De novo assembly and functional annotation of *Citrus aurantifolia* transcriptome from *Candidatus* Liberibacter asiaticus infected and non-infected trees

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**Abstract**

Mexican lime (*Citrus aurantifolia*) belongs to the Rutaceae family and nowadays is one of the major commercial citrus crops in different countries. In Mexico, Mexican lime production is impaired by Huanglongbing (HLB) disease associated to *Candidatus* Liberibacter asiaticus (CLas) bacteria. To date, transcriptomic studies of CLas-Citrus interaction, have been performed mainly in sweet citrus models at symptomatic (early) stage where pleiotropic responses could mask important, pathogen-driven host modulation as well as, host antibacterial responses. Additionally, well-assembled reference transcriptomes for acid limes including *C. aurantifolia* are not available. The development of improved transcriptomic resources for CLas-citrus pathosystem, including

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both asymptomatic (early) and symptomatic (late) stages, could accelerate the understanding of the disease. Here, we provide the first transcriptomic analysis from healthy and HLB-infected *C. aurantifolia* leaves at both asymptomatic and symptomatic stages, using a RNA-seq approach in the Illumina NexSeq500 platform. The construction of the assembled transcriptome was conducted using the predesigned workflow Transflow and a total of 41,522 tentative transcripts (TTs) obtained. These *C. aurantifolia* TTs were functionally annotated using TAIR10 and UniProtKB databases. All raw reads were deposited in the NCBI SRA with accession numbers SRR10353556, SRR10353558, SRR10353560 and SRR10353562. Overall, this dataset adds new transcriptomic valuable tools for future breeding programs, will allow the design of novel diagnostic molecular markers, and will be an essential tool for studying the HLB disease.

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### Specifications Table

| Subject                  | Plant Biology |
|--------------------------|---------------|
| Specific subject area    | Transcriptomics|
| How data were acquired   | Illumina NexSeq500 sequencing platform |
| Data format              | Raw and analyzed |
| Parameters for data collection | Leaves of *C. aurantifolia* were collected from shadow-greenhouse at the Experimental Station Tecoman-INIFAP, Tecoman, Colima, Mexico. The samples were taken at four conditions: asymptomatic CLas-infected plants (early stage of HLB disease), symptomatic CLas-infected plants (late stage of HLB disease), and mock-inoculated control plants at both time points, early and late stages of the HLB infection. |
| Description of data collection | The RNA was sequenced separately (2 × 150 bp) on an Illumina NexSeq500 instrument MID-Output in Langebio-CINVESTAV, Mexico. |
| Data source location     | Instituto Politécnico Nacional, CIIDIR-Unidad Sinaloa. Guasave, Sinaloa, Mexico |
| Data accessibility       | NCBI SRA accession numbers of SRR10353562, https://www.ncbi.nlm.nih.gov/sra/SRR10353562, SRR10353560 https://www.ncbi.nlm.nih.gov/sra/SRR10353560, SRR10353558 https://www.ncbi.nlm.nih.gov/sra/SRR10353558, SRR10353556 https://www.ncbi.nlm.nih.gov/sra/SRR10353556 for raw reads; https://data.mendeley.com/datasets/99tbnvjshh/1 for the assembled and annotated transcriptome. |

### Value of the Data

- This data provides the first transcriptome of *Citrus aurantifolia* that merges the information from CLas-infected and non-infected leaves tissue.
- The de novo assembled transcriptome is useful as a reference transcriptome to other scientists working in the prediction and functional annotation of differentially expressed genes in Mexican lime and other acid limes.
- The RNA-seq dataset is available as raw sequence reads that can be further processed and analyzed by scientists.
- This data could be useful for citrus breeding programs and the designing of novel diagnostic tools.

### 1. Data

The transcriptome assembly and its annotation for *Citrus aurantifolia* leaves were generated from RNA samples including four conditions: asymptomatic CLas-infected plants (early stage of HLB disease, 8 weeks post inoculation, wpi), symptomatic CLas-infected plants (late stage of HLB disease, 16 wpi) and mock-inoculated control plants at both time points, 8 and 16 wpi. The four cDNA libraries were sequenced using the Illumina NexSeq500 platform, resulting in a total of 110, 572, 474 raw reads.
After removal of low-quality reads, eight different de novo assemblies were generated. Based on the structural annotation statistics of the full set of tentative transcripts, the best de novo assembled transcriptome is then presented in Table 2. This assembly was functionally annotated by TAIR 10 database, and with the plant division for UniProtKB (Fig. 1) and then Gene Ontology (GO) identity was assigned (Fig. 2).

Raw RNA-seq reads and the de novo transcriptome assembly can be accessed at the NCBI with the following accession numbers: SRR10353562 for the RNA-Seq of asymptomatic Mexican lime infected with CLas (8 wpi), SRR10353558 for the RNA-Seq of symptomatic Mexican lime infected with CLas (16 wpi) and SRR10353560 and SRR10353556 for the RNA-Seq of mock-inoculated Mexican lime (negative control plants) at 8 and 16 wpi, respectively.

### 2. Experimental design, materials, and methods

#### 2.1. Plant materials

Mexican lime (C. aurantifolia) plants on alemow (C. macrophylla) rootstock were kept in a pathogen free shadow-greenhouse at Experimental Station Tecoman-INIFAP, Tecoman, Colima, Mexico. Forty-five 9 months-old Mexican lime (C. aurantifolia) plants were CLas-inoculated by grafting with budwood from HLB-infected Mexican lime trees as inoculum source, and other fifteen plants were inoculated with budwood from healthy Mexican lime plants as negative control plants (mock-inoculated). Following inoculation, plants were kept in a shadow-greenhouse and fertilized if necessary. Foliar tissue including complete leaves and petioles (8 leaves) were collected from individual plants, at 8 and 16-weeks post inoculation (wpi) (asymptomatic/early, and symptomatic/late stages, respectively). For each sampled plant, 4 complete leaves were grinded with mortar and pestle in liquid nitrogen (for RNA-Seq analysis). The remaining 4 leaves were used for dissection of central midribs and petioles (tissue where bacteria is mainly located) for bacteria detection, and liquid nitrogen grinded. Finally, the tissue powder was stored at −80 °C prior to use. To quantify the CLas bacterial titer in grafting inoculated C. aurantifolia plants, an absolute quantitative PCR (qPCR) assay was performed [1]. Total DNA was extracted from the powdered tissue from midribs and petioles (about 200 mg) from individual plants, using a previously described CTAB protocol [2,3]. Bacterial titer quantification of the forty-five CLas-infected plants was carried out at both 8 and 16 wpi. Five CLas-infected plants with a similar bacterial titer at each time point (2.2 ± 1.1 × 10^2 bacterial cells/100 ng of total DNA at 8 wpi and 1.2 ± 0.8 × 10^4 bacterial cells/100 ng of total DNA at 16 wpi) and the corresponding five mock-inoculated control plants (at 8 and 16 wpi), were selected as tissue source for the RNA-seq analysis. The grinded leaf tissue from the five plants selected for each condition and time point, were pooled for the RNA extraction.

#### 2.2. RNA sequencing, de novo assembly and transcript annotation

Total RNA was isolated according to TRIzol® protocol (Sigma-Aldrich) from tissue of complete leaves. The yield and quality of the RNA was verified by assessing the A_{280}/A_{260} ratio by Nanodrop 2000

| Library        | Combined number of reads (paired-end) | Raw reads | Clean reads |
|----------------|---------------------------------------|-----------|-------------|
| L8wpHLB+1      | 28,409,266                            | 23,860,630|             |
| L8wpHLB-3      | 29,239,848                            | 24,967,772|             |
| L16wpHLB+5     | 26,237,006                            | 22,153,278|             |
| L16wpHLB-7     | 26,686,354                            | 23,111,948|             |
| **Total reads**| **110,572,474**                       | **90,093,628**|   |
Table 2
Structural annotation statistics of the full set of tentative transcripts Oases K-35/55 CD-Hit, the best de novo assembled transcriptome.

| Property                        | Tentative transcripts | Percentage |
|---------------------------------|-----------------------|------------|
| Total tentative transcripts     | 41,680                |            |
| Tentative transcripts           | 41,522                | 100.00%    |
| Tentative transcripts >500pb    | 35,735                | 86.06%     |
| With orthologue                 | 34,123                | 82.18%     |
| Different orthologue IDs        | 16,426                | 48.14%     |
| Complete transcripts            | 26,614                | 77.99%     |
| Different complete transcripts  | 13,294                | 38.96%     |
| ncRNA                           | 405                   | 0.98%      |
| Without orthologue              | 6994                  | 16.84%     |
| Coding (all)                    | 1438                  | 20.56%     |
| Coding > 200bp                  | 1438                  | 20.56%     |
| Coding > 500bp                  | 1084                  | 15.50%     |
| Unknown (all)                   | 5556                  | 79.44%     |
| Artifacts                       | 158                   | 0.38%      |

**Status**

|                             | Sure | Putative |  |
|-----------------------------|------|----------|---|
| Complete                    | 19,013 | 7601     | 45.79% | 18.31% |
| C-terminus                  | 781   | 2769     | 1.88%  | 6.67%  |
| N-terminus                  | 1967  | 607      | 4.74%  | 1.46%  |
| Internal                    | 1385  |  | 3.34%  |
| Coding                      | 296   | 1142     | 0.71%  | 2.75%  |
| ncRNA                       | 405   |          | 0.98%  |
| Unknown                     | 5556  |          | 13.38% |

**Fig. 1.** Number of annotated tentative transcripts of the *C. aurantifolia* transcriptome using different public databases.
Spectrophotometer, and RNA integrity was determined using a 2100 Bioanalyzer RNA Nanochip (Agilent, CA, USA). Total RNA from selected plants for each condition was pooled in equimolar ratio to construct each cDNA libraries. The cDNA libraries with fragments ≈ 500 base pairs (bp) were constructed according to the manufacturer’s instructions using the TruSeq Stranded mRNA Sample Preparation kit (Illumina, San Diego, CA), and sequenced separately (2 × 150 bp) on an Illumina NexSeq500 instrument MID-Output by Langebio-CINVESTAV, Irapuato facilities (Mexico). A total of 110, 572, 474 (more than 25 million reads for each library) raw reads were generated. Quality of raw reads was assessed with FastQC tool with default parameters and then pre-processed to remove adapter and contaminant sequences and low quality reads using SeqTrimNext [4]. After removal of low-quality reads, 90, 093, 628 clean reads (i.e. 85 % of raw reads) were then assembled to generate eight C. aurantifolia transcriptomes using TransFlow [5] which is a modular assembling framework that combines different assembling strategies to finally select the most accurate de novo transcriptome. Assembling strategies of clean reads was based on two assemblers based on Bruijn algorithms: Oases [6] and SOAPdenovo [7] using K-mer parameters of 35, 55, and combined. Additionally, to reduce redundancy, the resulting contigs obtained with Oases K-mers 35/55 and SOAPdenovo K-mers 35/55

Fig. 2. GO category distribution of AgriGO annotated C. aurantifolia Tentative Transcripts.
were clustered with CD-HIT [8] and then reconciled with Minimus [9] using the default parameters. Completeness and quality of the eight assemblies were analyzed as described for TransFlow [5] to identify the best transcriptome assembly based on principal component analysis (PCA). The best assembling strategies correspond to the ones having the shortest distance to the A. thaliana (TAIR10) reference transcriptome. Finally, structural annotation of the complete set of TTs from Oases K-mers 35/55 CD-Hit assembling was obtained using Full-LengtherNext [5], preferentially annotating with A. thaliana proteome from TAIR 10 database, and then with the plant division of UniProtKB. The best transcriptome consisted of 41,522 Tentative Transcripts (TTs). The protein-transcript orthology was assessed using Full-LengtherNext (5). Gene ontology (GO) enrichment was analyzed by AgriGO version 2.0 [10]. Enriched GO terms were selected using Singular Enrichment Analysis (SEA) with the A. thaliana reference genome as background (TAIR10_2017). The over-represented terms in the three categories biological process, cellular component, and molecular function were filtered by statistical information using an FDR-adjusted p-value of $<0.05$.

Acknowledgments

This work was supported by Instituto Politécnico Nacional, Mexico (SIP20194949) and by Ministerio de Economía, Industria y Competitividad/FEDER, Spain (AGL2016-75819-C2-1). APAL tanks CONACyT and BEIFI IPN programme.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2020.105198.

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