kiwifruits in response to Pseudomonas syringae pv. Actinidiae

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

Psa inoculation

1 Psa was cultured on beef peptone medium for 24 h at 20°C.

2 The microbial concentration of Psa was diluted to 10⁸ colony-forming units (cfu)/ml prior to inoculation.

3 For inoculation, the detached shoots were surface sterilized with chlorine and then cut into 10 cm shoots and the ends of the shoots were dipped in candle wax to reduce dehydration.

4 A wound was made with a file about 1-1.5 cm from each end of the shoot and Psa was added to the wound with a pipette.

5 The inoculated shoots were placed in trays, which were placed in an artificial climate incubator at 20°C and 80% relative humidity for 12 h day/night cycles.

RNA extraction

6 Shoot samples were taken from the inoculated segments 0.5-1 cm away from the wound point at 0, 12, 24, 48, and 96 h after inoculation (hai). The samples were immediately placed in liquid nitrogen and stored at -80°C for RNA extraction and further analysis.

7 Total RNA was extracted using the RNA prep Pure Plant Kit (Polysaccharides & Polyphenolics-rich), following the manufacturer's protocol.

cDNA library construction

8 RNA concentration was measured using the Qubit RNA Assay Kit in a Qubit 2.0 Flurometer (Life Technologies, CA, USA) and RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). Following RNA quantification and qualification, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads and the mRNA was then broken into fragments using a fragmentation buffer. First strand cDNA was synthesized using random hexamer primers, and second strand cDNA synthesis was subsequently performed using buffer, dNTPs, DNA Polymerase I, and RNase H. The library fragments were purified with AMPure XP beads (Beckman Coulter, Beverly, USA), and USER enzyme was used with size-selected, adaptor-ligated cDNA before PCR. PCR was performed to enrich the purified cDNA libraries.

Sequencing read mapping and identification of DEGs

9 the library preparations were sequenced on an Illumina HiSeq platform.

10 Raw reads in FASTQ format were generated by base calling. Clean reads were obtained by removing reads with adapters, reads containing more than 10% ploy-N (where N refers to unknown bases), and low quality reads. All subsequent analyses were based on the clean data, which were aligned to the reference genome using the TopHat v2.0.12 .

11 Gene expression levels were calculated using the FPKM method (expected number of fragments per kilobase of transcript sequence per millions of base pairs sequenced) using HTSeq v0.6.1 .

12 DEGs were analyzed using the DESeq R package (1.18.0) .

13 The P-values were adjusted using Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted P-value <0.05 found by DESeq were assigned as differentially expressed .

Gene ontology

14 Gene ontology enrichment analysis of DEGs was implemented using the GOseq R package. Gene ontology terms with corrected P <0.05 were considered significantly enriched in DEGs.

KEGG pathway analysis

15 KOBAS software was used to test the enrichment of DEGs in KEGG (Kyoto Encyclopedia of Gene and Genomes) pathways .

Gene co-expression network analysis

16 Gene dendrograms were constructed with colors based on the correlations between the expression of genes. These were used to build clustering trees, and to divide the modules.

17 the correlation between modules and samples was also analyzed by weighted gene co-expression network analysis.

Quantitative real-time PCR analysis

18 qRT-PCR was performed on a RT-PCR instrument (Roche 480, Basle, Switzerland).

19 Template cDNA was synthesized using a qPCR-RT Kit (TOYOBO)

20 The 2×SYBR Green I RT-PCR Master Mix (Roche) was used as a fluorescent reporter.

21 The reaction was performed with the following program: pre-incubation at 95°C for 5 min, and then amplification followed by 40 cycles of 95°C for 10s, 60°C for 20s, and 72°C for 20s. The relative gene expression levels were calculated using the 2-ΔΔc(t) method.