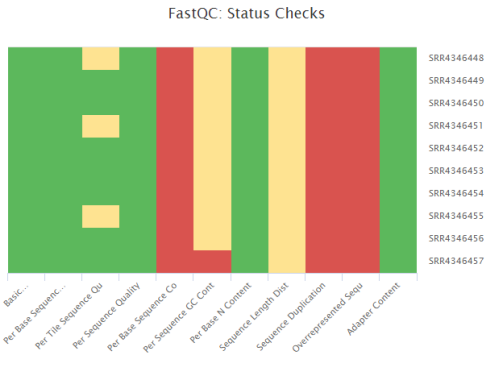
1-Data selection and processing

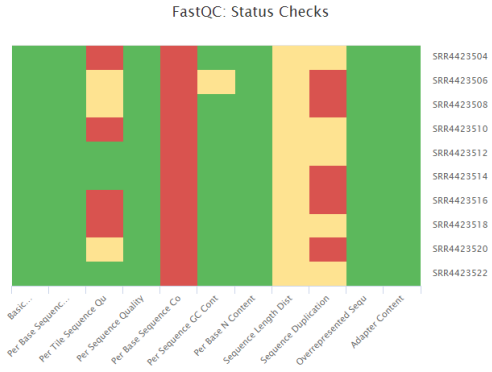
Gene expression of miRNA and mRNA datasets related to root-knot nematode (Meloidogyne incognita) infected tomato (Solanum lycopersicum) roots were identified by searching the Gene Expression Omnibus (GEO) database [1]. Using the GSE accession number of identified datasets, we obtained the corresponding BioProject accession number. The BioProject accession number PRJNA345485 comprises miRNA sequencing files, while mRNA sequencing files are associated with BioProject accession number PRJNA348521 **(Table).** The both of RNAseq datasets includes data for 5 stages.

GEO ID	BioProject	Platforms	Normal (N) vs Infected (F) (Number of Stages x replicates)	RNA type
GSE87651	PRJNA345485	Illumina HiSeq 2000 (Solanum lycopersicum)	N (5 x 1) vs F (5 x 1)	miRNA
GSE88763	PRJNA348521	Illumina HiSeq 2000 (Solanum lycopersicum)	N (5 x 2) vs F (5 x 2)	mRNA

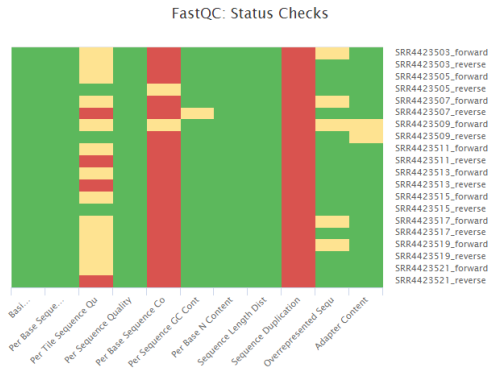
To obtain the FASTQ-formatted files, we utilized the Faster Download and Extract Reads in FASTQ tool available on the Galaxy platform (version 2.11) [2]. Subsequently, the quality of the processed clean reads was assessed using the FastQC tool (v0.11.9) provided on the platform **(Fig)**. For miRNA gene expression analysis, the miRNA analysis section of the "Whole transcriptome analysis of Arabidopsis thaliana" workflow template was followed [3]. The MiRDeep2 Mapper tool was used to preprocess the miRNA reads, followed by the MiRDeep2 Quantifier tool to map the deep sequencing reads to predefined miRNA precursors. The mature miRNA sequences and the miRNA stem loop sequences were obtained from miRbase database [4]. For mRNA data processing and annotation, the "1: RNA-Seq reads to counts" workflow template was employed [5]. The mRNA reads were aligned to the reference genome using the HISAT2 tool and then assigned to genes using the featureCounts tool **(Fig).** Finally, both miRNA and mRNA datasets were mapped to the tomato reference genome last version ITAG4.0 with complete annotation last version ITAG4.1 obtained from the Sol Genomics Network (SGN) database [6].



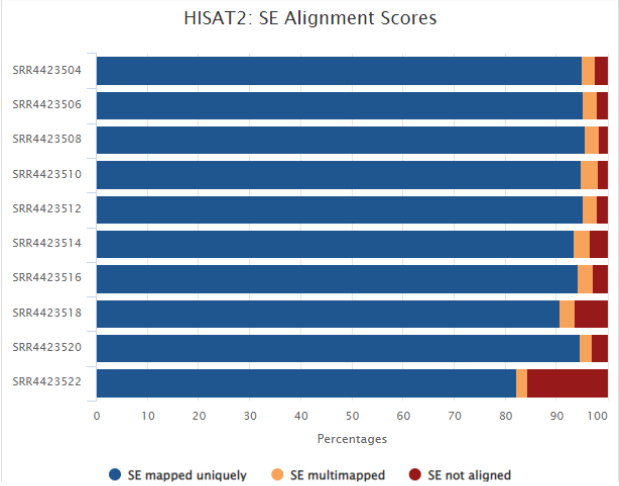
All miRNA processed data



mRNA single-end processed data



mRNA paired-end processed data



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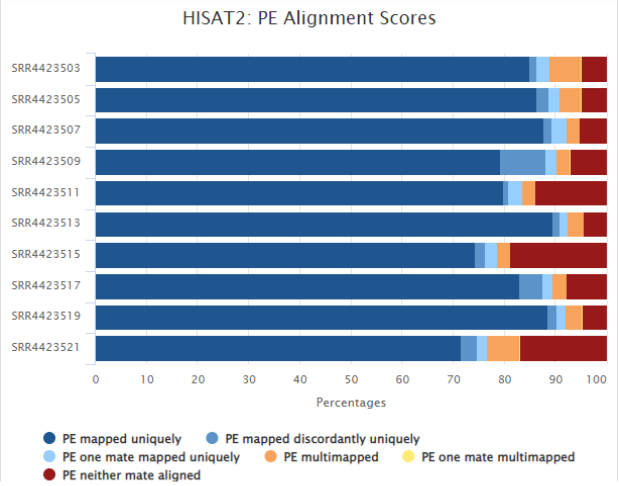

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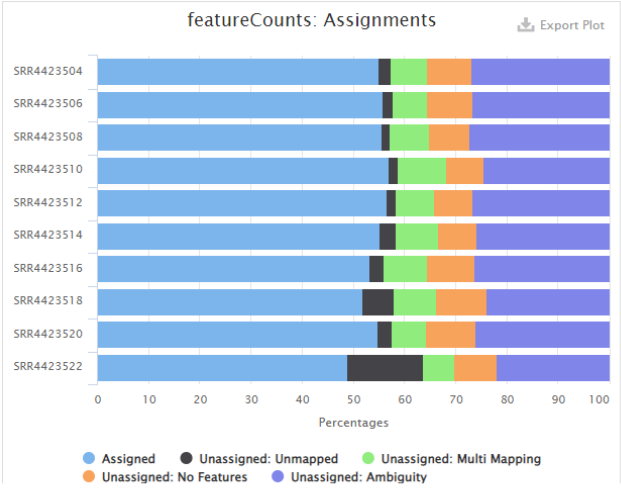
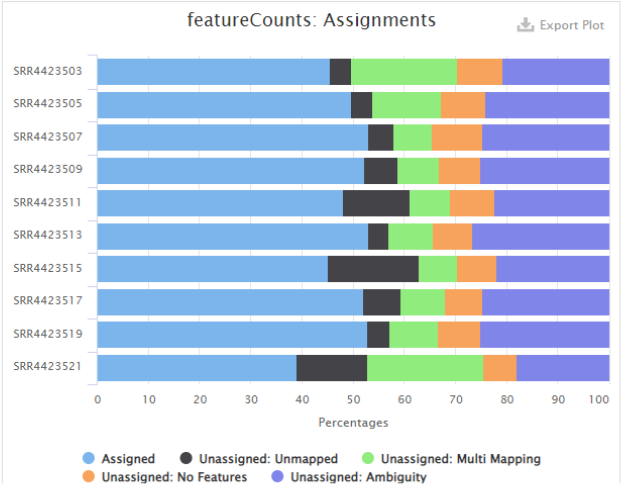


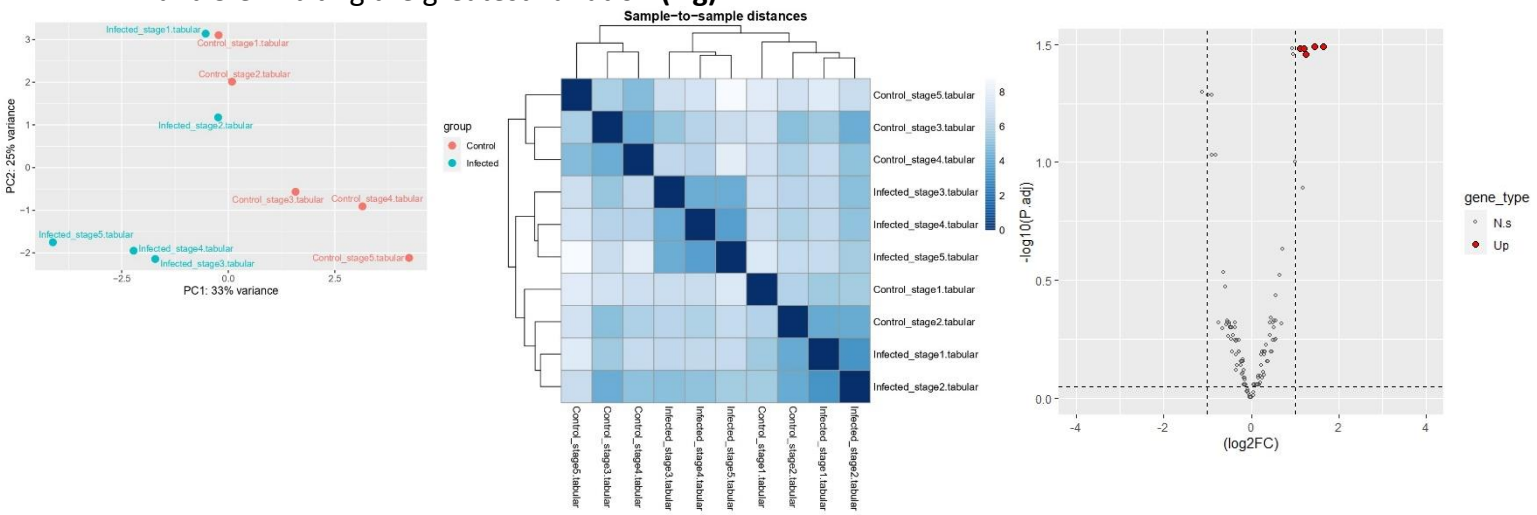
mRNA single-end data



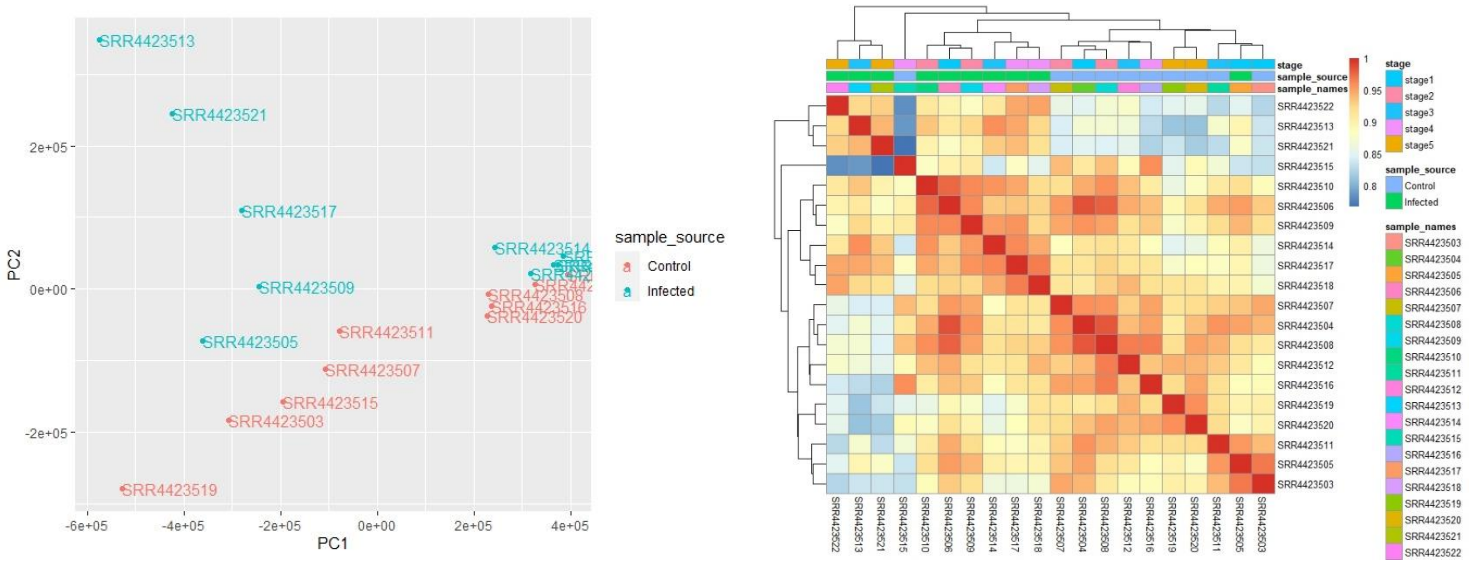
mRNA single-end data

2- Differential expression Analysis

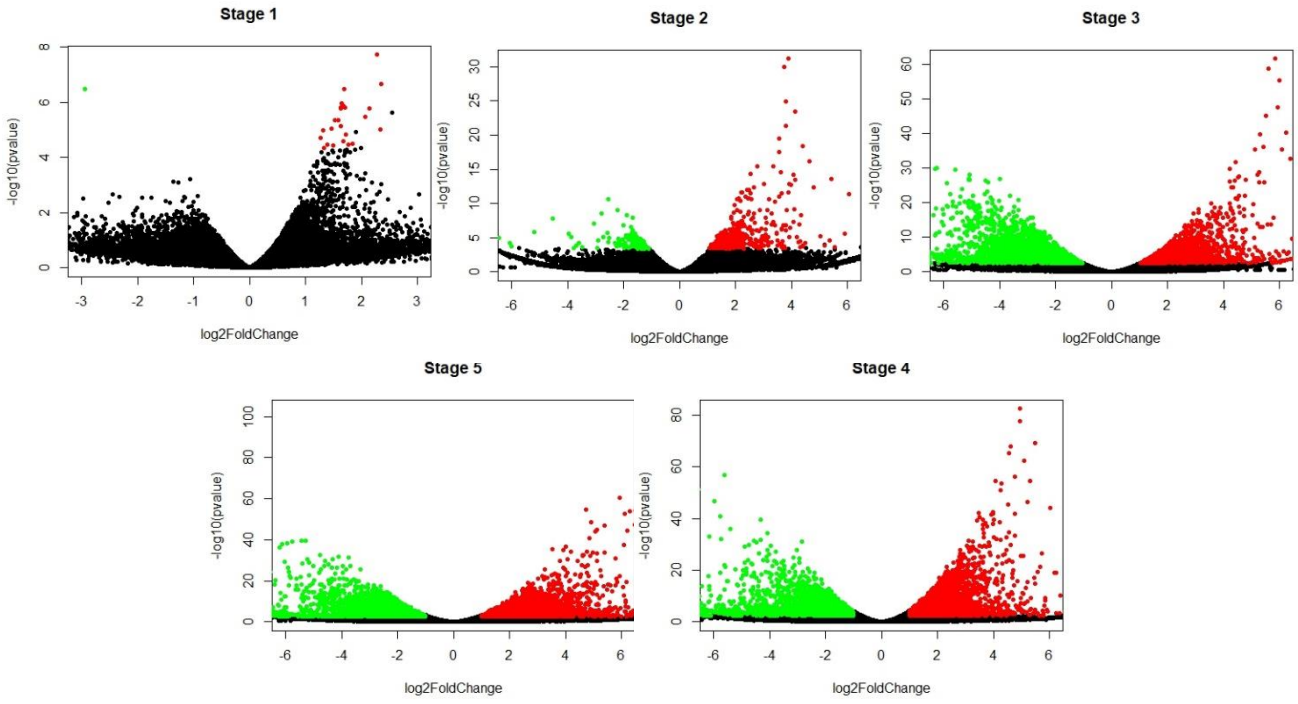
The lack of biological replicates in the miRNA dataset prevented separate differential expression analyses for each stage of tomato root infection by Meloidogyne incognita. As an alternative, we pooled the five normal samples across the five stages and compared them with the five infected samples across the same stages. Although this approach did not allow us to investigate stage-specific changes, it facilitated the identification of differentially expressed miRNAs. To address the limitations of the current miRNA dataset, we need to generate our own miRNA sequencing data to identify differentially expressed miRNAs across various stages of Meloidogyne incognita infection in tomato roots. We performed miRNA gene expression analysis using DESeq2 (v1.34.0) on the Galaxy platform (v2.11.40.7+galaxy2), and mRNA gene expression analysis using the DESeq2 package (v1.36.0) in the R language (v4.2.1) [7]. To control the false discovery rate, we applied the Benjamini-Hochberg (BH) method to adjust the raw P-values [8]. The thresholds for identifying differentially expressed miRNAs and mRNAs were set at an adjusted P-value <0.05 and |log2 fold change (FC)| ≥ 1. Although we observed upregulation of miR9474-5p, miR9474-3p, miR164a-5p, miR164a-3p miR164b-5p, and miR9472-5p, it is currently unclear at which developmental stages these miRNAs are upregulated **(Fig).** Therefore, further investigation is necessary to determine the stage-specific changes. To ensure the accuracy of our findings, it is important to note that a previous study has also reported upregulation of miR164a at stages 3 and 5 using qRT-PCR analysis [9]. For mRNA analysis, our analysis revealed an increase in the numbers of both upregulated and downregulated genes across the developmental stages when comparing normal tomato roots with those infected by Meloidogyne incognita. However, it is important to note that there was noticeable variance among biological samples, with stages 3, 4, and 5 exhibiting the greatest variation **(Fig).**



miRNA DESeq2 analysis



mRNA DESeq2 analysis



mRNA DESeq2 analysis

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