

Integrated transcriptome and translome analyses reveal the early regulatory network of *Brassica napus* roots in response to the growth-promoting rhizobacterium *Pseudomonas simiae* WCS417

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Abstract

Interactions between plant roots and complex microbial communities are critical for plant environmental adaptation. *Pseudomonas simiae* WCS417, a Gram-negative plant growth-promoting rhizobacterium (PGPR), is a model organism in plant-microbe interaction research and featured in over 750 studies since the 1990s. However, the transcriptome dynamics induced by WCS417 remain poorly understood. This study employed an integrated multi-omics approach, combining transcriptome (RNA-seq) and translatome (RNC-seq) analyses, to systematically investigate the transcriptional and translational regulatory networks in *Brassica napus* roots during early colonization by WCS417. Our results demonstrate that WCS417 significantly promotes lateral root formation, suppresses primary root elongation, and increases plant biomass. At the molecular level, WCS417 inoculation triggered extensive changes in gene expression and translation at 30 minutes and 6 hours post-inoculation, affecting key processes including phytohormone signaling, cell wall remodeling, immune responses, and abiotic stress adaptation. Notably, although transcript levels of some immune-related genes were downregulated, their translation efficiency was significantly enhanced, suggesting that plants maintain basal immunity while facilitating symbiotic establishment. Furthermore, WCS417 dynamically regulated genes involved in nitrogen/phosphorus uptake and core low-temperature response transcription factors in *Brassica napus* roots. These findings reveal a multi-layered regulatory mechanism by which WCS417 optimizes root system architecture and balances immunity with growth in *Brassica napus*, providing new insights into plant-microbe interactions.

Introduction

Plants, as immobile organisms, have their roots constantly exposed to a highly complex soil microbial environment comprising diverse microbial communities, including beneficial, pathogenic, and commensal microorganisms (Yu et al. 2019b). These microbial interactions have significant impacts on plant growth, development, immune responses, and environmental adaptation. To survive under such dynamic conditions, plants have evolved complex adaptive strategies, among which the selective recruitment of beneficial microbes stands out as a critical mechanism (Zhang et al. 2023; Wang and Song 2022). This process involves the modulation of root morphology and physiological traits in response to microbial stimuli (Vacheron et al. 2018; Contreras-Cornejo et al. 2009). Central to plant-microbe interactions is the recognition of microbe-associated molecular patterns (MAMPs), which trigger precise developmental reprogramming to facilitate symbiotic associations (Hacquard et al. 2017; Stringlis et al. 2018). Notably, these interactions induce dynamic remodeling of root architecture, characterized by the suppression of primary root elongation and the stimulation of lateral root proliferation. Such structural modifications ultimately enhance the plant's capacity for resource acquisition, thereby optimizing water and nutrient uptake efficiency (Vacheron et al. 2018; Motte et al. 2019; Koevoets et al. 2016).

Since its isolation from wheat roots in 1988, *Pseudomonas simine* WCS417 has been recognized as an important plant growth-promoting rhizobacterium (PGPR) (Pieterse et al. 2020). It effectively suppresses

soil-borne diseases caused by fungi, bacteria, and nematodes, such as take-all and Fusarium wilt, while also enhancing tolerance to abiotic stresses, including drought and salinity (Nel et al. 2006; Canchignia et al. 2017; M. Leeman and Schippers 1996; Ran et al. 2005). To date, it has been extensively studied and validated in more than 750 research studies (Pieterse et al. 2020), while the effect of WCS417 on *B. napus* has not been investigated to date.

WCS417 has been shown to markedly alter root system architecture, thereby improving water and nutrient uptake efficiency in many plant species (Aparicio et al. 2025; Zhou et al. 2025; Stringlis et al. 2018). In model plants such as *Arabidopsis thaliana*, WCS417 colonization induces profound remodeling of root architecture, such as inhibition of primary root elongation, enhanced lateral root formation, and promotion of root hair development (Stringlis et al. 2018). These morphological alterations are orchestrated through complex interactions with auxin signaling pathways (Zamioudis et al. 2013). Mechanistic studies reveal that WCS417 employs multiple synergistic strategies to influence plant growth. First, the bacterium synthesizes phytohormone, such as indole-3-acetic acid (IAA), which modulates the expression of auxin response factors (ARFs) to regulate the initiation and development of lateral root primordia (Zamioudis et al. 2013). Second, volatile organic compounds (VOCs) produced by WCS417 induce the expression of root-specific transcription factor MYB72 to regulate root development (Zamioudis et al. 2015; Zamioudis et al. 2014). Furthermore, both VOCs and microbial siderophores produced by WCS417 activate the MYB72-FIT1 signaling module, upregulating the expression of iron acquisition genes (*FRO2* and *IRT1*), thereby improving plant iron nutrition (Verbon et al. 2019; Verbon et al. 2017). Additionally, WCS417 secretions specifically regulate the expression of sugar transporter proteins (SWEET11 and SWEET12), suggesting a sophisticated mechanism for optimizing carbon allocation during symbiotic interactions (Desrut et al. 2020).

In plant roots, translational regulation serves as a critical mechanism of protein expression control that is not only involved in nutrient uptake, developmental processes, and adaptation to abiotic stress, but also plays a central role in root-microbe interactions (Li et al. 2024; Yang et al. 2025; Shang et al. 2025; Du et al. 2025; Wu et al. 2024). Recently developed protoplast-free single-nucleus RNA sequencing (snRNA-seq) has revealed that roots employ spatially partitioned and cell type-specific translational strategies to accurately distinguish beneficial from pathogenic microbes, maintain microbiome homeostasis, and coordinate immune responses. (Yang et al. 2025; Long et al. 2021). In the root meristematic zone, beneficial microbes significantly upregulate the expression of translation-related genes and promote the accumulation of ribosomal proteins and translation factors, thereby facilitating microbe-mediated growth promotion. In contrast, the mature zone employs localized translational mechanisms to activate immune responses, enabling rapid synthesis of defense-related proteins, including those involved in triterpenoid biosynthesis, to counteract pathogen invasion (Yang et al. 2025). This spatially specialized translatability allows the root system to simultaneously promote beneficial symbiosis and effectively suppress harmful infections. Recent advances in cell type-specific ribosome profiling have further elucidated the spatial heterogeneity of translational dynamics in different root zones upon microbial contact, highlighting the key role of translational regulation in root-microbe interactions (Froschel et al. 2021; Liu et al. 2022). Thus, by precisely controlling the spatiotemporal

production of proteins, translational regulation emerges as a core mechanism underlying root-microbial symbiosis, immune balance, and environmental adaptation.

Brassica napus (*B. napus*) is one of the most important oilseed crops worldwide. However, whether WCS417 could regulate root development and disease resistance in *B. napus* remains largely uninvestigated. Furthermore, although translational regulation is critical for microbe-root interactions, systematic studies of the root translome, particularly the molecular mechanisms underlying transient translational responses of plants during early colonization, remain relatively limited. In this study, we employed an integrated multi-omics approach, combining RNA-seq and RNC-seq (Ribosome Nascent-chain Complex sequencing) to systematically investigate the regulatory networks orchestrated by WCS417 at both transcriptional and translational levels during early colonization of *B. napus* roots.

Method

Plant material and growth conditions

Brassica napus seeds were surface sterilized with 75% ethanol for 8–10 min, followed by 50% bleach for another 2 min, and then washed three times with sterilized water. Afterward, seeds were germinated on ½ Murashige and Skoog (MS) agar plates with 0.5% sucrose in a growth chamber (22°C under a 16 h light/8 h dark period with light intensity at $450 \mu\text{molm}^{-2} \text{s}^{-1}$) for 2 days.

For measurement of plant biomass, Seedlings were transferred to ½ MS agar plates or natural soil, and then plants were grown at 21°C growth room under a 16 h light/8 h dark period with light intensity at $450 \mu\text{molm}^{-2} \text{s}^{-1}$.

For the RNC-seq, seedlings were grown in liquid ½ MS medium containing 2% (w/v) sucrose for 10 days (16 h Light/8 h Dark) and transferred to fresh liquid ½ MS medium without sucrose for 2 more days before inoculation.

Cultivation of *Pseudomonas simiae* WCS417 and treatment

Pseudomonas simiae WCS417 was cultured at 28°C on LB agar plates supplemented with $50 \mu\text{g mL}^{-1}$ of rifampicin. After 24 h of growth, cells were collected in 10 mM MgCl_2 , washed twice with 10 mM MgCl_2 by centrifugation for 5 min at 5000 g, and finally resuspended in ddH₂O. For RNC-seq, WCS417 was diluted to OD600 = 0.5 in 10mM MgCl_2 and then added to the liquid rhizosphere to a final OD600 of 0.05. Liquid ½ MS medium supplemented with an equal volume of 10mM MgCl_2 was used as a mock control.

Measurement of plant biomass and parameters of root architecture

For treatment with live WCS417 cells, *Brassica napus* seedlings were germinated on ½ MS agar plates with 1% sucrose. two-day-old *Brassica napus* seedlings were transferred to new agar-solidified ½ MS plates with 1% sucrose with or without WCS417 (final concentration OD₆₀₀ = 0.0005). Ten days after the start of the treatments, shoot fresh weight, primary root length, number of lateral roots, and hypocotyl length were determined.

Ribosome-nascent chain complex (RNC) extraction

RNC extraction was performed as previously described (Wang et al. 2013). with minor modifications. Briefly, roots were ground using a pre-chilled mortar with the addition of 2 mL of cell lysis buffer (200 mM Tris-HCl, pH 7.4, 35 mM MgCl₂, 200 mM KCl, 100 µg/mL cycloheximide, and 1% Triton X-100). After incubation on ice for 30 min, the lysates were centrifuged at 16,200 g for 20 min at 4°C. The supernatants were then layered onto 0.4 mL of a sucrose cushion buffer (1.75 M sucrose in gradient buffer). RNCs were pelleted by ultracentrifugation at 256,000 g for 3 h at 4°C (Merchante et al. 2016).

RNA-seq

The sequencing libraries were constructed following the Fast RNA-seq Lib Prep Kit V2 (ABclonal, #RK20306). Briefly, total RNA was isolated using TRNzol (TIANGEN, #DP424). Then, 1 µg of total RNA or RNC-mRNA was subjected to the polyA + mRNA isolation using the Poly(A) mRNA Capture Module beads (ABclonal, #RK20340) following the manufacturer's instructions. The mRNA was fragmented at 94°C for 15 min to obtain 200–250 bp inserts. First-strand cDNA was synthesized by First Strand Synthesis Enzyme Mix, and double-stranded cDNA was synthesized by Second Strand Master Mix using Second Strand & dA Buffer with dUTP *. The adapters were ligated by Ligase Mix T5. Ligated dscDNAs were purified and then amplified by 16 cycles of PCR with 2x PCR Master Mix and purified using AFTMag NGS DNA Clean Beads (ABclonal, #RK20257). Purified libraries were quantified by Qubit® 2.0 Fluorometer (Thermo Fisher, #Q33238). Libraries were barcoded, pooled, and sequenced in a HiSeq 2000 or 2500 machine (Paired-end 150-bp). High-quality reads that passed the Illumina quality filters were kept for the sequence analysis (Supplementary Table S1).

Data analysis

Both RNA-Seq and RNC-Seq reads were first processed using fastp (v1.01) to remove adapter sequences (Chen 2025). The resulting clean reads were then aligned to reference sequences of rRNA, tRNA, and other non-coding RNAs of *B. napus*, obtained from the Ensembl database, using Bowtie2 (v2.5.4) (Dyer et al. 2025; Langmead and Salzberg 2012) to eliminate the contaminations. Subsequently, the filtered RNA-Seq and RNC-Seq reads were mapped to the *B. napus* reference genome (ZS11 v0), downloaded from the BnIR database, using STAR (v2.7.11b) (Song et al. 2020; Yang et al. 2023; Dobin et al. 2013). Gene expression quantification was performed using the featureCounts tool within the Subread package (v2.0.2) (Liao et al. 2013).

Quality assessment of the processed datasets, including principal component analysis (PCA), enzymatic bias evaluation, gene coverage analysis, and sample reproducibility, was carried out using RiboShiny (v0.1), an integrated platform designed for comprehensive translation data analysis and visualization (Ren et al. 2025).

To identify differentially expressed genes (DEGs), the DESeq2 package (v1.4) in R was applied using the likelihood ratio test, followed by shrinkage of log₂ fold changes (LFC) via the lfcShrink function with the ashR estimator (Love et al. 2014). Genes with an adjusted *p*-value (*P*_{adj}) < 0.05 and |log₂(LFC)| ≥ 0.585 were classified as differentially transcriptionally regulated. Translation ratios (TR) and differentially translated genes (DTRGs) were identified using the RiboShiny (v0.1) pipeline.

Gene Ontology (GO) enrichment analysis for both DEGs and DTEGs was performed using the BnIR database, with a minimum count threshold of five genes per category.

Results

Developmental responses of *B. napus* seedlings to WCS417

In this study, *B. napus* cultivar Zhongshuang 11 (hereafter ZS11) was used to investigate the effects of WCS417 colonization on plant development. Two-day-old ZS11 seedlings were transferred to a ½ MS plate with or without WCS417 suspension. After 10 days of incubation in the greenhouse, the growth of ZS11 seedlings with or without WCS417 treatment was examined. We found that there is a 3.18-fold increase in lateral root number and a 50.1 ± 2.5% reduction in primary root length in the WCS417-treated seedlings compared with the control (Fig. 1A-C, Fig. S1). In addition, WCS417 inoculation led to significant increases in both root (35.9 ± 9.4%) and shoot (43.5 ± 7.9%) fresh weight (Fig. 1D-E, Fig. S1). These results align with earlier findings by Zamioudis et al. (2013) on WCS417-induced architectural remodeling in *Arabidopsis thaliana*, suggesting an evolutionary conservation of this PGPR's growth-promoting property within the Brassicaceae family (Zamioudis et al. 2013).

WCS417 induces a transient boost in global translation in rapeseed roots during early colonization

WCS417 colonization inhibits primary root growth, promotes lateral root development, and increases both roots and shoots biomass in *B. napus*. These phenotypic changes align with prior observations in model plants, such as *Arabidopsis thaliana* (Zamioudis et al. 2013). Although the mechanisms regulating WCS417-mediated root development and growth have been extensively characterized in earlier studies, the translational regulatory mechanisms triggered by its colonization of host roots, particularly the transient translational responses during early colonization, remain poorly understood.

To investigate the early translational response of rapeseed roots to *Pseudomonas simiae* WCS417, we performed polysome profiling at 30 minutes and 6 hours post-inoculation (He et al. 2024). The results

demonstrated a significant increase in the polysome-to-monosome (P/M) ratio at both time points compared to the control, indicating a marked enhancement of global translational activity. Notably, the comparable P/M ratios between the 30-minute and 6-hour samples suggest that this heightened translational state is rapidly established and maintained during early colonization, rather than representing a fleeting response.

RNA-seq and RNC-seq Quality Control Analysis

To investigate the role of translational regulation during early WCS417 colonization in root development, we performed an integrated analysis of transcriptome (RNA-seq) and translatome (RNC-seq) profiles. Two-day-old ZS11 seedlings were transplanted into liquid ½ MS medium supplemented with 2% sucrose and grown in the greenhouse for 12 days. Seedlings were then treated with a bacterial suspension of WCS417 at OD₆₀₀ = 0.05 for either 30 minutes or 6 hours. Each biological replicate consisted of root tissues pooled from 5–7 seedlings, with two independent replicates per condition.

The collected samples were subjected to cell lysis, ribosome isolation, and extraction of ribosome-protected mRNA fragments. Subsequently, corresponding RNA-seq and RNC-seq libraries were constructed for each sample using commercial kits, followed by high-throughput sequencing for transcriptomic and translatomic analysis. Stringent quality control procedures were applied to the raw sequencing data. Each sample yielded more than 34 million raw reads, with Q30 scores exceeding 94%. No adapter contamination or abnormal GC content was detected based on assessments with FastQC and MultiQC. High-quality reads were aligned to the ZS11 reference genome using STAR, with a median unique mapping rate of 80%. Gene coverage uniformity was high without notable 3' bias, indicating high RNA integrity and library preparation quality. Collectively, these results confirm that the sequencing data are of high quality and suitable for subsequent analysis.

After verifying library quality, we further evaluated the reliability of the RNA-seq and RNC-seq data. Inter-sample correlation analysis showed that the correlation coefficients among biological replicates within each group (control, 30-minute, and 6-hour treatments) were close to 1, indicating high intra-group reproducibility, while clear differences were observed between groups (Fig. S2C). Principal component analysis (PCA) further confirmed clear separation between control and treatment groups in the principal component space, demonstrating high reliability of the transcriptome data (Fig. S2D). Pearson correlation heatmaps of RNA-seq and RNC-seq data across the three time points revealed substantial changes in gene expression patterns following WCS417 inoculation. PCA results indicated that transcriptional and translational alterations occurred as early as 30 minutes after inoculation, with more pronounced changes at 6 hours after inoculation (Fig. S2D), suggesting that these responses are likely induced by WCS417 colonization.

WCS417 modulates multiple physiological processes in *B. napus* roots during early colonization

To systematically investigate changes in gene expression, we performed differential expression analysis at both the transcriptome and translome levels across all time points after normalization (Fig. 3, Table S1). Scatter plots comprehensively display the distribution of differentially expressed genes (DEGs) and differentially translated genes (DTGs) for each comparison group, including WCS417 treatment for 30 minutes versus control, 6 hours versus control, and 6 hours versus 30 minutes. Transcriptome analysis revealed that compared to the control, WCS417 treatment for 30 minutes induced up-regulation of 1,806 genes and down-regulation of 1,117 genes; after 6 hours of treatment, the numbers of up- and down-regulated genes were 2,328 and 2,907, respectively; while the comparison between the 6-hour and 30-minute treatment groups identified 2106 up-regulated and 3100 down-regulated genes. At the translome level, WCS417 treatment for 30 minutes versus control resulted in 1,762 up-regulated and 1,628 down-regulated genes; after 6 hours, 1,699 and 1,914 genes were up- and down-regulated, respectively; and the comparison between the two treatment time points showed 1,659 up-regulated and 2,645 down-regulated genes. Overall, WCS417 treatment induced extensive transcriptional and translational responses in the roots as early as 30 minutes, with the number of affected genes further increasing at 6 hours (Fig. 3, Table S2-S3).

In the analysis of DEGs and DTGs, genes related to plant growth, development, and nutrient uptake were significantly affected. Genes associated with lateral root development (e.g., *CLE1* and *WOX11*) (Nakagami et al. 2023; Ge et al. 2019) were upregulated in response to WCS417 (Fig. 3), which is consistent with the phenotype that WCS417 promotes lateral root formation in *B. napus* (Fig. 1A). On the other side, homologs of *PXMT1*, which could promote primary root elongation (Chung et al. 2016), were firstly upregulated at 30 mins of WCS417 treatment, but significantly downregulated at both transcriptional and translational levels at 6 hours of WCS417 treatment (Fig. 3), which is in line with the long term suppression of primary root elongation by WCS417 (Fig. 1B).

Concurrently, notable changes were detected in genes related to nutrient absorption. The expression and translation of several homologs of *NRT2*, a high-affinity nitrate transporter involved in nitrogen uptake (Jacquot et al. 2020), were significantly upregulated 6 hours after inoculation. Meanwhile, the expression and translation of *PHT3* homologs, which encode phosphate transporters (Mlodzinska and Zboinska 2016), also increased 6 hours after WCS417 inoculation. These results indicate that WCS417 colonization may enhance nitrogen and phosphate utilization and thereby promote plant growth (Fig. 1C, Fig. 3).

In addition to growth and development-related genes, WCS417 treatment significantly affects the expression of many abiotic stress-related genes. Genes associated with antioxidant, drought, and salt stress responses (e.g., *ERF53*, *ERF54*, and *OXS3*) exhibited elevated transcriptional and translational activity at both 30 minutes and 6 hours. The expression of *GAS2*, an enzyme catabolizing ABA to phaseic acid (PA) and putative 8'-carboxy-ABA (Lange et al. 2023), was consistently elevated at both time points. Interestingly, the expression of *DREB1B*, *DREB1C*, *DREB1D*, and *DREB1E* (Fowler et al. 2005; Vyse et al. 2022), core AP2/ERF family transcription factors in the low-temperature response pathway, was

consistently reduced at both time points (Fig. 3). The function of these TFs in plant-microbe interaction was largely unexplored and worth further investigation.

WCS417 infection also dynamically modulated the expression of immune-related genes. For instance, positive immune regulators such as *SEN1* (which acts downstream of the SA and JA signaling pathways) (Schenk et al. 2005) and *CBL7* (a calcium sensor involved in immune signal transduction) (Perez-Alonso et al. 2022) were consistently up-regulated at both the transcriptional and translational levels at the two time points examined, indicating that transient WCS417 infection activates plant immune responses. In contrast, the expression of *CYP94B1*, a negative regulator of JA signaling (Bruckhoff et al. 2016), was up-regulated at 30 minutes post-infection but down-regulated at 6 hours (Fig. 3). This expression pattern may help to alleviate the suppression of immune responses at later stages, thereby preventing excessive immune activation and facilitating a balance between growth and defense.

In summary, these results demonstrate that during early colonization, WCS417 modulates multiple physiological processes, contributing to the rebalancing of plant immunity and development.

WCS417 modulates immune responses in *B. napus* roots during early colonization

To further elucidate the regulatory patterns of gene expression during the symbiotic interaction between WCS417 and *B. napus* roots, we performed Gene Ontology (GO) enrichment analysis on the differentially expressed genes (Fig. 4, TableS4-S5). The results indicated that both the transcriptomic and translational data were significantly enriched in pathways related to immune response, root development, hormone signaling, and amino acid transport during WCS417 infection, with the notable observation that several pathways enriched in the RNC-seq data (such as the jasmonic acid-mediated signaling pathway) were also detected in the RNA-seq data, suggesting a coordinated regulatory process from transcription to translation.

Previous studies have indicated that although WCS417 is a non-pathogenic bacterium, its interaction with plant roots can still activate certain immune-related signaling pathways (Ruan et al. 2019; Hou and Tsuda 2022; Huang et al. 2016; van Loon et al. 2006; Janda et al. 2020; Kachroo et al. 2020; Miao et al. 2025; Shidore and Triplett 2017; Hacquard et al. 2017). Our results demonstrate that at the early stage of WCS417 inoculation (30 minutes), GO terms related to salicylic acid (SA), jasmonic acid (JA) signaling pathways were significantly upregulated (Fig. 4A-B). As core components of the plant defense system, these pathways are known to comprehensively regulate immune responses (Mao et al. 2007; Hofmann et al. 2006; Vorwerk et al. 2007; Yang et al. 2020). At the gene expression level, biosynthesis-related genes in the SA pathway (such as *WRKY28*) and the JA pathway (such as *AOC2*, *LOX3*, and *LOX4*) showed consistent upregulation at both transcriptional and translational levels (Fig. 3–4). These findings suggest that although WCS417 is a beneficial rhizobacterium, its initial colonization triggers an immune response in the plant, thereby inducing defense mechanisms to counteract WCS417 invasion.

In the GO enrichment analysis conducted at 6 hours post-WCS417 infection and in comparison with the 30-minute time point, immune-related responses, including the JA and ET signaling pathways (e.g., *LOX3*, *LOX4*, *PDF1.4*, *ERF1A*, *ERF12*, *ERF13*), exhibited a gradual downregulation trend (Chandler and Werr 2020; Yang et al. 2020) (Fig. 3-4B). This shift suggests a potential transition of the plant from a defensive state toward symbiotic adaptation. Notably, at 30 minutes post-inoculation, the transcription factor *ERF018*, which regulates JA biosynthesis, was already significantly downregulated at both transcriptional and translational levels (Huang et al. 2021) (Fig. 3). This observation implies that JA biosynthesis-related genes may be initially modulated at the translational level to fine-tune the immune response, followed by subsequent transcriptional and translational regulation of JA synthesis-related genes by 6 hours post-infection.

Concurrently, genes associated with processes critical for symbiosis were upregulated at both transcriptional and translational levels. These included genes involved in cell wall remodeling (e.g., *EXPA1*, *EXPA10*, *EXPA15*), root development (e.g., *EP3*, *SMB*, *CLE1*), amino acid transport (e.g., *GDU6*, *GDU1*, *LHT1*), and auxin biosynthesis (e.g., *YUC6*, *WEI8*) (Cha et al. 2015; Fang et al. 2016; Jacquot et al. 2020; Nakagami et al. 2023; Nakano et al. 2011; Pratelli et al. 2010; Tao et al. 2024; Wang et al. 2022) (Fig. 3-4B). These changes suggest that the plant promotes root structural reorganization and hormonal responses to accommodate WCS417 colonization.

To systematically investigate the coordinated and stage-specific regulation of transcription and translation across different time points, we employed UpSet plots to analyze gene sets that were either commonly or uniquely responsive at each time point (Fig. 5A-B). The results revealed a substantial number of differentially expressed genes (DEGs) shared between the two time points at both transcriptional and translational levels. These common DEGs likely represent a persistent and core response essential for the interaction, potentially forming a “core response module” critical for the establishment of symbiosis. In addition, each treatment group contained genes that were specifically co-regulated at the transcriptional and translational levels in a time-dependent manner, as well as genes altered exclusively at either level. This pattern indicates that distinct physiological processes are regulated at different stages, highlighting the dynamic and phase-specific nature of the host response during WCS417 colonization.

Furthermore, the presence of independent transcriptional and translational DEG sets in the UpSet plot suggests that WCS417 treatment induces an imbalance between transcription and translation for a subset of root genes. To further dissect the coordination between transcriptional and translational regulation, we generated a nine-quadrant scatter plot based on transcript-level changes and translational-level changes (Fig. 5C-E). Differential expression analysis was performed using DESeq2, and genes with an adjusted *p*-value > 0.05 were excluded.

The results showed that during WCS417 colonization from 30 minutes to 6 hours, most genes exhibited consistent trends in both transcriptional and translational changes, indicating a coordinated regulatory mode (Fig. 5C-E). However, a subset of genes displayed different changes, with misaligned mRNA

expression and translation levels, some even showing opposite trends (e.g., upregulated mRNA with decreased translation, or downregulated mRNA accompanied by enhanced translation). These genes are likely subject to post-transcriptional regulation, suggesting that WCS417 infection specifically modulates the translation efficiency of certain genes.

WCS417 fine-tunes the translational ratios of immune and root development genes.

To further elucidate the regulatory impact of WCS417 on translational control in *B. napus* roots, we compared translation ratios (TR) between untreated controls and roots treated for 30 minutes and 6 hours. After 30 minutes of WCS417 treatment, 3,223 genes exhibited significant changes in TR, with 3,021 exhibiting increased ratios and 202 showing decreased ratios, indicating that extensive translome reprogramming had already occurred during the early inoculation stage, suggesting a critical role of translational regulation in the initial establishment of symbiosis (Fig. 6A, Table S6). After 6 hours of treatment, the number of genes with altered TR declined to 1,486, of which 1,006 showed elevated TR and 480 showed reduced TR, reflecting a more convergent translational response as the host adapted to bacterial colonization (Fig. 6B, Table S6). Notably, comparison with the 30-minute treatment group revealed TR alterations in 2,061 genes at the 6-hour time point, with 772 genes exhibiting increased TR and 1,289 showing decreased TR (Fig. 6C, Table S6). These genes are likely involved in maintaining cellular homeostasis or executing core regulatory functions during sustained symbiosis. Collectively, these results demonstrate that WCS417 infection triggers dynamic reprogramming of translation efficiency.

Differential translation ratios (DTR) analysis revealed that WCS417 colonization triggers extensive translome reprogramming during early infection. At 30 minutes post-inoculation, the TR of multiple immune-related genes (e.g., *CRK11*, *HA5*, *GR2*, *ZRK10*, and *TIFY6B*) was significantly elevated, indicating rapid activation of host immune responses upon initial contact (Chen et al. 2003; Zhao et al. 2022; Zarei et al. 2017; Diplock et al. 2023). Concurrently, increased TR of cell wall-related factors *MYB54* and *PME5* suggested that cell wall remodeling represents an early physiological adaptation to bacterial colonization (Zhong et al. 2008; Etchells et al. 2012) (Fig. 6A).

As colonization progressed to 6 hours, the TR of *ERF13* (Yu et al. 2024), a key transcription factor in the JA/ET signaling pathway, was consistently suppressed, while the immune negative regulator *HA5* and *TIFY6B* showed enhanced TR, implying that plants may selectively suppress certain immune pathways to facilitate symbiotic establishment (Chini et al. 2007). Furthermore, significantly increased TR of the endoplasmic reticulum formation-related gene *BGLU18* and the amino acid transporter *CAT5* at 6 hours suggested reinforced secretory capacity and nitrogen utilization to support host-microbe interface formation (Bizan et al. 2025; Su et al. 2004) (Fig. 6B).

In the 6-hour versus 30-minute comparison, sustained elevation in TR was observed for the phosphate transporter *PHT4* (Cubero et al. 2009), root development-related gene *DOT4* (Petricka et al. 2008), and the sucrose efflux transporter *SWEET15* (Desrut et al. 2020), further supporting the role of WCS417 in enhancing nutrient acquisition and root development. Notably, tRNA methyltransferases *TRM8b* and

TRM4B, which are critical for tRNA stability, proper folding, and overall translational accuracy, also exhibited significantly increased TR (Fig. 6C). Their upregulation may systemically enhance global protein synthesis capacity during microbial colonization.

To assess systematic differences in TR across treatment groups, we first visualized the overall TR distribution using empirical cumulative distribution function (ECDF) curves and boxplots (Fig. S3). The results showed that compared to the control group, both the 30-minute and 6-hour treatment groups exhibited significantly higher overall TR levels, displaying a dynamic trend of initial increase followed by a slight decline (Fig. S3A). This indicates that WCS417 infection rapidly induces extensive translational reprogramming in roots during the early colonization stage. As the treatment duration extended to 6 hours, the overall translational activity gradually returned toward the control level, suggesting a stabilization of the translational state. Notably, due to the relatively limited difference in TE between the 30-minute and 6-hour groups, no significantly enriched pathways were identified in the 6-hour versus 30-minute comparison. In contrast, functional pathway enrichment associated with TE changes was primarily observed at the 30-minute post-inoculation time point.

GO enrichment analysis of differentially translated genes showed that at 30 minutes post-inoculation, immune-related processes such as JA and SA signaling pathways were significantly upregulated at the TR level, consistent with translational and translational expression trends. This confirms that transient WCS417 infection rapidly triggers translational activation of immune pathways. By 6 hours, TR of the SA pathway remained elevated, further indicating that plants enhance the translation of immune components to balance symbiotic establishment with basal defense (Fig. 6D, TableS7).

Validation of changes in mRNA abundance during early WCS417 colonization by qRT-PCR

To validate the reliability of our RNA-seq data, we selected key genes associated with immune response, root development, abiotic stress, and cold tolerance for qRT-PCR analysis. The results showed that the expression patterns of these genes were highly consistent with the transcriptome data (Fig. 7, Fig. S4), confirming the accuracy and reproducibility of our sequencing results. This validation provides reliable experimental support for subsequent in-depth analyses based on the transcriptome data, thereby strengthening the credibility of the multi-omics integration findings in this study.

To assess the translational changes induced by WCS417 in plant roots, we isolated ribosome-bound mRNAs using a sucrose cushion method and quantified the relative abundance of specific genes in this fraction by qPCR (Fig. 8A-B). The results revealed significant differences in the level of target ribosome association genes across treatment time points, whereas the control gene (*ACTIN*) remained stable across groups (Fig. 8B, Fig. S5). These findings demonstrate that WCS417 colonization triggers extensive translational reprogramming in the roots of *B. napus*.

Discussion

Plant Growth-Promoting Rhizobacteria (PGPR) are well-known for their ability to enhance plant growth (Kloepper et al., 1980). The mechanisms underlying PGPR-mediated growth promotion have been extensively studied (Lugtenberg and Kamilova 2009; Verbon and Liberman 2016). Among them, *P. simiae* WCS417, a representative PGPR strain, efficiently colonizes plant roots and modulates rhizosphere development through multiple mechanisms to promote plant growth. (Zamioudis et al. 2013; Pieterse et al. 2020). Our study demonstrated that colonization of *B. napus* roots by WCS417 resulted in increased lateral root formation, reduced primary root length, and a significant increase in plant fresh weight (Fig. 1).

We focused particularly on the transcriptional and translational regulatory mechanisms by which WCS417 influences root development in *B. napus*. Previous studies showed that WCS417 primarily modulates root architecture through auxin-dependent pathways, such as upregulating the expression of endogenous auxin biosynthesis genes (e.g., *YUCCA* family) and altering the localization and abundance of PIN proteins (e.g., PIN1, PIN2) to modify auxin distribution gradients, thereby promoting lateral root initiation and root hair formation (Zamioudis et al. 2013). Additionally, it activates Auxin Response Factors (ARFs) to drive the expression of downstream root development-related genes (Zamioudis et al. 2013). Consistently, we found that auxin-related genes such as *YUC6*, *WEI8*, and *PIN3* showed significant upregulation at both transcriptional and translational levels at 6 hours post-WCS417 inoculation (Fig. 3–4).

Moreover, WCS417 may regulate root architecture through cell wall remodeling and lignification (Marzorati et al. 2023). This study revealed that at 6 hours post-inoculation, cell wall-related genes (e.g., *EXPA1*, *EXPA10*, *EXPA15*) and root development-related genes (e.g., *EP3*, *SMB*, *CLE1*) were significantly upregulated at the transcriptional and translational levels (Fig. 3–4). This suggests that WCS417 may facilitate structural reorganization by previously regulating genes associated with root morphogenesis. Meanwhile, we observed a significant downregulation in both the transcription and translation of the primary root elongation factor *PXMT1* after six hours of infection, which may contribute to the observed inhibition of primary root growth (Fig. 1, 3).

The flagellin epitope (flg22₄₁₇) of WCS417 activates MAMP-triggered immunity (MTI) in plants (Gomez-Gomez et al. 1999; Millet et al. 2010; Pel and Pieterse 2013). However, the bacterium secretes gluconic acid and other metabolites to acidify the rhizosphere microenvironment, thereby suppressing immune responses, and simultaneously exhibits tolerance to antimicrobial coumarins produced via the MYB72 pathway (Yu et al. 2019a; Yu et al. 2019b; Stringlis et al. 2018). These synergistic mechanisms enable WCS417 to successfully colonize the host while evading immunity, ultimately establishing a stable mutualistic relationship (Gomez-Gomez et al. 1999; Pel and Pieterse 2013; Millet et al. 2010; Stringlis et al. 2018). Our findings reveal that immune pathways were upregulated at both transcriptional and translational levels at 30 minutes post-WCS417 inoculation, but were downregulated by 6 hours, a shift that may facilitate bacterial colonization (Fig. 3–5). Notably, however, the translation efficiency of genes associated with these immune pathways (e.g., *CRK11*, *HA5*, *GR2*, *ZRK10*) was elevated at 30 minutes, and GO enrichment analysis still indicated upregulation of the SA immune pathway at 6 hours (Fig. 6).

This suggests that although the plant attenuates immune-related gene expression to accommodate WCS417, it simultaneously enhances the translation efficiency of certain immune components to partially compensate for reduced transcript abundance, thereby maintaining basal defense capacity against potential pathogenic threats.

In this study, we observed significant alterations in the expression of abiotic stress-related genes during colonization by WCS417. Specifically, positive regulators associated with drought and salt tolerance (e.g., *ERF53* and *ERF54*) exhibited consistent upregulation at both transcriptional and translational levels, suggesting that WCS417 may enhance plant pre-adaptation to drought and salinity stress by activating these transcription factors (Fig. 3). In contrast, the expression of core regulators of the low-temperature response pathway (e.g., *DREB1B*, *DREB1C*, *DREB1D*, and *DREB1E*) was persistently downregulated during the treatments (Fig. 3). This observation suggests that WCS417 may suppress the cold response pathway—or employ unexplored functions unrelated to cold tolerance but relevant to plant-microbe interactions—to reallocate plant resources, thereby prioritizing metabolic processes essential for establishing symbiosis.

The functions of these transcription factors in plant-microbe interactions have not yet been systematically investigated. The observed downregulation of core cold-response factors during symbiosis establishment suggests that they may represent a novel regulatory node for balancing cold tolerance and mutualistic benefits in plants. Further elucidating the specific roles and regulatory mechanisms of these transcription factors within plant-microbe interaction networks will help reveal new strategies employed by plants to coordinate biotic and abiotic stress responses with microbial assistance and provide a theoretical basis for using beneficial microorganisms to improve crop stress tolerance.

In summary, our findings demonstrate that during the early colonization of *B. napus* roots by WCS417, the host rapidly initiates transcriptional and translational reprogramming to respond to bacterial invasion, followed by gradual adjustments to stabilize the symbiotic association. This process involves multi-layered regulations, including auxin signaling, cell wall remodeling, immune balance, and abiotic stress, reflecting the complex and efficient molecular adaptation strategies employed in plant-PGPR interactions. However, the upstream regulators governing the immune-development balance—particularly those operating at the translational level, such as key receptors, protein kinases, transcription factors, and other core genes determining mRNA translation efficiency—remain unknown, and it is essential to investigate whether they function as central hubs that prioritize growth over defense. Future research should employ single-cell transcriptome sequencing technologies to elucidate the spatial architecture of gene expression and to decipher how WCS417 modulates post-transcriptional processes in specific cell layers, such as the root epidermis or cortex.

Declarations

Author Contribution

L.Z. performed all the experiments, analyzed the data, and wrote the draft. J.L. analyzed the data. W.W. and Z.Z. conceived the experiments and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Figures

Fig. 1

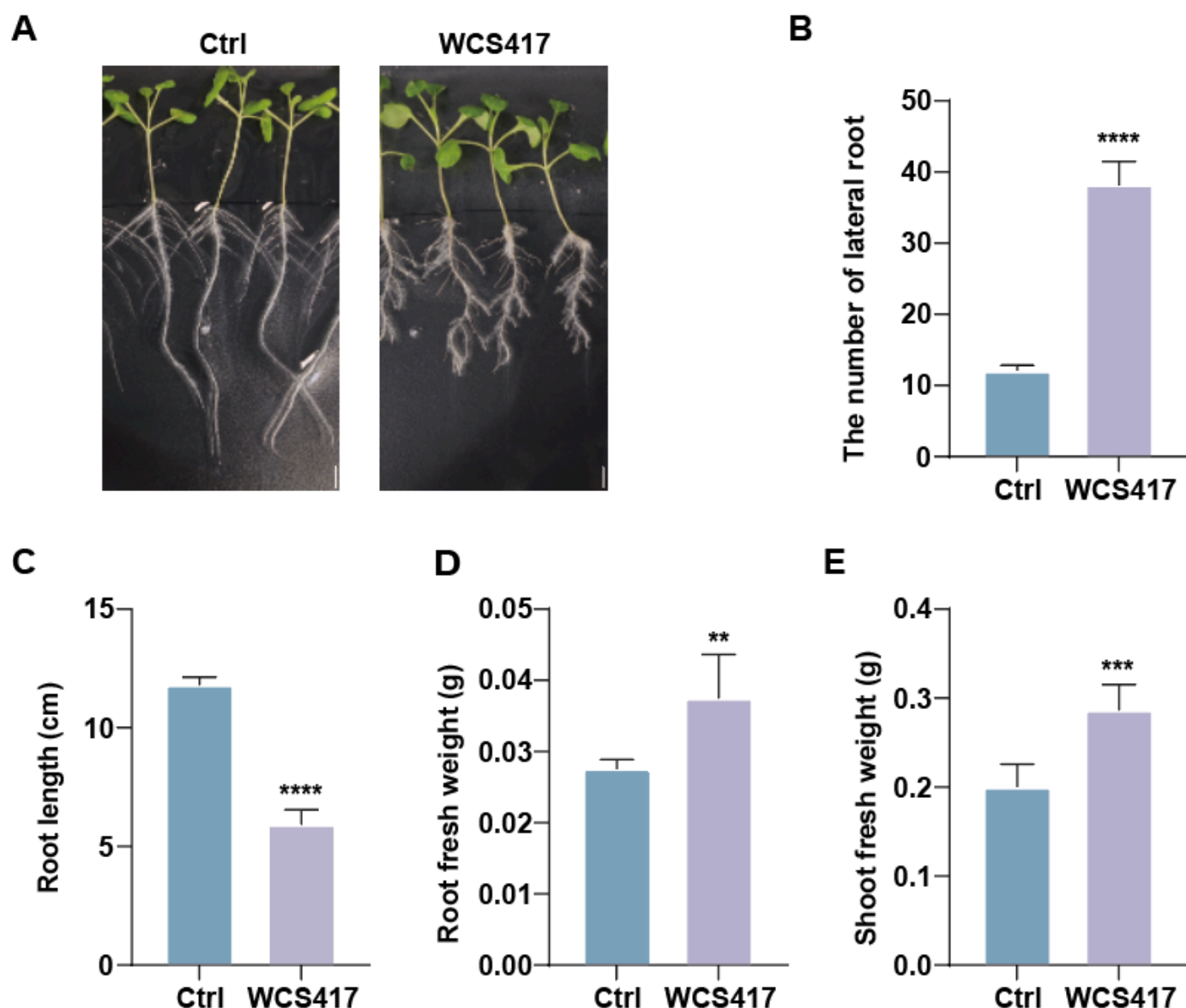


Figure 1

Developmental responses of *B. napus* seedlings to WCS417

A. WCS417 induces changes in root architecture. **B-E.** Quantification of growth parameters in seedlings with or without WCS417 inoculation. (B) Lateral root number. (C) Primary root length. (D) Root fresh weight. (E) Shoot fresh weight. Significance analysis was calculated using GraphPad Prism 8 with an unpaired two-tailed Student's *t*-test. Error bars represent the standard deviation of six biological replicates. *n* = 6; Asterisks indicate significant differences between the two sets of data. ** *P* < 0.01, *** *P* < 0.001, **** *P* < 0.0001. Scale bar, 1 cm.

Fig. 2

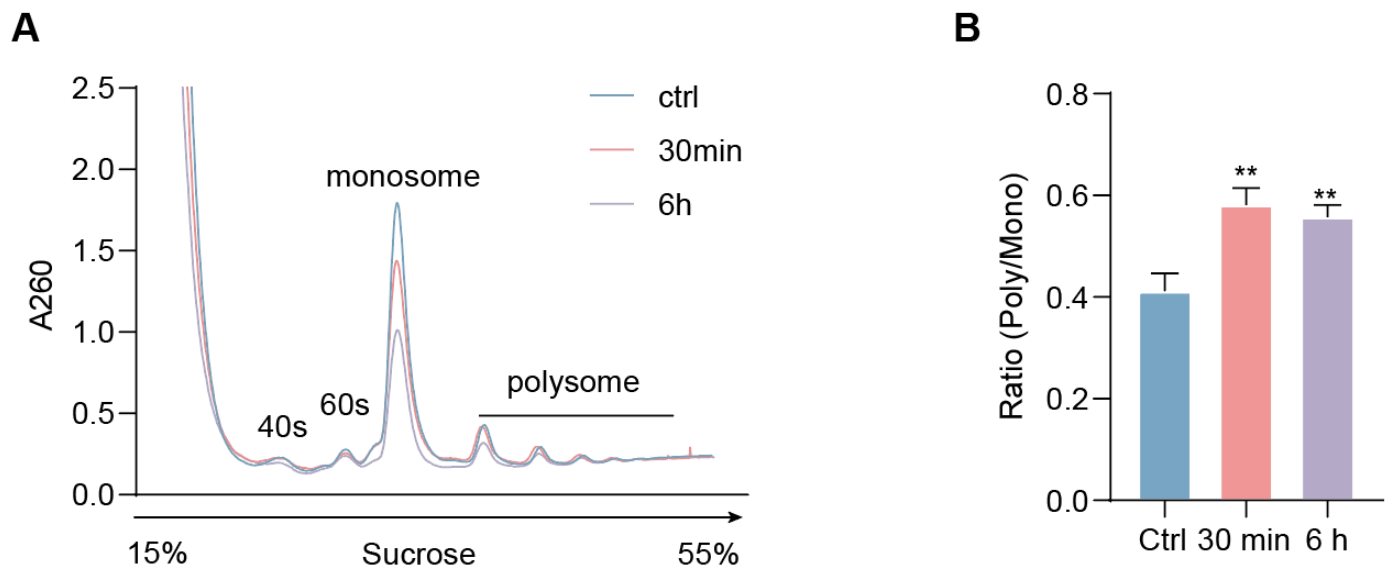


Figure 2

Polysome profiling to detect global translation in *B. napus* roots before and upon WCS417 infection at 30 minutes and 6 hours.

A. Polysome profiling analysis. **B.** Quantification of the polysome-to-monosome (P/M) ratio. Significance analysis was calculated using GraphPad Prism 8 with an unpaired two-tailed Student's *t*-test. Error bars represent the standard deviation of three biological replicates. *n*= 3; Asterisks indicate significant differences between the two sets of data. ** *P* < 0.01, *** *P* < 0.001, **** *P* < 0.0001.

Fig. 3

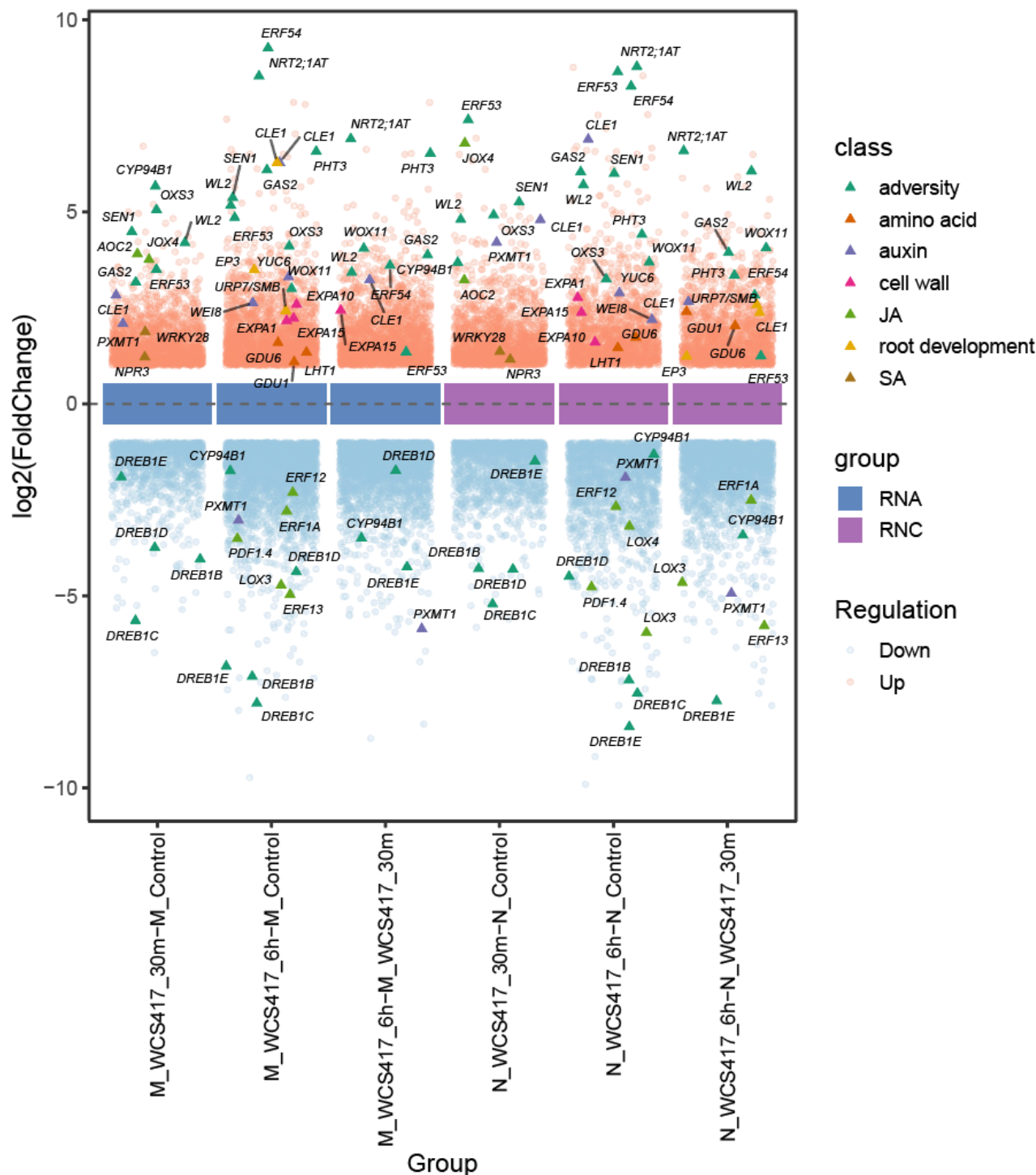
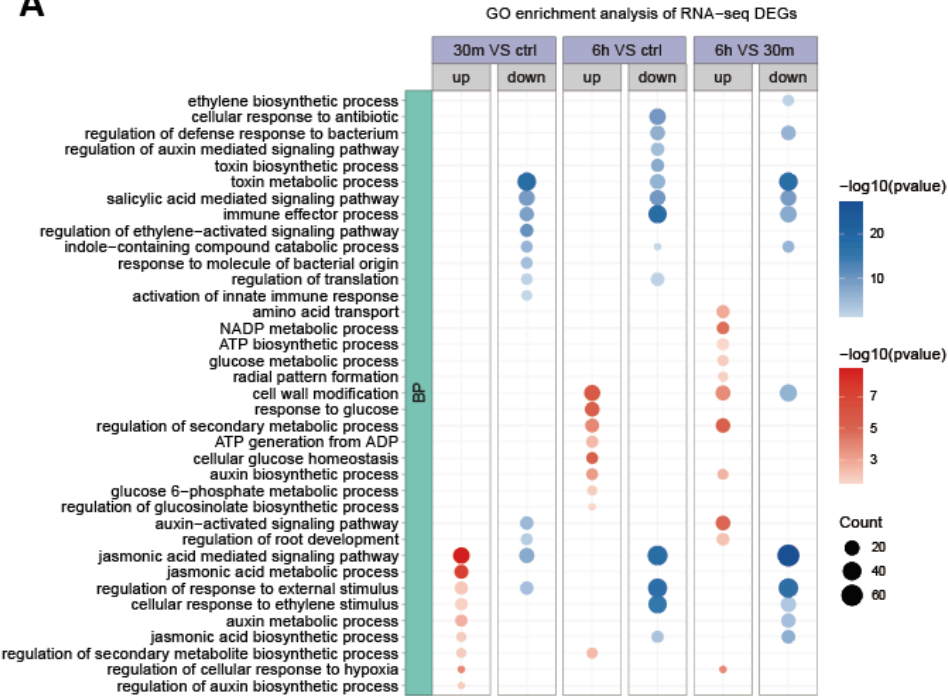


Figure 3

Volcano plots display differentially expressed genes (DEGs) and differentially translated genes (DTGs) from RNA-seq and Ribo-seq data in *B. napus* roots under control versus WCS417 treatment at 30 minutes and 6 hours. Genes related to immunity, stress response, and root development are highlighted with red circles. The dashed lines indicate the thresholds for significance (absolute fold change = 2, adjusted P -value [Padj] = 0.05).

Fig. 4

A



B

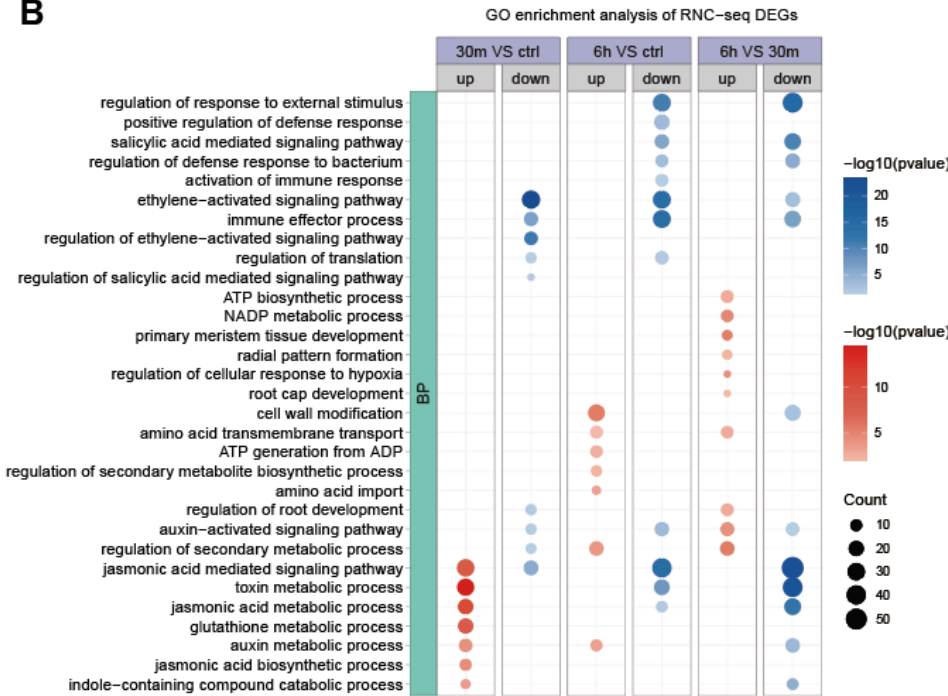


Figure 4

GO functional annotation of differentially expressed genes in the transcriptome and translome.

A. GO enrichment analysis of differentially expressed genes (DEGs) from the transcriptome. B. GO enrichment analysis of differentially translated genes (DTGs) from the translome. The Y-axis represents the GO term names. The X-axis represents the different time-point comparisons of WCS417

treatment. The dot size corresponds to the number of genes enriched in the respective GO term, and the dot color intensity represents the $-\log_{10}$ (adjusted P -value). Adjusted P -values were calculated using the clusterProfiler package with the Benjamini-Hochberg (BH) correction method.

Fig. 5

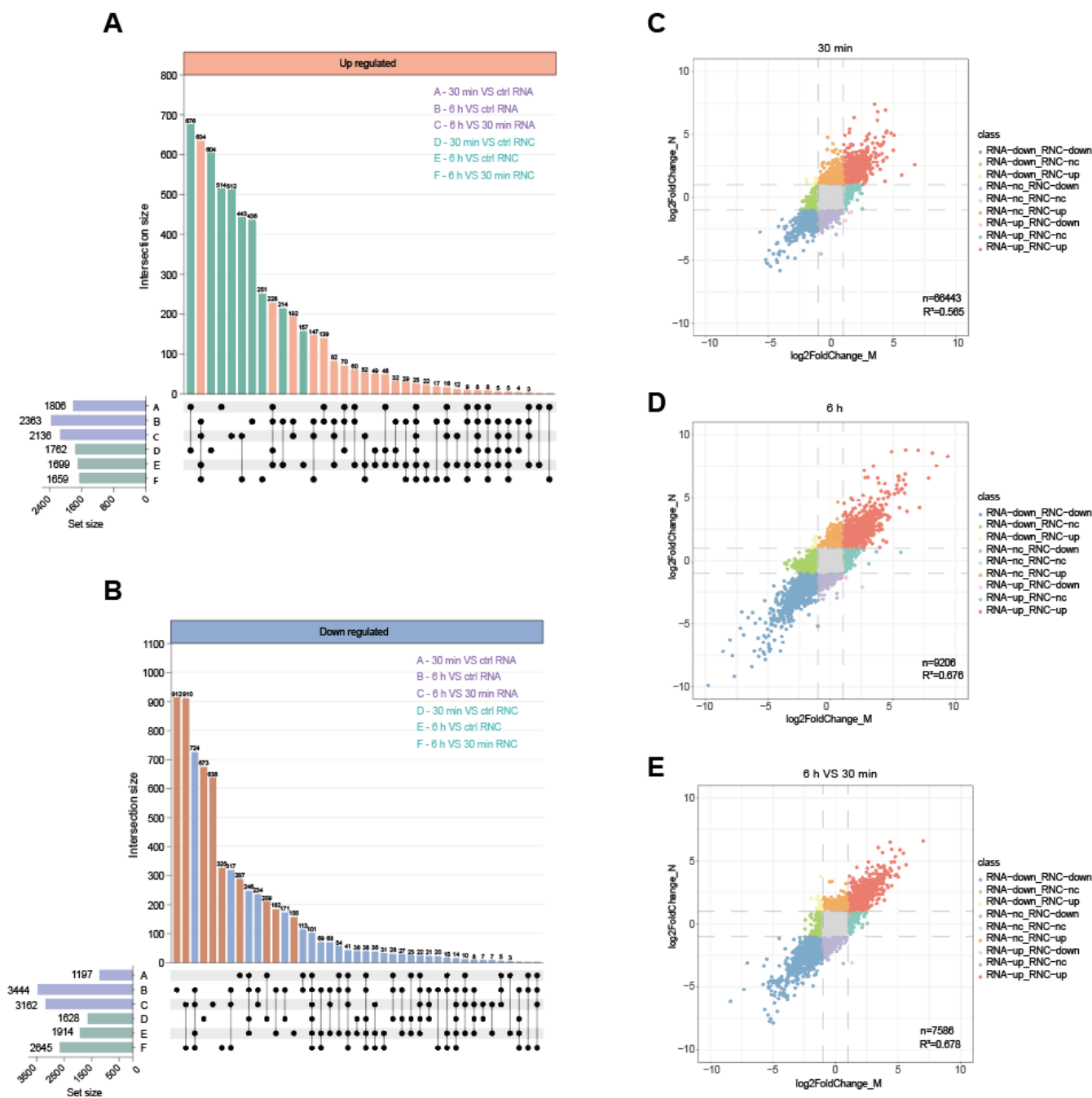


Figure 5

Analysis of DEGs and DTEs from RNA-seq and RNC-seq data across different time points of WCS417 infection.

A-B. Upset plots showing the common and unique DEGs (upregulated or downregulated) across different time points in the RNA-seq (A) and RNC-seq (B) datasets. The top bar chart represents the number of upregulated (orange) or downregulated (blue) genes. The central vertical bars indicate the size of each specific intersection set; the height of each bar corresponds to the number of items (e.g., genes) in the intersection shown directly below it. The bottom matrix shows the composition of each intersection: each column represents an original set (time point comparison), filled dots represent items in each original set. All intersections are sorted by the number of items they contain, from largest to smallest. The green and dark red colors correspond to gene sets exhibiting specific transcriptional, translational, or coordinate functions that the set participates in the intersection above, and empty spaces indicate non-participation. The left-side bar chart displays the total number of transcriptional-translational activities at distinct time points. **C-E.** Scatter plots showing the correlation between transcriptional and translational changes for genes. The grey horizontal and vertical lines represent the significance thresholds (± 1 log₂ fold change) for identifying genes with significant changes at the mRNA level (transcriptome) and the translation level (translatome), respectively.

Fig. 6

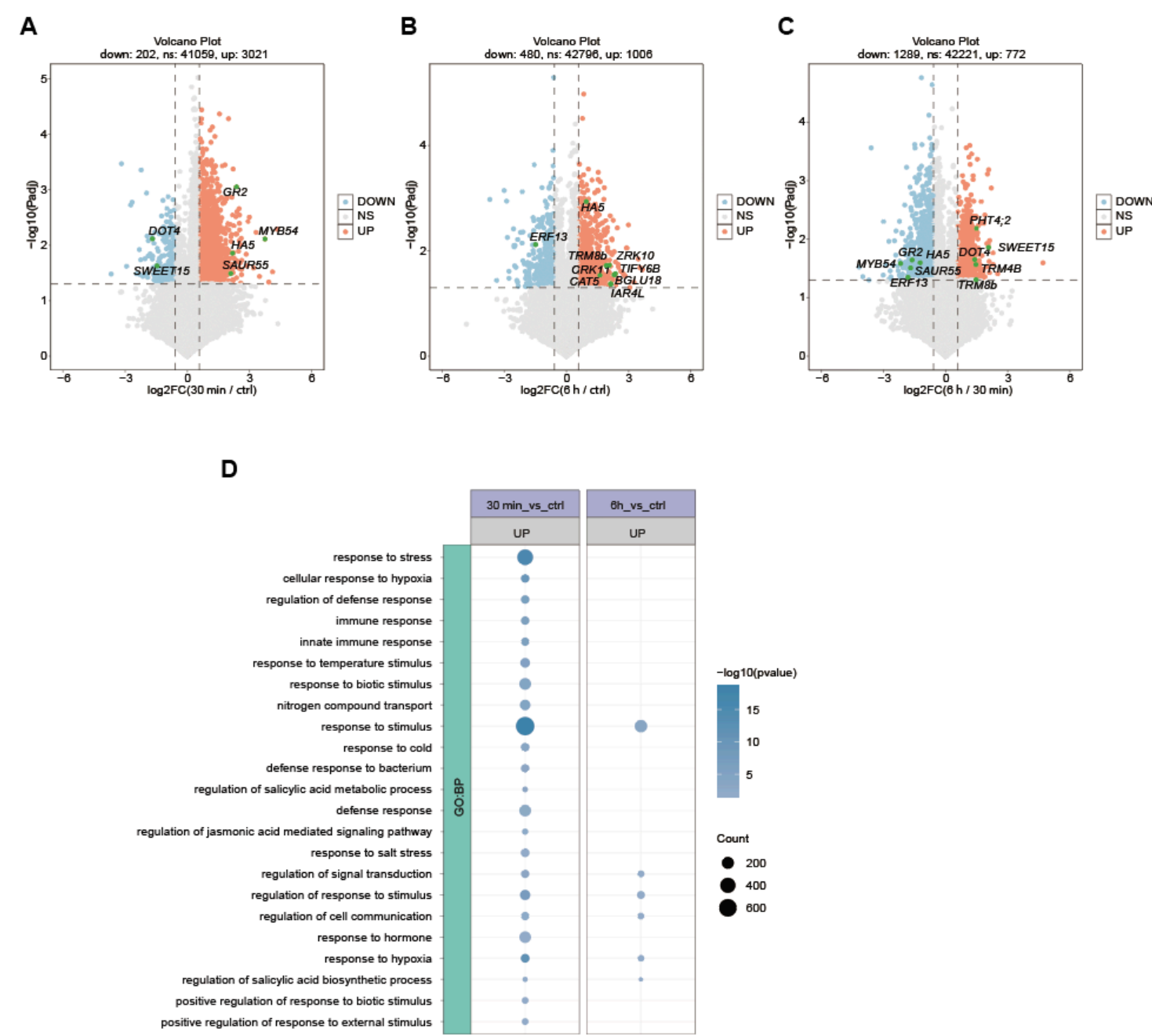


Figure 6
Analysis of differentially translated genes with altered translation Ratios (DTRGs) in *B. napus* roots under WCS417 treatment.

A-C. Volcano plots displaying DTEGs at different time points post-WCS417 treatment. The dashed lines indicate the thresholds for significance (fold change = 1.5, adjusted P -value [Padj] = 0.05). **D.** GO enrichment analysis of DTEGs. The Y-axis represents the GO term names. The X-axis shows the different

WCS417 treatment time-point comparisons. The dot size corresponds to the number of genes enriched in the respective GO term, and the dot color intensity represents the $-\log_{10}$ (adjusted P -value). Adjusted P -values were calculated using the clusterProfiler package with the Benjamini-Hochberg (BH) correction method.

Fig. 7

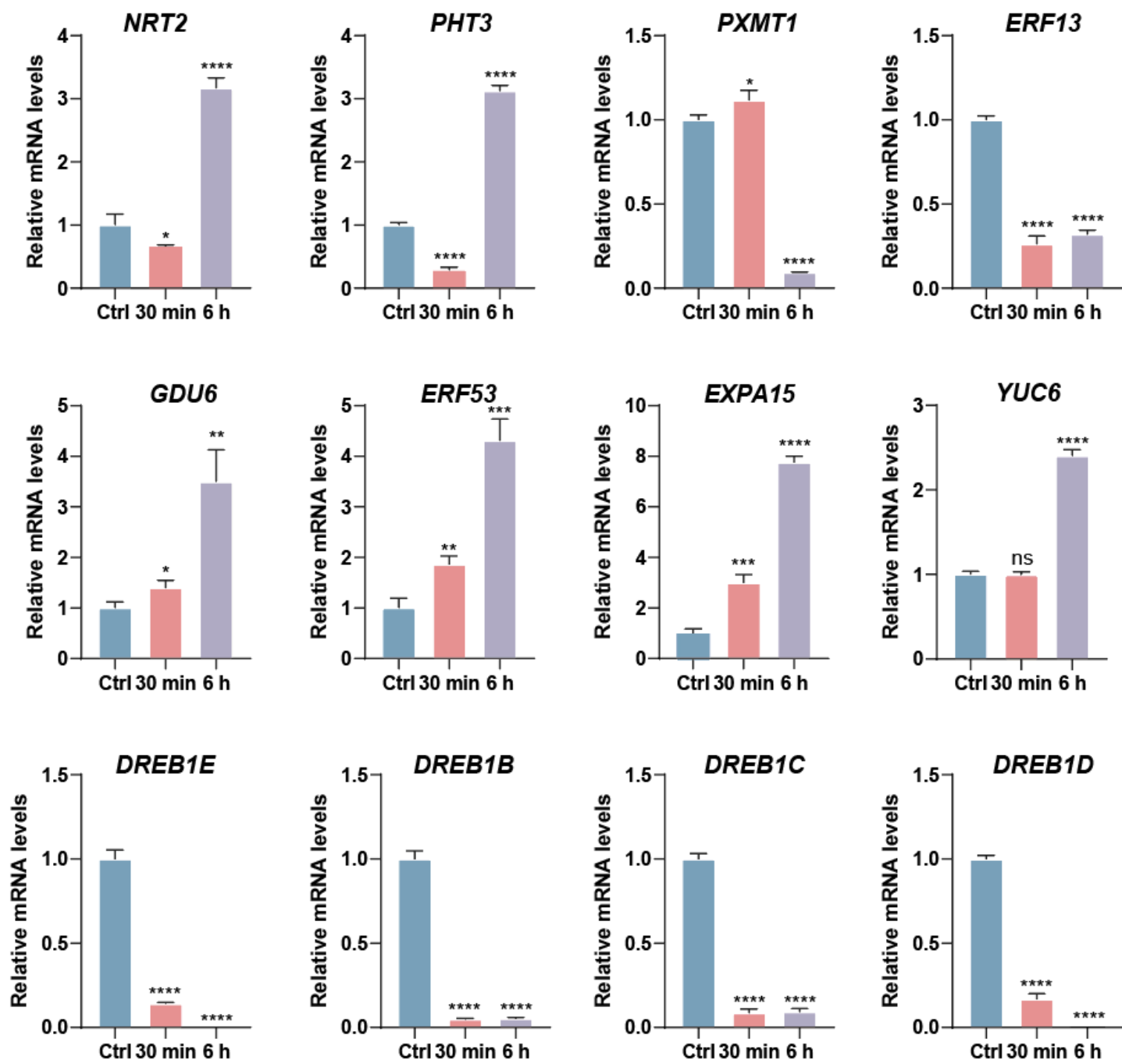


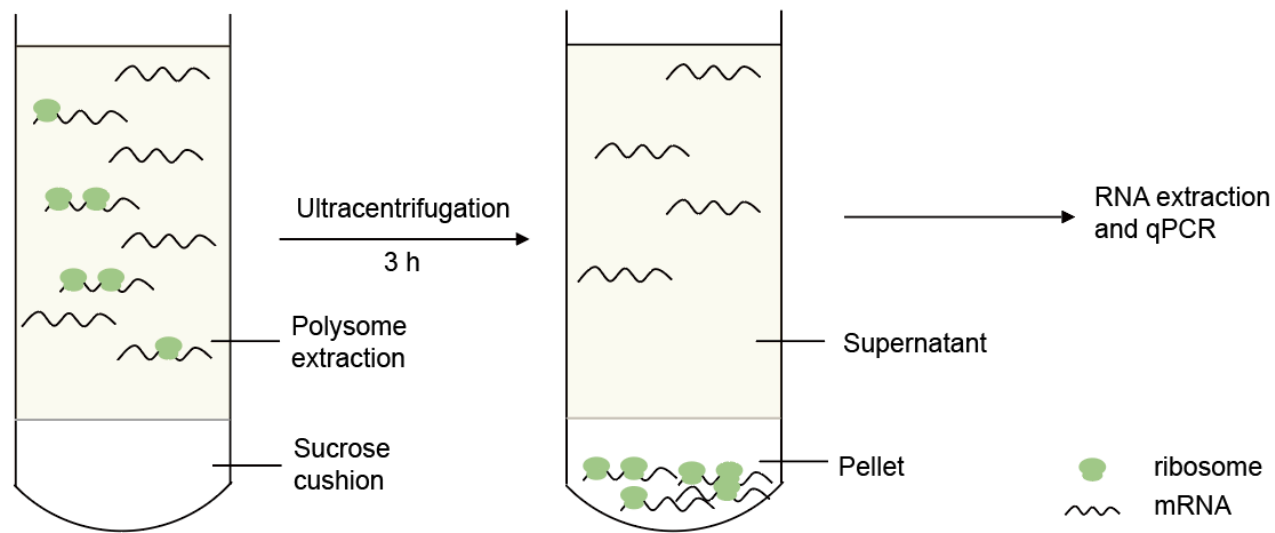
Figure 7

qRT-PCR analysis validates the expression patterns of key genes in response to WCS417 colonization. Significance analysis was calculated using GraphPad Prism 8 with an unpaired two-tailed Student's t -

test. Error bars represent the standard deviation of three biological replicates. $n = 3$; Asterisks indicate significant differences between the two sets of data. * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$, **** $P < 0.0001$.

Fig. 8

A



B

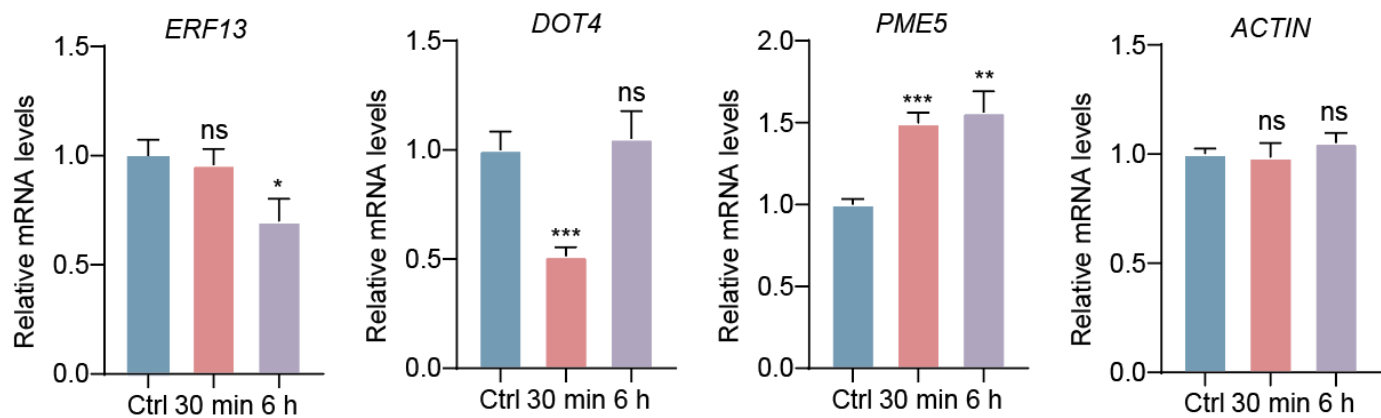


Figure 8

Ribosome occupancy of target mRNAs during WCS417 infection

A. Sucrose cushion schematic diagram. **B.** qPCR analysis to determine the percentage of mRNAs with altered translation rates (TR) in the sucrose cushion pellet fraction. *ACTIN* is the control. Significance analysis was calculated using GraphPad Prism 8 with an unpaired two-tailed Student's t -test. Asterisks indicate significant differences between the two sets of data. Error bars represent the standard deviation of three biological replicates. $n = 3$; *, $P < 0.05$; **, $P < 0.01$.

Supplementary Files

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- [TableS2RNAseqdifferentialexpressiongenes.xlsx](#)
- [TableS3RNCseqdifferentialtranslationgenes.xlsx](#)
- [TableS4DEGSGOenrichment.xlsx](#)
- [TableS5DTGSGOenrichment.xlsx](#)
- [TableS6Differentialtranslationefficiencygenes.xlsx](#)
- [TableS7DTEGsGOenrichment.xlsx](#)
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