T1 at 10^4 cfu/ml concentration. The differentially expressed gene (DEG) analysis of RNA-Seq data identified a diverse list of genes that play a defence role in *QM/RPL10*-mediated nonhost resistance. When *NbRPL10A* was silenced in *N. benthamiana*, molecular-function associated genes were highly represented in the DEGs in both control and nonhost pathogen

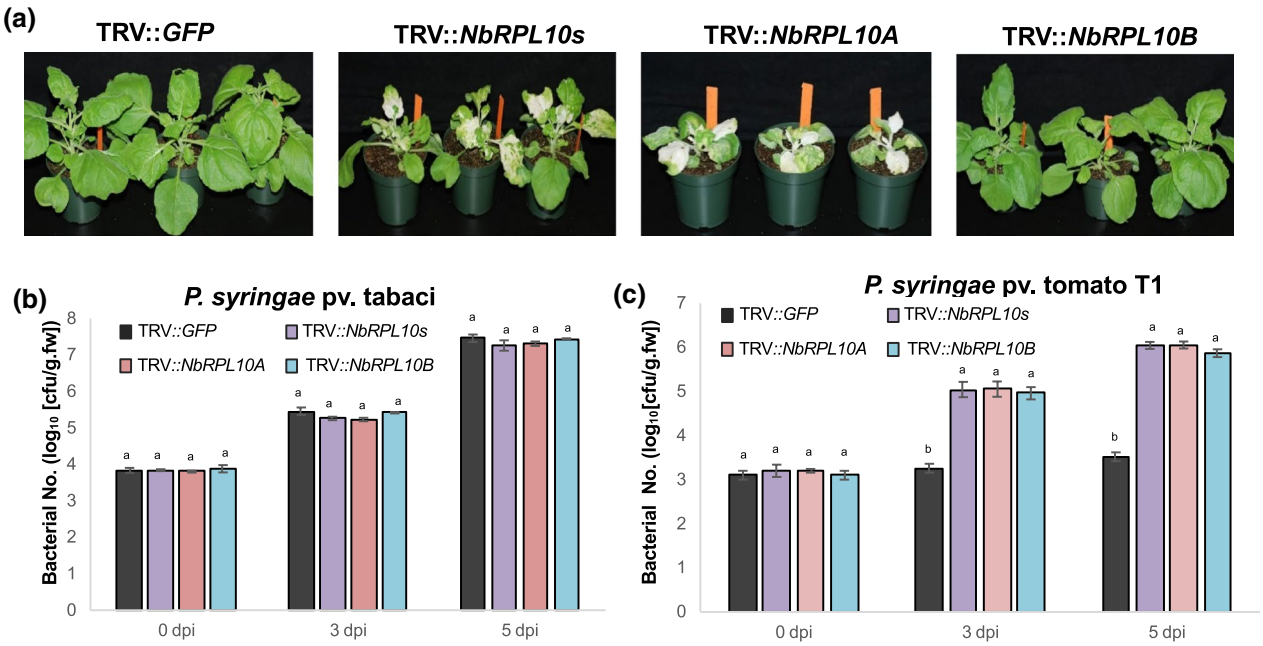

FIGURE 1 Silencing *NbQM/NbRPL10* in *Nicotiana benthamiana* enhances multiplication of nonhost pathogen *Pseudmonas syringae* pv. *tomato* T1. (a) Visualization of developmental changes in *N. benthamiana* plants individually inoculated with TRV::*NbRPL10s* (silences both *NbRPL10A* and *NbRPL10B*), TRV::*NbRPL10A*, TRV::*NbRPL10B*, and TRV::*GFP* (control; *GFP* does not have any sequence similarity to plant DNA and therefore will not cause gene silencing). Three weeks after TRV inoculation, *N. benthamiana* plants were vacuum-infiltrated with host pathogen *P. syringae* pv. *tabaci* or nonhost pathogen *P. syringae* pv. *tomato* T1 at 10^4 cfu/ml concentration. Photographs were taken 3 days postinoculation (dpi). (b) and (c) Quantification of host and nonhost bacterial multiplication in TRV::*NbRPL10*-silenced and TRV::*GFP* inoculated plants at 0, 3, and 5 dpi. Bars represent average values of three biological replicates and experiments were repeated three times with similar results. Error bars indicate standard error. Different letters above the bars indicate a significant difference from two-way analysis of variance at $p < .05$ with Tukey's HSD means separation test ($\alpha = .05$) within a time point among respective control and silenced plants.

treatment. Following this group, the genes that encode proteins having catalytic, biosynthetic, and transferase activities were highly represented among DEGs. Interestingly, the expression of genes associated with morphology, cell proliferation, nuclear chromosomes, and embryonic development were not affected (Figure S2a,b).

In the *NbRPL10A*-silenced plants, 2,928 and 3,134 genes were up- or down-regulated, respectively, when compared to the control plant without any pathogen inoculation. On nonhost pathogen infection (12 hr postinoculation, hpi), 2,844 and 2,708 genes were up- or down-regulated, respectively, in *NbRPL10*-silenced plants when compared to control. There were 619 and 577 genes that were up- or down-regulated commonly between 0 and 12 hpi samples (Figure 2a). We compared the DEGs to assess the differences in control and *NbRPL10A*-silenced *N. benthamiana* plants in response to nonhost pathogen *P. syringae* pv. *tomato* T1. Interestingly, we observed that more genes were differentially regulated in response to the nonhost pathogen in control plants than the *NbRPL10*-silenced plants (Figure 2b). These results suggest that *NbQM/NbRPL10A*-silenced plants were muted to some extent in inducing defence responses when compared to control. The data also suggest that the expression of these genes could be directly or indirectly regulated by NbQM/NbRPL10.

2.3 | Genes involved in pathogen signalling and protein translational processes were differentially expressed in *NbQM/NbRPL10A*-silenced *N. benthamiana* plants

The DEGs from the transcriptome profiling experiment described above were mapped using MapMan analysis to identify the key pathways that are differentially regulated due to *NbQM/NbRPL10A* silencing in *N. benthamiana* and to identify the pathways responsible for compromised disease resistance against nonhost pathogen *P. syringae* pv. *tomato* T1 (Figure 2c,d). Many genes associated with abiotic stress response, redox state, signalling, proteolysis, and cell wall mechanisms were up-regulated, indicating that the plants were experiencing stress in *NbRPL10A*-silenced plants without the nonhost pathogen infection compared to nonsilenced control plants (Figure 2c). The redox genes like *Thioredoxin*, *Ascorbate peroxidase*, and *Glutaredoxin* were up-regulated more than 2.5-fold, and others like *Dehydroascorbate reductase*, *2-oxoglutarate dependent dioxygenase*, and so on were up-regulated more than 2-fold. Furthermore, the transcription factor-encoding genes *WRKY41*, *WRKY53*, *MYB*, and *Dof Zinc finger* were up-regulated more than 3-fold. In addition, leucine-rich repeat (LRR) genes, MAP kinase-encoding genes,

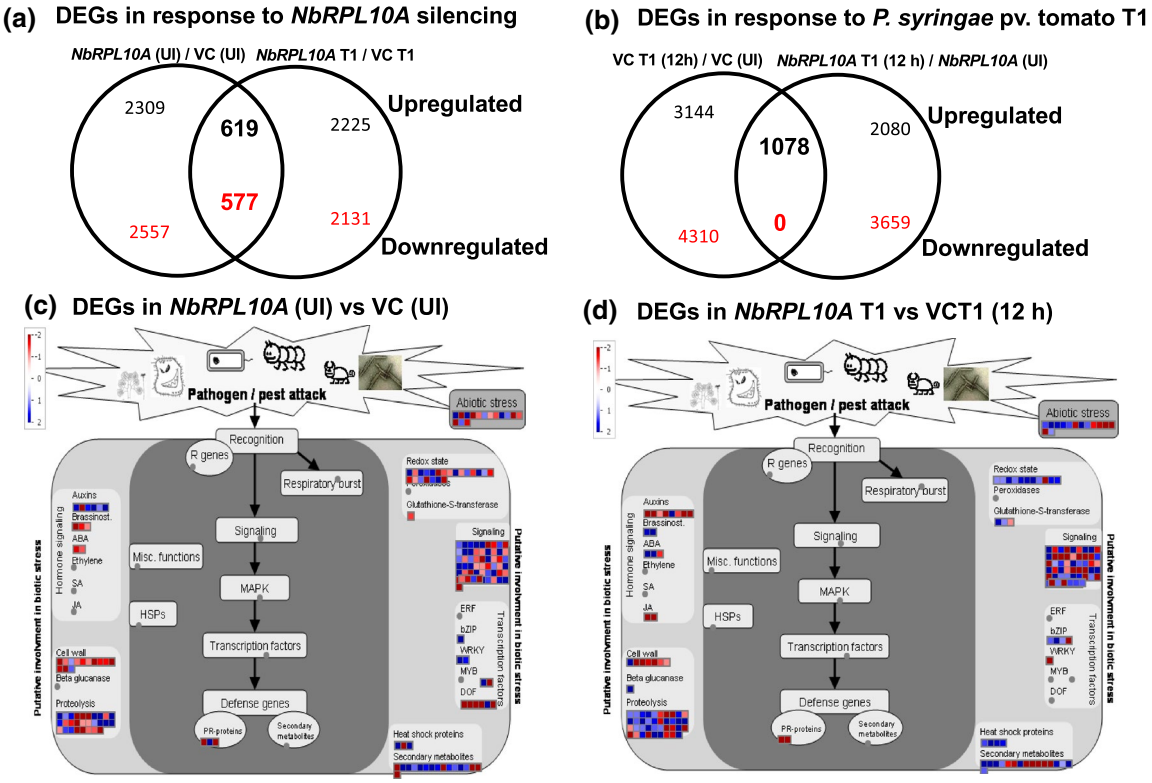

FIGURE 2 Transcriptome analysis of *NbRPL10A*-silenced *Nicotiana benthamiana* plants treated with nonhost pathogen. Two-week-old *N. benthamiana* plants were inoculated with TRV::*GFP* (vector control-VC, UI- uninfected) or TRV::*NbRPL0A* constructs. Three weeks after TRV inoculation, the plants were vacuum-infiltrated with nonhost pathogen *Pseudomonas syringae* pv. *tomato* T1 at 10^4 cfu/ml concentration. Leaf samples were collected 12 hr after pathogen inoculation, RNA was isolated and subjected to RNA-Seq experiment. (a) Venn diagram showing up- and down-regulated genes in *NbRPL10A*-silenced plants over vector control uninfected (VC UI) samples and 12 hr after pathogen treatment. (b) Venn diagram showing up- and down-regulation of genes in *NbRPL10A*-silenced or VC plants 12 hr after pathogen treatment compared to uninfected. (c) MapMan analysis of differentially regulated genes in uninfected *NbRPL10A* silenced plants compared to uninfected VC. (d) Differential expression of genes in *NbRPL10A*-silenced plants compared to VC 12 hr after nonhost pathogen inoculation

and genes involved in calcium signalling were up-regulated more than 2-fold (Data S1). Interestingly, when the *NbRPL10A*-silenced plants were infected with a nonhost pathogen, the transcripts of genes that are associated with abiotic stress (early responsive to dehydration [*ERD3*, *ERD15*], *Dehydration-responsive element-binding* [*DREB*], *basic zipper* [*bZIP*], and *WRKY41*), redox state (*Glutaredoxin*), proteolytic mechanisms (genes encoding C3HC4-type RING finger family and LysM domain containing proteins), phytohormone signalling (*Lipoxygenase LOX3*, *Allene oxide synthase- AOS*, *Biogenic amine synthase BAS1*), pathogen defence (genes encoding LRR, trypsin and protease inhibitor family proteins) and cell-wall-associated genes (*UDP-gluconic acid deacetylase*, *Epimerase*, *Lyase* and a gene-encoding polygalactorunase inhibiting protein) were significantly less when compared to nonsilenced control (Data S1). These results suggest that, unlike the nonsilenced control, *NbRPL10A*-silenced plants were not able to induce expression of several stress- or defence-associated genes upon infection with a nonhost pathogen. This could be due to the regulatory role of QM/RPL10 in controlling the expression of many genes associated with stress or defence response.

Furthermore, we identified 17 up-regulated and 13 down-regulated genes associated with plant defence responses in uninfected *NbRPL10A*-silenced plants compared to nonsilenced control plants. Upon nonhost pathogen infection, nine defence-related genes were up-regulated and 17 defence-related genes were down-regulated in *NbRPL10A*-silenced plants compared to nonsilenced control (Figure 3a and Data S2). As expected, many DEGs between the *NbRPL10A*-silenced plant and the nonsilenced control plant were associated with protein translation mechanisms (Figure 3 and Table 1), with 238 up-regulated and 855 genes down-regulated in uninfected *NbRPL10A*-silenced plants compared to the control. Upon nonhost pathogen infection, there were 148 translation-associated genes up-regulated and 286 genes down-regulated in *NbRPL10A*-silenced plants compared to the control (Table 1 and Data S2).

In *NbRPL10A*-silenced plants without any pathogen infection, the transcripts that are involved in organelle organization, cellular metabolic process, oxidation reduction, small molecule metabolic process, response to diverse stimulus, and signalling genes were highly represented in the down-regulated gene set compared to the control (Figure S3). Similarly, genes that are involved in cell death, regulation of immune system process, positive regulation of biological organellar process, ribonucleoprotein complex, protein complex biogenesis, photosynthesis, secondary metabolite synthesis, and so on were up-regulated in uninfected *NbRPL10A*-silenced plants (Figure S4). Upon inoculation with nonhost pathogen *P. syringae* pv. *tomato* T1, genes involved in cell death, regulation of immune response, signalling, response to diverse stresses, flavonoid synthesis, redox mechanisms, and salicylic acid (SA) and jasmonic acid (JA) metabolism were highly represented in the down-regulated set in *NbRPL10A*-silenced plants when compared to the control (Figure S5). The genes that are involved in systemic acquired resistance and redox metabolism were also represented in the up-regulated gene set in *NbRPL10A*-silenced plants on nonhost pathogen infection compared to the control, but were fewer in number compared to down-regulated genes (Figure S6). The transcriptome data clearly suggest that QM/RPL10 regulates several genes involved in plant defence signalling and translation mechanisms (Figure S7). The transcriptome data further revealed down-regulation of *Phytoene synthase* and *Phytoene desaturase* genes in *NbRPL10A*-silenced plants when compared to

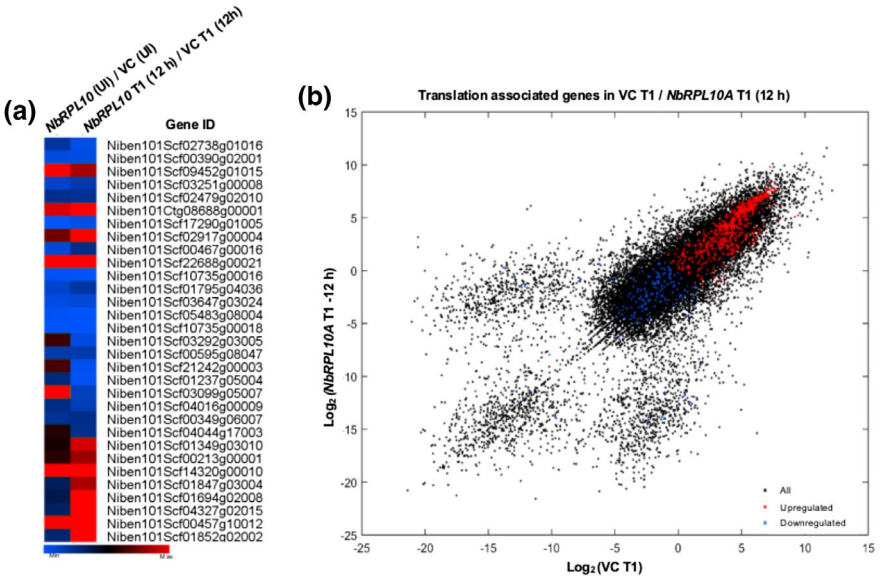

FIGURE 3 Plant defence and protein translation-associated genes that are differentially expressed in *NbRPL10A*-silenced *Nicotiana benthamiana* plants. A select number of genes from the data presented in Figure 2 is shown here. (a) Heat map showing differentially expressed genes that are known to play a role in plant defence. The map was generated using multiple expression viewer (MeV). (b) Distribution of differentially expressed protein translation-associated genes from the whole genome RNA-Seq data. A total of 6,817 genes (black dots) were used for analysis, out of which 419 genes were up-regulated (red) and 494 genes were down-regulated (blue) in *NbRPL10A*-silenced plants

TABLE 1 Differentially expressed genes related to defence and translation mechanisms

Processes		NbRPL10 (UI)/VC (UI)	NbRPL10 A T1/VC T1 (12h)	VC T1 (12h)/VC (UI)	NbRPL10A T1 (12h)/NbRPL10 (UI)
Defence response	Up-regulated	17	9	23	31
	Down-regulated	13	17	13	16
Translation	Up-regulate	238	148	201	208
	Down-regulated	855	286	527	33
Total	Up-regulated	2,927	2,843	4,221	3,157
	Down-regulated	3,133	2,707	4,310	3,659

the control, which correlates with the photobleaching phenotype observed in silenced plants (Figure 1a).

2.4 | Overexpression of *AtRPL10A* in *Arabidopsis* reduces susceptibility to host bacteria and RNAi, and mutants show compromised resistance to nonhost pathogens

To determine if the function of QM/RPL10 in nonhost disease resistance is conserved in other plant species, we identified *qm/rpl10 Arabidopsis* mutants and generated *AtRPL10A* overexpression lines in *Arabidopsis*. We also generated RNAi lines that can silence all the family members (*RPLs*) and lines that can specifically silence *AtRPL10A* or *AtRPL10B*. Homozygous lines were made for all transgenic and mutant lines, and gene overexpression or down-regulation was confirmed by quantitative reverse transcription PCR (RT-qPCR) (Figure S8). *Arabidopsis Atrpl10b* mutants were embryo-lethal. Therefore, *AtRPL10B RNAi* lines were generated. All the *Arabidopsis* mutants and transgenic lines mentioned above along with wild-type (Col-0) were inoculated with nonhost pathogens *P. syringae* pv. *tabaci*, *P. syringae* pv. *tomato* T1, and host pathogen *P. syringae* pv. *tomato* DC3000 by flood inoculation using 10^5 cfu/ml bacterial concentration. Overexpression of *AtRPL10A* resulted in less accumulation of host pathogen *P. syringae* pv. *tomato* DC3000 and produced fewer disease symptoms (Figure 4a,b). Similar to *N. benthamiana* results, *AtRPL10A*, *AtRPL10B*, and *AtRPLs* RNAi lines were susceptible to nonhost pathogen (Figure 4a,c,d). In addition, *Atrpl10a* and *Atrpl10c* mutants were also susceptible to nonhost pathogens *P. syringae* pv. *tabaci* and *P. syringae* pv. *tomato* T1 (Figure 4a,c,d). These results suggest that QM/RPL10 also plays a positive role in *Arabidopsis* nonhost resistance against bacterial pathogens.

2.5 | RPL10 interacts with other ribosomal proteins involved in translation mechanisms

To understand the molecular mechanism of QM/RPL10-mediated nonhost resistance, the *Arabidopsis AtRPL10A* was cloned into yeast two-hybrid (Y2H) bait vector pDEST32 and screened against the *Arabidopsis* stress-induced library (Lee *et al*., 2017). Several putative RPL10A-interacting proteins were identified from this screen (Table S2). Among them, chloroplast, cytoplasmic-intracellular-component-associated, and ribosomal proteins were identified. Many of these proteins have binding function, enzymatic activity, and other molecular functions (Figure S9). The ribosomal proteins such as AtRPL23, AtRPL30, AtRPS30, and RNA-recognition motif (AtRRM) were found to interact with AtRPL10A in Y2H assay. Furthermore, to understand the role of protein translational mechanisms in plant defence, *Arabidopsis* ribosomal protein-encoding full-length genes of *AtRPL23*, *AtRPL30*, *AtRPS30*, and *AtRRM* were cloned in pDEST22 and we confirmed their encoded protein interaction with AtRPL10A by Y2H assay. The X-Gal assay on triple-dropout media further confirms their interaction (Figure 5a). Furthermore, the *Arabidopsis* proteins interacting with AtRPL10A were predicted by in silico analysis using STRING protein–protein interaction networks by functional enrichment analysis (https://string-db.org). The analysis indicated that several ribosomal proteins potentially interact with AtRPL10A along with the identified proteins from Y2H assay (Figure 5b).

The AtRPL10B- and AtRPL10C-interacting proteins were also predicted (Figure S10a,b), and we found that 13 common proteins, including AtRPL23, interact with all the RPL10s, that is, 10A, 10B, and 10C (Figure S10c and Table S3). Three proteins are common between RPL10A and 10B, seven are common with 10A and 10C, and eight are common with 10B and 10C. A few unique proteins were also predicted to specifically interact with individual RPL10 proteins (Figure S10 and Table S3), suggesting their independent functions. These observations are consistent with a peptidyl transferase centre in the ribosome structure where RPL10 is found to play a role in assembly and rotation of the ribosome and therefore has to interact with many proteins. Based on the ribosome structure, several proteins, such L3, L5, L12, L20, L21, L23, L40, NMD3, and a few others, are in close proximity to RPL10. Some of these proteins were identified in our in silico and Y2H analyses (Tables S2 and S3).

2.6 | Silencing of QM/RPL10-interacting ribosomal protein-encoding genes in *N. benthamiana* showed varied response to nonhost pathogen infection

To evaluate if the *N. benthamiana* homologs of *Arabidopsis* QM/RPL10-interacting proteins play a role in nonhost disease resistance, we identified *N. benthamiana* cDNA clones from VIGS phenomics and the functional genomics database (Senthil-Kumar *et al*.,

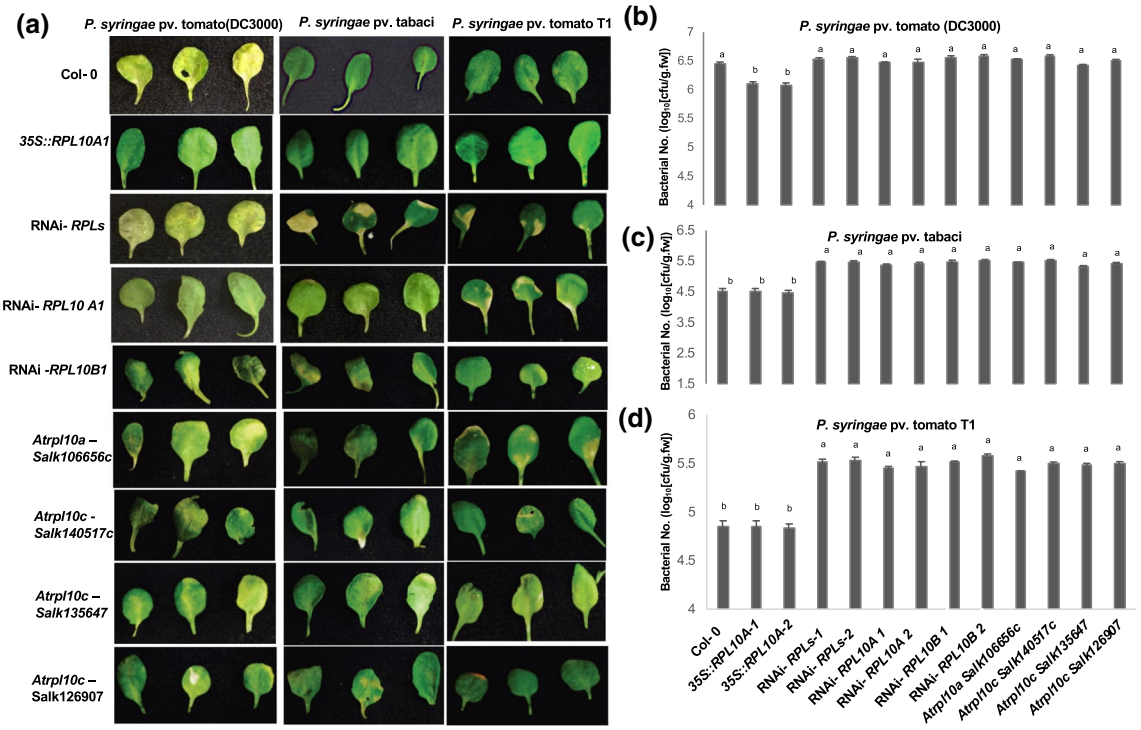

FIGURE 4 *AtRPL10* RNAi and *Atrpl10* mutant plants compromise nonhost disease resistance, and *AtRPL10A* overexpression plants show less susceptibility to host pathogen. Four-week-old *Arabidopsis AtRPL10A* overexpressors (35S::*AtRPL10A-1* and 35S::*AtRPL10A-2*), *AtRPL10s* RNAi (RNAi-*AtRPL10s-1*and RNAi-*AtRPL10s*-2), *AtRPL10A* RNAi (RNAi- *AtRPL10A-1* and RNAi-*AtRPL10A*-2), *AtRPL10B* RNAi (RNAi-*AtRPL10B-1* and RNAi-*AtRPL10B*-2), *rpl10a* mutant (Salk106656c), *rpl10c* mutants (Salk140517c, Salk135647, and Salk126907), and wild-type Col-0 were flood-inoculated with host pathogen *Pseudomonas syringae* pv. *tomato* DC3000 or nonhost pathogens *P. syringae* pv. *tabaci* or *P. syringae* pv. *tomato* T1 at 10^5 cfu/ml concentration. (a) Photographs of a few representative lines showing disease symptoms after inoculation with host and nonhost pathogens. Photographs were taken 3 days postinoculation (dpi). (b)–(d) Bacterial titre was assessed at 3 dpi from leaves of seedlings that were flood-inoculated with host and nonhost pathogens. Error bars represent the standard error for three biological replicates in three independent experiments. Different letters above the bars indicate a significant difference from two-way analysis of variance at $p < .05$ with Tukey's HSD means separation test ($\alpha = .05$)

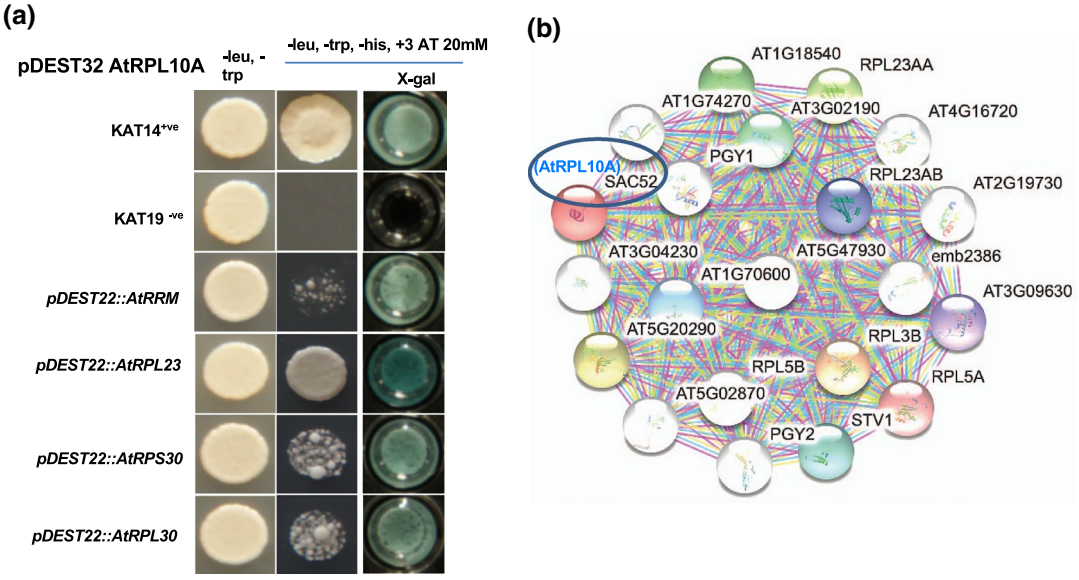

FIGURE 5 Identification of AtRPL10A-interacting proteins. (a) Yeast two-hybrid (Y2H) assay to confirm the protein–protein interactions of a few selected proteins that were identified from the Y2H library screen. The *AtRPL10A* full-length gene cloned in pDEST32 and *AtRPL23*, *AtRPL30*, *AtRPS30*, and *AtRRM* genes were cloned in pDEST22 and cotransformed into MaV203 yeast cells, then were grown on double (−leu, −trp) or triple (−leu, −trp, −his with 20 mM 3-AT) drop-out media. Krev1 RalGDS and Krev1 RalGDS-m1 were used as positive and negative controls, respectively. The X-Gal staining was done to confirm the interaction of the two proteins. (b) Representative image showing in silico prediction of AtRPL10A-interacting proteins using STRING online tool (https://string-db.org)

2018). *NbTI01C11* (*NbRPL30*), *NbME01F11* (*NbRPL23*), *NbME14G06* (*NbRPS30*), and *NbME39D07* (*NbRRM*) clones were recovered from the library, and VIGS was performed in 4-week-old *N. benthamiana* plants. Silencing of *NbRPL23* caused a variegated phenotype, and other selected genes when silenced caused stunted growth (Figure 6a). The silenced plants showed more than 50% down-regulation of their respective transcripts (Figure 6b). These plants were vacuum-infiltrated with host pathogen *P. syringae* pv. *tabaci* or with the nonhost pathogen *P. syringae* pv. *tomato* T1 at 10^4 cfu/ml concentration (Figure 6b). The *N. benthamiana*-silenced plants did not show any difference in host bacterial multiplication when compared to control plants (Figure 6c). However, all the silenced plants showed significantly higher multiplication of nonhost pathogen *P. syringae* pv. *tomato* T1 when compared to the nonsilenced control (Figure 6d). These results suggest that most of the ribosomal proteins that potentially interact with QM/RPL10 also play a role in nonhost resistance against a bacterial pathogen in *N. benthamiana*.

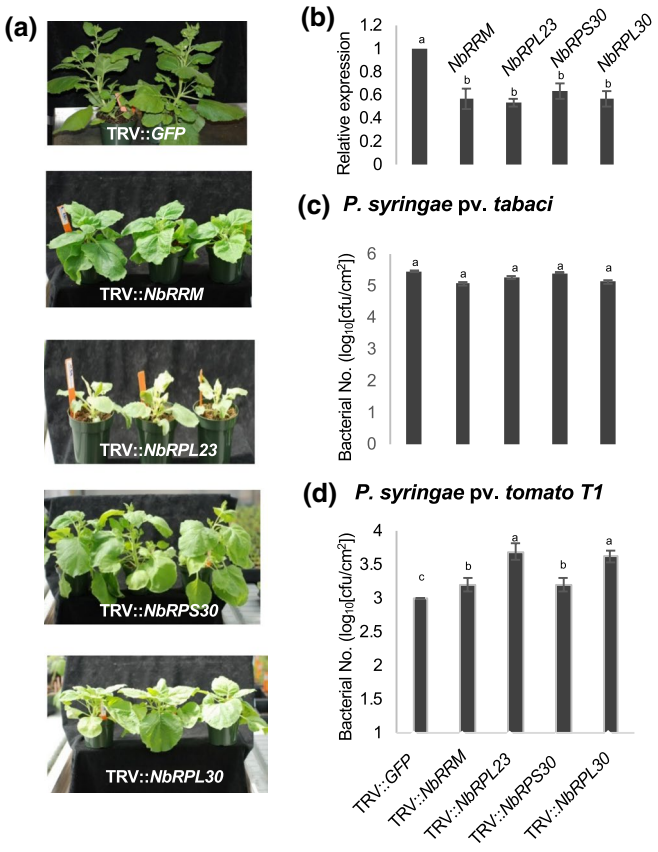

FIGURE 6 Silencing of RPL10A-interacting ribosomal protein-encoding genes in *Nicotiana benthamiana* compromises nonhost disease resistance. (a) Altered developmental phenotypes (dwarf, variegated, etc.) of *N. benthamiana* 3 weeks after inoculation with TRV::*NbRPL23* or TRV::*NbRPL30* or TRV::*NbRPS30* or TRV::*NbRRM* or TRV::*GFP* (control). (b) Expression of respective genes in specific virus-induced gene silenced *N. benthamiana* plants using quantitative reverse transcription PCR (c) Bacterial multiplication rate of host pathogen and (d) nonhost pathogen at 3 days postinoculation (dpi) on silenced plants. Three weeks after TRV inoculation, *N. benthamiana* plants were vacuum-infiltrated with host pathogen *Pseudomonas syringae* pv. *tabaci* and nonhost pathogen *P. syringae* pv. *tomato* T1 at 10^4 cfu/ml concentration. Bars represent the average of three biological replicates. Experiments were repeated three times with similar results. Error bars indicate standard error. Different letters above the bars indicate a significant difference from two-way analysis of variance at $p < .05$ with Tukey's HSD means separation test ($\alpha = .05$)

2.7 | *Arabidopsis* QM/RPL10-interacting ribosomal protein-encoding mutants show compromised resistance to nonhost pathogens

The *Arabidopsis* mutant lines *Atrpl23*, *Atrpl30*, and *Atrrm* were obtained from the Arabidopsis Biological Resource Center and were made homozygous. These mutants, along with the wild type (Col-0), were flood-inoculated with host pathogen *P. syringae* pv. *tomato* DC3000 and nonhost pathogen *P. syringae* pv. *tomato* T1 or *P. syringae* pv. *tabaci* at 10^5 cfu/ml concentrations. *Arabidopsis rpl23c*, *rrm*, and *rpl30* mutants showed slightly reduced disease symptoms when treated with host pathogen *P. syringae* pv. *tomato* DC3000 (Figure 7a). However, the bacterial multiplication assay did not show any significant difference when compared to the wild type (Figure 7b). On the contrary, all the tested mutants showed compromised nonhost resistance against *P. syringae* pv. *tomato* T1 and *P. syringae* pv. *tabaci* (Figure 7a). The mutants showed increased bacterial multiplication with both the nonhost pathogens tested when compared to the wild type (Figure 7c,d). Taken together, these results suggest that in addition to QM/RPL10, some of their interacting proteins are also required for nonhost disease resistance against bacterial pathogens in *N. benthamiana* and *Arabidopsis*.

3 | DISCUSSION

Plant immunity against pathogens relies on the capacity of plant cells to detect pathogens and activate defence responses. Plants do not possess specialized defence cells, as most animals do. Some bacterial pathogens have evolved to evade plant defence by hijacking host cellular machinery to cause disease (Wang *et al*., 2007; Jones *et al*., 2016). However, the majority of potential plant pathogens (nonhost pathogens) cannot infect most plants due to a broad-spectrum nonhost disease resistance mechanism (Heath, 2000; Mysore and Ryu, 2004). Using a VIGS-based forward genetics screen, we identified and characterized several genes involved in nonhost disease resistance (Rojas *et al*., 2012; Senthil-Kumar and Mysore, 2012; Wang *et al*., 2012; Kaundal *et al*., 2017; Lee *et al*., 2017). Previously, we reported that when silenced, two ribosomal genes *NbRPL12* and *NbRPL19* compromised nonhost disease resistance against *P. syringae* pv. *tomato* T1 (Nagaraj *et al*., 2016). In addition, many ribosomal protein-encoding genes have been shown to be important for gene-for-gene mediated resistance using VIGS-mediated forward genetic screens (Lu *et al*., 2003; Gabriels *et al*., 2006). Many plant defence-related transcriptome profiles show differential regulation of

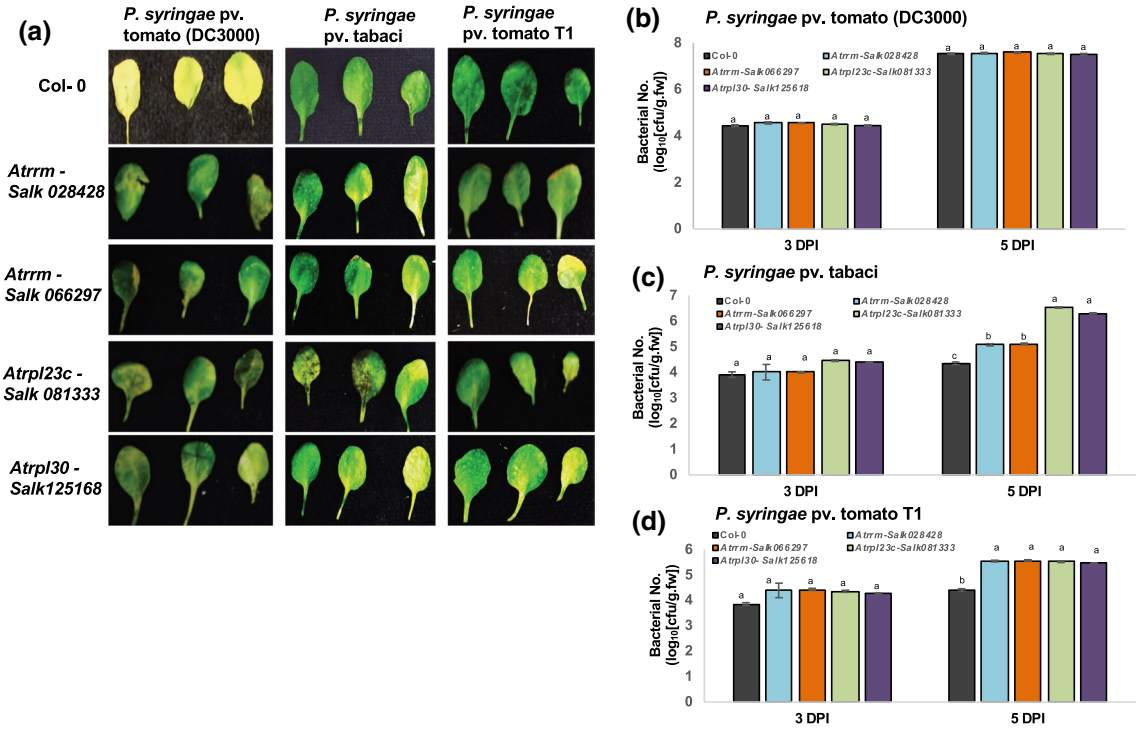

FIGURE 7 Response of *Arabidopsis* RPL10A-interacting ribosomal protein-encoding gene mutants to host and nonhost pathogens. Four-week-old *Arabidopsis* mutants *Atrrm*, *Atrpl23*, and *Atrpl30* were flood inoculated with nonhost pathogens *Pseudomonas syringae* pv. *tabaci*, *P. syringae* pv. *tomato* T1, and host pathogen *P. syringae* pv. *tomato* DC3000 at 10^5 cfu/ml concentration. (a) Leaves of host and nonhost pathogen inoculated *Arabidopsis* mutants and wild-type (Col-0) were detached 3 days postinoculation (dpi) and photographs were taken. (b)–(d) Bacterial accumulation at 3 and 5 dpi was measured from leaves that were floodinoculated with *P. syringae* pv. *tomato* DC3000 or *P. syringae* pv. *tabaci* or *P. syringae* pv. *tomato* T1. Bars represent the average of three biological replicates in three independent experiments. Error bars represent the standard error. Different letters above the bars indicate a significant difference from two-way analysis of variance at $p < .05$ with Tukey's HSD means separation test ($\alpha = .05$) within a time point among respective wild-type and mutant lines

ribosomal protein-encoding genes (Vemanna *et al*., 2019; Solano-De La Cruz *et al*., 2019). However, the role of ribosomal proteins in plant defence was not further characterized.

The involvement of protein translation machinery in plant defence is poorly studied. Some reports have shown that many ribosomal proteins are known to play extraribosomal functions, such as the involvement in plant–bacteria or plant–virus interactions (Gabriels *et al*., 2006; Yang *et al*., 2009). We identified *NbME23C12*, encoding QM/RPL10, in *N. benthamiana* that when silenced showed compromised nonhost disease resistance against *P. syringae* pv. *tomato* T1. The VIGS fragment predicted for *NbRPL10A* using newly designed webserver PssRNAit has identified fewer and precise off-targets (Ahmed *et al*., 2020). The RNA-Seq analysis revealed several defence-related DEGs in *NbRPL10*-silenced plants in the response to the nonhost pathogen *P. syringae* pv. *tomato* T1. For example, *Flagellin sensitive 2* (*Fls2*) homolog was down-regulated in *NbRPL10*-silenced plants when compared to nonsilenced control. Arabidopsis *fls-2* mutant is hypersusceptible to the host pathogen *P. syringae* pv. *tomato* DC3000 (Zheng and He 2010). *Arabidopsis fls-2* mutant has also been shown to compromise nonhost resistance against several bacterial pathogens (Ishiga *et al*., 2011). We speculate that PAMP-mediated plant defence is compromised in *NbRPL10A*-silenced plants. RNA-Seq data also indicated that many Ca^{2+} signalling-associated genes were differentially regulated in *NbRPL10*-silenced plants when compared to the control. Genes such as *Calcium-dependent protein kinase* (*CDPK* or *CPK)6*, *CPK3*, and genes encoding the calmodulin binding EF motif family were up-regulated, and genes like *CPK13*, *CDPK19*, *Salt overlay sensitive* (*SOS3*), *Receptor lectin kinase* (*RLK*), *Wall associated kinase* (*WAK2*), and a gene-encoding calmodulin-related binding protein were significantly down-regulated in *NbRPL10*-silenced plants. The PAMP signalling genes were induced at early stages of infection and subsequently down-regulated in *NbRPL10*-silenced plants with respect to nonhost pathogen. This is consistent with calcium signalling, where it is up-regulated in response to PAMPs at early stages of infections and subsequently declines to a steady state (Blume *et al*., 2000; Ranf *et al*., 2008). We speculate that PAMP-signalling mediated nonhost disease response is compromised in the *NbRPL10*-silenced plants. However, it is intriguing that despite down-regulation of PAMP signalling genes, the *NbRPL10*-silenced plants did not compromise basal resistance to the host pathogen. Perhaps silencing of these genes is not enough to compromise basal resistance against the host pathogen but sufficient to compromise nonhost resistance.

The genes encoding AAA^+-ATPase family proteins were up-regulated 10-fold in *NbRPL10*-silenced plants, which could lead to dissociation of macromolecular complexes such as H^+-ATPase involved in defence response (Kaundal *et al*., 2017). Multiple MAP kinases were

down-regulated in *NbRPL10*-silenced plants, and transcription factor-encoding genes like *WRKY41* and *C3H4-Zinc* finger family genes were also down-regulated. Down-regulation of *Egg binding receptor (EBR1)* or *C3H4 type ring finger E3 ligase* led to a hypersensitive response against bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (You *et al*., 2016). Down-regulation of *Suppressor of acaulis (SAC) 51* encoding bHLH transcription factor in *sac52/rpl10* mutants resulted in a dwarf phenotype in *Arabidopsis* (Imai *et al*., 2008), indicating that RPL10 has an extraribosomal role in the regulation of transcription factors.

The phytohormone JA precursor enzyme LOX3- and AOS-encoding genes are highly down-regulated in response to nonhost pathogen *P. syringae* pv. *tomato* T1. We expect that if JA is low, SA could accumulate to provide resistance against biotrophic and hemibiotrophic pathogens (Zhang *et al*., 2018). However, *NbRPL10*-silenced plants showed compromised nonhost disease resistance against the hemibiotrophic pathogen *P. syringae* pv. *tomato* T1, suggesting that the phytohormone-independent defence pathway is regulated by *NbRPL10*. In addition, we did not find down-regulation of SA biosynthetic or signalling genes in *NbRPL10*-silenced plants. Furthermore, down-regulation of many genes involved in the response to wounding also correlates with the expression of genes in association with systemic acquired resistance or SA-mediated resistance (Park *et al*., 2007). Interestingly, the *Proline dehydrogenase 2* (*PDH2*) gene was down-regulated in *NbRPL10*-silenced plants. Down-regulation of proline biosynthesis genes in *Arabidopsis* and *N. benthamiana* causes compromised nonhost disease resistance (Senthil-Kumar *et al*., 2013). The *pdh* yeast mutants were rescued by *QM* overexpression under paraquat stress (Chen *et al*., 2006). The role of QM/RPL10 in the regulation of proline biosynthesis or metabolic pathway in plants needs further study.

NbRPL10-silenced plants showed a variegated or photobleaching phenotype, which could be due to the down-regulation of *Phytoene synthase* (*Psy1*) and *Phytoene desaturase* (*PDS*) genes (Figure S6). The silencing of *PDS* through VIGS results in a photobleaching phenotype (Senthil-Kumar and Mysore, 2011). Similarly, *Psy1*-RNAi lines of oncidium hybrid orchid showed reduced chlorophyll content (Liu *et al*., 2014). The reduced expression of *pyrabactin* 1 (*PYR1*), an ABA receptor, in response to nonhost pathogen *P. syringae* pv. *tomato* T1 correlates with the expression of *PDS1* and *PSY1* in *NbRPL10*-silenced plants. The down-regulation of *PYR1* in *NbRPL10*-silenced plants may disrupt immune responses associated with ABA signal transduction and could occur at the level of Ca^{2+} signalling (Kim *et al*., 2010). Genes involved in Ca^{2+} signalling were also down-regulated in *NbRPL10*-silenced plants. Other defence-associated genes such as *MLO-like protein 6*, *MLP-like 43*, and *Defensin* genes were also down-regulated in *NbRPL10*-silenced plants in response to nonhost pathogen. In contrast, genes encoding peptidyl-prolyl-cis-trans isomerases were up-regulated in *NbRPL10*-silenced plants. In *Arabidopsis*, elevated expression of *Peptidyl-prolyl-cis-trans isomerases* showed defence against *Xanthomonas campestris* infection (Mokryakova *et al*., 2014).

Approximately 900 genes associated with protein translation mechanisms were differentially regulated in *NbRPL10*-silenced plants, indicating that QM/RPL10 could be a central regulator of gene expression as well as of protein translation mechanisms. Eukaryotic elongation, initiation factors, ribosomal proteins *L-29*, *L-30*, *L-23*, *L-3*, *S-4*, *S-3*, *L-38*, *EIF-3C*, *L-5*, *S-4*, *S-11*, *S-30*, *S-17*, *S-13*, *L-5*, *L-17*, *L-2*, *L-24*, *S-13*, and so on were highly down-regulated with or without pathogen responses in *NbRPL10*-silenced plants. Similar observations were reported in *F. oxysporum* f. sp. *vanillae* infection in vanilla (Solano-De La Cruz *et al*., 2019), *X. oryzae* pv. *oryzae* infection in rice (Moin *et al*., 2016; Saha *et al*., 2017; Vemanna *et al*., 2019), and *P. syringae* pv. *tomato* T1 infection in tomato (Mysore *et al*., 2002) wherein genes associated with ribosomal proteins were up-regulated. These data substantiate that ribosomal proteins play an intrinsic role in plant defence mechanisms. It is possible that QM/RPL10 plays a transcriptional role in regulating translational mechanisms and defence-associated genes. The interaction of QM/RPL10 with MYB transcription factor and regulation of defence genes against virus infection (Carvalho *et al*., 2008; Zorzatto *et al*., 2015) substantiates this theory. The *Arabidopsis rpl10a* mutant plants showed reduced translation efficiency, and also specific protein translation is affected in individual *Atrpl10* mutants (Falcon-Ferreyra *et al*., 2010). Based on this report we can hypothesize that the DEGs in the *NbRPL10A*-silenced plants were due to reduced *RPL10A* protein and not due to an unusual ribosome. The gene expression reprogramming in RPL10-R98S mutation has shown profound structural, biochemical, and translational fidelity defects that cause cancer in humans (Sulima *et al*., 2014). Similarly, RPL12 and RPL19 proteins are known to be required for proper functioning of a number of factors involved in ribosome biogenesis and protein synthesis (Plafker and Macara, 2002).

The *Arabidopsis* plants overexpressing *AtRPL10A* showed reduced susceptibility to host pathogen *P. syringae* pv. *tomato* DC3000 when compared to wild-type Col-0. Because the *AtRPL10A* overexpression lines did not confer complete resistance, the infected plants will eventually die but can survive longer than wild-type plants. In contrast, the mutants or RNAi lines of At*RPL10A*, *B*, and *C* compromised resistance against nonhost pathogens *P. syringae* pv. *tabaci* and *P. syringae* pv. *tomato* T1. This further confirms that QM/RPL10 family proteins have conserved mechanisms to confer nonhost resistance against bacterial pathogens in *N. benthamiana* and *Arabidopsis*. We identified RPL30, RPS30, RPL23, and RRM proteins that interact with AtRPL10A. Silencing the homologs of these genes in *N. benthamiana* or *Arabidopsis rpl23c*, *rpl30*, and *rrm* mutants showed higher nonhost pathogen growth when compared to the wild type, suggesting that these ribosomal proteins may have an extraribosomal function, including a role in plant immunity. The nonhost bacterial growth in *NbRPL10*-silenced or *Atrpl10* mutants was higher than in other ribosomal gene-silenced plants. This further confirms that RPL10 is a central regulator of defence and the RPL10A-interacting proteins studied have a minor role in nonhost resistance. Induction of RPL23 with other ribosomal proteins was reported in spinach and resurrection plant *Tortula ruralis* under desiccation stress (Thomas *et al*., 1988; Wood *et al*., 2000). RPL23 promotes multidrug resistance in gastric cancer cells by suppressing drug-induced apoptosis, and overexpression of *RPL23* induces *Glutathione-S transferase* in

yeast (Shi *et al*., 2004). The eukaryotic RPS30 that is conserved from yeast to humans showed antimicrobial activity within the cytosolic fraction. RPS30 peptide fragments kill mycobacteria through autophagy mechanisms (Ponpuak *et al*., 2010). Posttranslation modification (sumoylation) of RPL30 by SUMO protease protein, suppressor of mat3-7 (SMT7), regulates the cell size checkpoint function, supporting the importance of the extraribosomal functions of ribosomal proteins (Lin *et al*., 2020). Glycine-rich protein 7 (GRP7) belonging to the RRM protein family is ADP-ribosylated by the *P. syringae* pv. *tomato* DC3000 secreted effector protein, ADP-ribosyl transferase hopU1. The *Arabidopsis grp7* mutant plants were more susceptible than the wild type against host pathogen *P. syringae* pv. *tomato* DC3000 (Fu *et al*., 2007). Overall, our study clearly demonstrates that QM/RPL10 is involved in the regulation of many genes that are important for bacterial defence and translation mechanisms. Our results provide a novel strategy by altering the expression of ribosomal protein-encoding genes to confer disease resistance in crop plants.

4 | EXPERIMENTAL PROCEDURES

4.1 | Gene constructs, bacterial strains, and plant materials

Agrobacterium tumefaciens GV3101 containing TRV2::*23C12* (*NbME23C12* http://vigs.noble.org) was grown at 28°C in a Luria Bertani (LB) medium supplemented with rifampicin (10 μg/ml) and kanamycin (50 μg/ml). VIGS was carried out by mixing a 1:1 ratio of *Agrobacterium* strains containing TRV1 and TRV2 as described (Senthil-Kumar and Mysore, 2014). *P. syringae* strains were grown in King's B medium at 30°C supplemented with rifampicin (10 μg/ml), kanamycin (50 μg/ml), or streptomycin (50 μg/ml) when needed. The TRV2::*RPL10s*, TRV2::*RPL10A*, TRV2::*RPL10B*, TRV2::*RRM* (*NbME39D07*), TRV2::*RPL23* (*NbME01F11*), TRV2::*RPL30* (*NbTI01C11*), TRV2::*RPS30* (*NbME14G06*), or TRV2::*GFP* constructs were developed and used for VIGS as described (Senthil-Kumar and Mysore, 2014).

Arabidopsis T-DNA insertion mutants *rpl10c*-Salk140517c, Salk135647, Salk126907-1, *rpl23*-Salk 081333, *rpl30*-Salk 099025, *rpl30*-Salk 12246, *rrm*-Salk 028428, and Salk 066297 were obtained from the Arabidopsis Biological Resource Center and identified homozygous lines for further analysis. Full-length *AtRPL10A* cDNA was cloned into a pMDC32 binary vector under the CaMV 35S promoter and RNAi constructs for *AtRPL10A* and *AtRPL10B* were developed in pH7GWIW G2 (II) vector (Karimi *et al*., 2007) and mobilized into *A. tumefaciens* GV3101. The floral dip method of transformation (Clough and Bent, 1998) was used to develop transgenic lines that were screened on hygromycin (25 μg/ml) and Basta (25 μM).

4.2 | Disease assays in *N. benthamiana* and *Arabidopsis*

Three weeks after TRV inoculation, the silenced and nonsilenced plants were vacuum-infiltrated with *GFPuv* (Wang *et al*., 2007) expressing host (*P. syringae* pv. *tabaci*) or nonhost pathogen (*P. syringae* pv. *tomato* T1) and tissue was collected 8 hr postinoculation (hpi). To determine the bacterial titre, leaf samples at 0, 3, and 5 dpi from four biological replicates were collected using a 1 cm^2 core borer. Leaf samples were ground, subjected to serial dilution, plated on King's B agar supplemented with appropriate antibiotics and incubated at 28°C for 2 days for bacterial colony counting.

Flood inoculation assay was used to infect *Arabidopsis* plants. Four-week-old *Arabidopsis* plants grown in Murashige and Skoog (MS) plates were incubated for 1 min with 40 ml of bacterial suspension as described (Ishiga *et al*., 2011). Symptoms were observed after 5 days. To quantify bacteria, the entire rosette was harvested, ground, and serially diluted as described previously (Ishiga *et al*., 2017). Leaf samples from three biological replicates were collected using a core borer and the bacteria were quantified in a similar fashion to that described above for *N. benthamiana*.

4.3 | Differential gene expression analysis by RNA-sequencing

The leaf samples from three biological replicates from 4-week-old *NbRPL10A*-silenced *N. benthamiana* were harvested at different times after infection with *P. syringae* pv. *tomato* T1 and immediately frozen in liquid nitrogen. The total RNA was extracted and RNA samples with high quality (RIN > 7.5 assessed by Agilent 2100 Bioanalyzer) were used for Illumina library preparation following the manufacturer's instructions (Illumina). Paired-end reads were generated from each sample having a minimum of two replicate (eight) libraries sequenced with Illumina 70 HiSeq 2000. After adapter sequences and low-quality reads were removed, the remaining reads were aligned to the annotated *N. benthamiana* reference transcriptome (https://solgenomics.net/organism/1490/view) and the gene-wise raw counts were calculated. The differential analysis was carried out using DE seq analysis. Genes with more than 2-fold change in expression and a *p* value of 0.05 were considered DEGs. Data from all the samples have been uploaded into our GEA universal gene expression atlas platform (https://bioinfo.noble.org/vigs/) for data normalization, visualization, and differential expression analysis using RNA-Seq analysis software. To predict the pathways and functions of DEGs, the *N. benthamiana* RNA-Seq information was BLAST analysed against the *Arabidopsis* genome and the *Arabidopsis* IDs were used for agriGO analysis (http://bioinfo.cau.edu.cn/agriGO/) and MapMan analysis (https://mapman.gabipd.org) to predict the pathways and functions of DEGs.

4.4 | RT-qPCR

Tissue was collected 3 weeks after TRV inoculation to test the down-regulation of *NbRPL10* and other ribosomal protein-encoding gene transcripts in *N. benthamiana*-silenced plants. Leaf tissue was collected 3 weeks after germination to determine the expression of *AtRPL10A*, *AtRPL10B*, and *AtRPL10C* in *Arabidopsis*

wild type (Col-0), *AtRPL10A* overexpression, and RNAi or mutant plants. Total RNA was extracted according to the manufacturer's instructions using a Qiagen total RNA extraction kit. The cDNA was synthesized by oligo (dT) primers using Molony murine leukaemia virus reverse transcriptase (Thermo Fisher Scientific) according to the manufacturer's instructions. The real-time RT-qPCR was performed with a Sigma KicQStart SYBR green kit. The conditions for PCR were as follows: 95°C for 2 min, 25 cycles of denaturation at 94°C for 45 s, annealing for 30 s at 58°C, polymerization for 45 s (72°C), followed by plate reading at 72°C for 5 min, estimation of melting curve from 50°C to 95°C, and incubation at 72°C for 4 min. The primers used in the study are given in Table S1.

4.5 | Yeast two-hybrid assay

Yeast two-hybrid assays were performed following the manufacturer's protocol using the ProQuest Two-Hybrid System (Thermo Fisher Scientific). *AtRPL10A* was fused to the *GAL4* DNA-g binding domain in pDEST32 as the bait construct. The *Arabidopsis* mixed elicitor treated cDNA library was used for screening (Lee *et al*., 2018). Based on the screening the full-length genes of AtRRM1, AtRPL30, AtRPL23, and AtRPS30 were used as prey proteins and fused to the GAL4 activation domain in pDEST22. Bait and prey constructs were cotransformed into yeast MaV203 competent cells. Positive clones were identified by their ability to grow on synthetic defined medium minus leucine/tryptophan/histidine (triple-dropout medium) or leucine/tryptophan/histidine/uracil (quadruple-dropout medium) containing 20 mM 3-aminotriazole. Liquid media contained X-Gal was used to confirm the interactions.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

VIGS clones are available at the Noble Research Institute *VIGS* Database at https://vigs.noble.org/line2.php?id=NbME23C12 as NbME23C12. RNA-Seq data are available at https://bioinfo.noble.org/vigs/ as VIGS: NicotianaBenthamiana_Niben101 Gene Expression Atlas and Network Analysis.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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