Nutrient Uptake in Huanglongbing-affected Sweet Orange: Transcriptomic and Physiological Analysis

Faisal Shahzad, Changpin Chun, Arnold Schumann, and Tripti Vashisth

University of Florida/Institute of Food and Agriculture Science, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850

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ABSTRACT. Since the advent of Huanglongbing [HLB (*Candidatus Liberibacter asiaticus*)] in Florida, several preliminary reports have emerged about the positive effects of mineral nutrition on the performance of HLB-affected citrus (*Citrus* sp.) trees. HLB-affected trees are known to undergo significant feeder root loss. Therefore, studies have focused on foliar nutrient application instead of soil-applied nutrients speculating that the HLB-affected trees root systems may not be competent in nutrient uptake. Some studies also suggest that HLB-affected trees benefit from micronutrients at higher than the recommended rates; however, the results are often inconclusive and inconsistent. To address this, the goal of the present study was to evaluate the nutrient uptake efficiency and the quantitative and qualitative differences in nutrient uptake of HLB-affected trees. HLB-affected and healthy sweet orange (*Citrus sinensis*) trees were grown in a 100% hydroponic system with Hoagland solution for 8 weeks. The trees were deprived of any fertilization for 6 months before the transfer of trees to the hydroponic solution. Altogether, the four treatments studied in the hydroponic system were healthy trees fertilized (HLY-F) and not fertilized (HLY-NF), and HLB-affected trees fertilized (HLB-F) and not fertilized (HLB-NF). HLY-F and HLY-NF trees were found to have similar levels of leaf nutrients except for N, which was found to be low in nonfertilized trees (HLY and HLB). Both HLB-F and HLB-NF trees had lower levels of Ca, Mg, and S compared with HLY trees. In addition, HLB-NF trees had significantly lower levels of micronutrients Mn, Zn, and Fe, compared with HLY-NF trees. The hydroponic solution analysis showed that HLB-F and HLY-F trees had similar uptake of all the nutrients. Considering that HLB-affected trees have a lower root-to-shoot ratio than healthy trees, nutrient uptake efficiency per kilogram of root tissue was significantly higher in HLB trees compared with HLY trees. Under nutrient-deficient conditions (day 0) only nine genes were differentially expressed in HLB roots compared with HLY roots. On the other hand, when fertilizer was supplied for ≈ 1 week, ≈ 2300 genes were differentially expressed in HLB-F roots compared with HLY-F roots. A large number of differentially expressed genes in HLB-F were related to ion transport, root growth and development, anatomic changes, cell death, and apoptosis compared with HLY-F trees. Overall, anatomic and transcriptomic analyses revealed that HLB-affected roots undergo remarkable changes on transitioning from no nutrients to a nutrient solution, possibly facilitating a high uptake of nutrients. Our results suggest the roots of HLB-affected trees are highly efficient in nutrient uptake; however, a small root mass is a major limitation in nutrient uptake. Certain micronutrients and secondary macronutrients are also metabolized (possibly involved in tree defense or oxidative stress response) at a higher rate in HLB-affected trees than healthy trees. Therefore, a constant supply of fertilizer at a slightly higher rate than what is recommended for micronutrients and secondary macronutrients would be beneficial for managing HLB-affected trees.

Currently, HLB affects 90% of commercial citrus (*Citrus* sp.) orchards (Singerman and Useche, 2016) and is the biggest challenge for the Florida citrus industry. With the epidemic of HLB, FL orange production has dropped by $\approx 70\%$ (U.S. Department of Agriculture, 2019) in the past 15 years. HLB is a bacterial disease caused by the fastidious phloem-restricted bacterium *Candidatus Liberibacter asiaticus* (CLAs). Once CLAs is transmitted to a citrus tree, the sieve pores are plugged and phloem disruption is observed (Kim et al., 2009). The disruption of vascular function, loss of root mass, and altered mineral nutrition in CLAs-infected trees lead to arrested tree and fruit growth, increased fruit drop, a decline in production, and could eventually lead to tree death (Bové, 2006; Halbert and Manjunath, 2004; Tang et al., 2019). Visible symptoms of HLB typically include yellowing of leaves, blotchy mottle, chlorotic patterns of

leaves resembling those induced by Zn and Fe deficiencies, shoot dieback, and reduced tree height (Bové, 2006).

There is no cure for HLB yet; therefore, citrus growers often face challenges for both replanting and managing their mature orchards. Several reports on the use of enhanced nutritional products to extend the vigor and productivity of HLB-affected trees by commercial citrus growers have been documented in the past several years (Spann and Schumann, 2012; Vashisth and Vincent, 2018). In a recent greenhouse study, HLB-affected sweet orange (*C. sinensis*) leaves had lower concentrations of K, Ca, Mg, Cu, Fe, Zn, Mn, and B compared with leaves from healthy trees (Morgan et al., 2016). The same study also reported using three times the recommended rates of Mn and Zn (for healthy citrus by University of Florida) resulted in the improvement of health of HLB-affected trees (Morgan et al., 2016). Zambon et al. (2019) reported the therapeutic effects of high rates of Mn (three to four times higher than recommended rates) on CLAs reduction in HLB-affected trees, thereby improving the productivity of HLB-affected sweet orange. Recently, in addition to enhanced fertilization, soil acidification for HLB-affected trees has become recommended. Irrigated Florida soil has a high pH due to high bicarbonate levels; this makes certain nutrients unavailable for uptake. Therefore, some citrus growers are opting

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F.S. and C.C. contributed equally to the manuscript.

T.V. is the corresponding author. E-mail: tvashisth@ufl.edu.

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for soil acidification and are observing improvement in yield and health of the trees (Morgan and Graham, 2019). Florida soil already had a high pH pre-HLB, but nutrient availability has become an issue since HLB's onset. Therefore, such field and scientific observations on nutrients, especially secondary macronutrients and micronutrients, raises a question about the nutrient uptake potential and nutrient requirement in HLB-affected trees. It is well known that HLB-affected trees undergo significant root loss on CLas infection (Graham et al., 2013). The feeder root dieback starts before visible symptoms appear on the canopy (Johnson et al., 2014). Overall, the root-to-shoot ratio decreases in HLB-affected trees. Hamido et al. (2017a, 2017b) showed that reduced water use and uptake in HLB-affected trees, but increasing the frequency of irrigation for HLB-affected trees can improve water uptake and use. Such findings raise numerous questions about feeder root performance and nutrient uptake efficiency in HLB-affected trees. To circumvent the impaired, HLB-affected roots for nutrient uptake many growers have switched to foliar nutrient application (Giles, 2011; Morgan et al., 2016). The nutrient uptake and required amount of nutrients in healthy citrus are well studied, where soil-application of all nutrients with supplemental foliar application of micronutrients is highly recommended (Obreza and Morgan, 2008). However, understanding nutrient uptake capacity and the potential of HLB-affected roots and if HLB-affected trees have a preferential uptake/requirement of certain nutrients is still lacking. Therefore, the objectives of this study were 1) to determine the nutrient uptake efficiency of HLB-affected trees, 2) to investigate the qualitative and quantitative differences in nutrient uptake among HLB-affected and healthy trees, and 3) to determine the molecular regulation of nutrient uptake in HLB-affected trees.

Materials and Methods

TREE MATERIAL AND EXPERIMENTAL DESIGN. Two-year old 'Midsweet' sweet orange budded on 'Kuharske Carrizo' citrange (*C. sinensis* × *Poncirus trifoliata*) rootstock potted (pot dimensions: 10.2 × 10.2 × 35.6 cm) in commercial growing medium (mixture of peat/perlite/vermiculite at 3:1:1 by volume) were used in this study. To obtain HLB-positive trees, the trees were budded with CLas-infected buds, whereas the healthy trees were mock-inoculated with healthy buds. Three months after inoculation, trees were confirmed for CLas infection by quantitative real-time polymerase chain reaction (qRT-PCR), as described by Vashisth and Livingston (2019). Uniform looking HLB-positive and healthy trees were selected and were kept in greenhouse for 6 months where they were not fertilized to create nutrient-deficient conditions. Throughout the experiment (including plant preparation time) the plants were grown under natural light conditions in the greenhouse; the temperature and relative humidity of the greenhouse fluctuated between 22 and 25 °C and 60% to 80%, respectively. At the end of 6 months, both HLB-affected and healthy trees were transferred to a 100% hydroponic system [deionized (DI) water] with continuously running air pumps. Black 5-L buckets with lids were used for hydroponic setup. The lids of the buckets were cut out to fit the tree trunk and the trees were suspended in the buckets with help of a trellis. The experimental setup was a completely randomized block design (n = 4), where four trees were considered as one replicate. Trees were given 1 week for acclimatization to the hydroponic system before beginning the experiment. After 1 week, Hoagland

solution (fertilizer) was added to a set of HLB-affected and healthy trees, whereas the other set of trees continued in the hydroponic system with no Hoagland solution (without fertilizer). Hoagland solution was prepared by mixing the reagents as follows: Group A (macronutrients): 5 mM Ca(NO₃)₂·4H₂O, 5 mM KNO₃, 2 mM MgSO₄·7H₂O, and 1 mM KH₂PO₄; Group B (micronutrients): 46 μM H₃BO₃, 9 μM MnCl₂·4H₂O, 0.8 μM ZnSO₄·7H₂O, 0.4 μM CuSO₄·7H₂O, 0.02 μM (NH₄)₆MoO₄·4H₂O, 0.02 μM FeSO₄·7H₂O, and 0.02 μM EDTA-Na₂. Group A and B solutions were diluted 1:100 and 1:1000 (v:v), respectively, in the DI water. The water level was maintained at the 3.5-L mark in the bucket throughout the experiment (measured and replenished every 3 d). The four treatments: 1) healthy trees with fertilizer solution (HLY-F), 2) HLB-affected trees with fertilizer solution (HLB-F), 3) healthy trees without fertilizer solution (HLY-NF), and 4) HLB-affected trees without fertilizer solution (HLB-NF) were evaluated for the following variables during the course of 8 weeks.

TREE BIOMASS AND GROWTH. Trees were destructively harvested and leaf, shoot, and root fresh and dry weights were measured at the end of the experiment. Dry weight was measured by drying the samples at 68 °C for ≈72 h in a convection oven (Thermo Fisher Scientific, Waltham, MA). Increase in the number of leaves, tree height, and stem diameter was determined by calculating the difference from the time of adding fertilizer solution (day 0) to the end of the experiment and is expressed as a percentage change. Leaf chlorophyll index (SPAD value) was measured for five same-age leaves on days 0, 7, 14, 28, 42, and 56 using a chlorophyll concentration meter (MC-100; Apogee Instruments, Logan, UT).

PHYSICAL, CHEMICAL, AND NUTRIENT PROFILE OF GROWING MEDIA AND LEAVES. A digital pH/conductivity meter (Orion Star A215; Thermo Fisher Scientific) was used to measure the pH and electrical conductivity (EC) (millisiemens per centimeter) of the hydroponic solution on days 0, 7, 14, 28, 42, and 56 of the experiment.

On days 0, 28, and 56, 20-mL samples of the hydroponic solutions were collected. Leaves were collected at the end of the experiment and were washed using 1% (v/v) anionic detergent (Liqui-Nox; AlcoNox, White Plains, NY), rinsed with DI water, followed by drying at 68 °C for 48 h, and then were milled using a grinder (Fex IKA A11; IKA-Werke, Staufen, Germany). The ground leaf and hydroponic solution samples were sent to Waters Agricultural Laboratories (Camilla, GA) to perform a standard nutrient analysis. Based on the nutrient concentration in the hydroponic solution, the nutrient uptake efficiency of roots (milligrams nutrient per kilogram of root) was calculated for HLB-F and HLY-F trees using the formula:

$$\text{nutrient uptake efficiency} = \left[\frac{\text{mineral nutrient concentration (milligrams per liter) in hydroponic solution on day 0} - \text{mineral nutrient concentration (milligrams per liter) in hydroponic solution on day 56}}{\times 3.5 \text{ L}} \right] / \text{fresh root biomass (kilograms)}.$$

ROOT ANALYSIS. At the end of the experiment, feeder roots of HLB-F and HLY-F trees were categorized in new and old

growth. Different root variables such as length, diameter, volume, and surface area were calculated using WinRHIZO software (version 2012b; Regent Instruments, Quebec City, QC, Canada).

For anatomic analysis, ≈ 0.5 -cm-long feeder roots were collected at five different locations from the root system on days 0 and 14. Root samples were fixed in a 50% ethyl alcohol, 10% formalin, and 5% acetic acid solution and stored at 4 °C until further analysis. Paraffin protocol was followed from Ruzin (1999) with slight modifications. Briefly, samples were rinsed in DI water with graded series of ethanol, embedded in paraffin, and sectioned (10 μ m) using a microtome. After removal of paraffin from the sections, Toluidine Blue (0.1% of aqueous solution) was used for staining. Samples were analyzed under a charge-coupled device digital camera (Omax 14.0 MP; Olympus, Tokyo, Japan) attached to an epifluorescence microscope (BX61; Olympus). Image analysis was done using ImageJ software (National Institutes of Health, Bethesda, MD) (Schindelin et al., 2012).

TRANSCRIPTOME ANALYSIS. Transcriptome analysis was performed on two treatments: HLY-F and HLB-F. Feeder roots were collected from the same order of roots on days 0, 3, and 9. The root samples were immediately flash frozen in liquid nitrogen and stored in -80 °C until further analysis. Total RNA was extracted from 100 mg of root samples using modified TRIzol protocol (Chomczynski and Sacchi, 1987). The quantity and quality of extracted RNA were evaluated using a spectrophotometer (Epoch 2 Microplate; BioTek Instruments, Winooski, VT) and denaturing formaldehyde 1.2% agarose gels (Rio, 2015), respectively. For global transcriptome analysis using RNA sequencing (RNAseq), the samples were sent to the Interdisciplinary Center for Biotechnology Research, University of Florida (Gainesville). The illumina TruSeq mRNA protocol (Illumina, San Diego, CA) was used for the transcript sequencing. The *C. sinensis* genome v1.1 (Wu et al., 2014) from the Phytozome database was used as a reference genome for transcript sequencing. Differential gene expression (DEG) was analyzed using RSEM v1.2.31 (Li and Dewey, 2011) with a cutoff of the absolute value of $\log_2$ fold change (LFC) greater than 1. A false discovery rate (FDR)-corrected probability value of less than or equal to 0.05 was used as the threshold. It should be noted that the DEGs presented in the current study were significantly upregulated or downregulated in HLB-F roots compared with HLY-F roots.

Multiple tools were used to study the biological significance from the results of RNAseq. MapMan software [version 3.5.1.R2 (Thimm et al., 2004; Usadel et al., 2005)] was used to identify the biochemical or physiological processes represented by DEGs; the percentage of DEGs in each functional category (BIN) is reported over total number of DEGs. To get statistically significant ($P < 0.05$) and nonredundant gene ontology (GO) terms for individual upregulated and downregulated DEGs, gene sets from treatments at each time points were annotated using AgriGO in tandem with REVIGO (Supek et al., 2011;

Tian et al., 2017), respectively. In addition, the enzymes encoded by DEGs involved in the metabolic pathways were determined by using the Kyoto Encyclopedia of Genes and Genomes (KEGG) Mapper tool (Kanehisa et al., 2017).

RELATIVE GENE EXPRESSION ANALYSIS. To validate the results of RNAseq analysis, qRT-PCR was used to determine the relative expression of eight selected genes including *senescence-associated gene 20* (SAG20), *ethylene response factor 1* (ERF1), *calcium dependent calmodulin* (CAM8), *2-oxoglutarate and Fe dependent oxygenase superfamily protein* (OG-FE), *auxin-responsive family protein* (ABF3), and *zinc transporter 10 precursor* (ZIP10). The two normalization genes used were *actin* (ACT) and DIM1 homolog/YLS8 (DIM). Complementary DNA synthesis, qRT-PCR reaction, and gene expression calculations were done as described in Vashisth and Livingston (2020). List of genes, GenBank accession number, and primers used are presented in Supplemental Table 1.

STATISTICAL ANALYSIS. All the data were analyzed using a one-way or two-way analysis of variance in Sigma Plot (version 12; Systat Software, San Jose, CA). Mean separation among treatments was performed using Tukey's honestly significant test at $\alpha \leq 0.05$.

This experiment was conducted twice to validate the findings; however, the transcriptomic and anatomical analysis was performed only on the second experiment due to limited resources. The greenhouse and nutrient analysis results of the two experiments were similar; thus, the results presented herein are from the second experiment.

Results

Tree biomass and growth

At the end of the experiment, both HLB-F and HLB-NF trees had significantly lower total biomass as well as individual root, shoot, and leaf biomass compared with HLY-F and HLY-NF trees (Table 1). The root, shoot, and leaf biomass were not different among HLB-F and HLB-NF or HLY-F and HLY-NF trees. HLY-F trees had the highest increase in leaf number and HLB-F, HLB-NF, and HLY-NF had the similar increase. There was no significant increase in the growth of fertilized (both HLB and HLY) trees compared with nonfertilized trees (both HLB and HLY; Table 1). At the beginning of the experiment, HLB-affected trees showed a 30% lower SPAD value compared with healthy trees (Fig. 1). The SPAD value for HLY-F

Table 1. Growth parameters of Huanglongbing (HLB)-affected and healthy (HLY) sweet orange trees grown in hydroponic solution with fertilizer (F) and no-fertilizer (NF) for 56 d.

Parameter	HLB-F	HLY-F	HLB-NF	HLY-NF	P value
Fresh root biomass (g) ^a	11.2 b ^y	24.3 a	13.3 b	22.7 a	0.001
Fresh shoot biomass (g)	15.9 b	25.1 a	17.3 b	23 a	0.007
Fresh leaf biomass (g)	6.5 b	15.1 a	7 b	9 a	0.040
Fresh whole tree biomass (g)	33.6 b	64.4 a	37.7 b	54.7 a	0.002
Change in number of leaves ^x	18.9 b	22.5 a	11.2 b	12.3 b	0.005
Increase in tree height (%)	10.5	12.3	7.4	8.1	NS
Increase in stem diameter (%)	2.1	3.0	1.2	1.3	NS

^aFresh root biomass, fresh shoot biomass, fresh leaf biomass, and fresh whole tree biomass are absolute values measured at the end of experiment (56 d).

^yMeans followed by different letters indicate statistically significant differences among different treatments for given parameter using Tukey's honestly significant difference test at $\alpha = 0.05$.

^xNumber of leaves, increase in tree height, and increase in stem diameter are calculated by considering the difference in measurement between day 56 and day 0.

Fig. 1. Average leaf chlorophyll index expressed as SPAD value for Huanglongbing (HLB)-affected and healthy (HLY) sweet orange trees grown under fertilized (F) and nonfertilized (NF) hydroponic conditions during the course of experiment (from day 0 to 56). Before day 0 both HLB and HLY plants were grown in nutrient-deficient conditions for 6 months.

did not change during the experiment but increased in HLB-F trees over 8 weeks. HLB-F and HLY-F had same SPAD value at the end of the experiment. The SPAD value of HLB-NF and HLY-NF decreased by 24% and 43%, respectively, from the start until the end of the experiment.

Physical, chemical, and nutrient profile of growing medium and leaves

No significant differences were found in the pH of the hydroponic solution of HLB and HLY trees (F and NF) at the end of the experiment (data not shown). EC decreased by 40% during the experiment as the fertilizer depleted from the hydroponic solution; 2.06 and 2.08 $\text{mS}\cdot\text{cm}^{-1}$ to 1.25 and 1.27 $\text{mS}\cdot\text{cm}^{-1}$ in HLB-F and HLY-F, respectively.

No significant difference was observed in water use among HLB-F and HLY-F trees. There was also no difference in water use for HLB-NF and HLY-NF trees; however, the water use in HLB-F and HLY-F trees was significantly higher than HLB-NF and HLY-NF trees, respectively. Mineral nutrient analysis of the hydroponic solution was performed on days 0, 28, and 56 to determine the quantitative and qualitative differences in the nutrient uptake of HLB-F and HLY-F trees; all the nutrients in HLB-F and HLY-F trees were at the same concentration on day 0 of the experiment. Overall, the nutrient concentrations decreased in the hydroponic solution of both HLB-F and HLY-F as the experiment progressed, suggesting nutrient uptake by the trees. At the end of the experiment, all nutrients in the HLB-F and HLY-F growing media solutions were at similar concentrations. Interestingly, on day 28, the P uptake in HLB-F trees was lower than in HLY-F trees as suggested by the P concentration in the hydroponic solution (Supplemental Fig. 1), although it was similar on day 56. Manganese and Fe concentrations in the hydroponic solution of both HLB-F and HLY-F trees decreased abruptly by day 28 and on day 56 very little ($<0.05 \text{ mg}\cdot\text{L}^{-1}$) was found, suggesting a rapid uptake of these nutrients by the trees.

At the end of the experiment, the nutrient analysis of the leaves showed that HLB-NF trees had a significantly lower concentration for most of the nutrients as compared with HLY-

NF (Fig. 2). In HLY-F and HLY-NF, the concentration for 9 of 10 nutrients was the same. HLY-NF trees had lower N concentrations than HLY-F; a similar trend was seen for HLB-F and HLB-NF trees. Overall, the nutrient concentrations in HLB-F trees and HLY-F trees were equivalent except for the secondary macronutrients Ca, Mg, and S, where a significant effect on tree health (disease) was seen. Both HLB-F and HLB-NF trees were lower in Ca, Mg, and S than HLY-F and HLY-NF trees. A significant interaction between tree health and fertilization was observed in K, Mn, Fe, and Zn, where HLB-NF trees had lower concentrations of the respective nutrients than HLB-F, HLY-F, and HLY-NF trees. No significant differences were found in P and B concentrations in HLB-F, HLB-NF, HLY-F, and HLY-NF trees.

At the end of the experiment, nutrient uptake efficiency was calculated as milligram of nutrient per kilogram of root tissue in both HLB-F and HLY-F. On average, HLB-F trees had 2- to 3-fold higher ($P < 0.05$) nutrient uptake efficiency for both macro- and micronutrients than HLY-F trees (Fig. 3). The concentrations of all the nutrients in the hydroponic solution of HLB-F and HLY-F trees were similar at the end of the experiment, which reinforces the nutrient uptake efficiency results.

Root analysis

HLB-F trees had a lower average feeder root diameter ($\leq 2 \text{ mm}$), root volume, and root length than HLY-F trees (Table 2), but no significant difference was observed in the root diameter and volume of new feeder root growth in HLB-F and HLY-F. However, the length of newly grown feeder roots in HLB-F trees was significantly shorter than new growth in HLY-F. Old feeder roots of HLB-F were lower in diameter, volume, and length than HLY-F roots. According to an ImageJ analysis for day 0, HLB-F root had a lower vascular bundle diameter and a lower ratio of vascular bundles to root diameter (0.28) than HLY-F trees (0.39) (Fig. 4). Both HLB-F and HLY-F roots showed an increase in vascular bundle diameter when grown under the fertilizer condition. An increase was observed in the ratio of vascular bundles to root diameter of 1.7-fold in HLB-F and 1.4-fold in HLY-F on day 14 compared with day 0. The mean diameter of vascular bundles and vascular bundles to the diameter ratio was significantly higher in HLY-F trees than HLB-F trees on day 14 (Fig. 4), although when the increase in the ratio of vascular bundle to diameter between days 0 and 14 was evaluated, the increase in HLB-F trees was significantly higher than HLY-F trees.

Transcriptomic analysis

A total of 9, 19, and 2324 DEGs were expressed in HLB-F and HLY-F roots on days 0, 3, and 9, respectively (Table 3). Notably, all 9 DEGs in HLB-affected roots were upregulated on day 0. In HLB-F trees, 5 and 14 DEGs were upregulated and downregulated on day 3, respectively. On day 9, 627 and 1697 DEGs were upregulated and downregulated in HLB-F, respectively, compared with HLY-F roots.

Due to a small number of DEGs in HLB-F and HLY-F roots on days 0 and 3, an enrichment analysis (either via MapMan or GO) could not be performed. Gene names with description and LFC of the nine DEGs in HLB-F roots on day 0 are presented in Table 4.

An overview comparing the metabolic processes of HLB-F roots and HLY-F roots on day 9 was obtained using the MapMan enrichment analysis (Fig. 5). The BINs that included

Fig. 2. Leaf mineral nutrient content of sweet orange trees with two disease indexes {DI [Huanglongbing (HLB)-affected and healthy (HLY)]} grown in hydroponic media of two fertilizer conditions {FC [fertilized (F) and nonfertilized conditions (NF)]} for 56 d. Before addition of fertilizer, both HLB and HLY trees were grown in nutrient-deficient conditions for 6 months. Different letters in each panel indicate significant difference based on Tukey's honestly significant difference test ($\alpha = 0.05$); NS = not significantly different.

more than 2% DEGs were BIN 35 with no assigned functions (29% DEGs), followed by protein (BIN 29; 12% DEGs), RNA (BIN 27; 11% DEGs), signaling (BIN 30; 9% DEGs), stress (BIN 20; 5% DEGs), transport (BIN 34; 5% DEGs), cell (BIN 31; 5% DEGs), DNA (BIN 28; 5% DEGs), development (BIN 33; 3% DEGs), hormone metabolism (BIN 17; 2% DEGs), and secondary metabolism (BIN 16; 2% DEGs). On day 9, GO enrichment analysis was performed using upregulated and downregulated DEGs in HLB-F roots compared with HLY-F roots (Fig. 6). For the upregulated DEGs

in HLB-F, the top 10 biological enriched GO categories were related to transport, cellular amino acid and derivative metabolic process, oxoacid metabolic process, organic acid biosynthesis processes, phenylpropanoid metabolic process, response to carbohydrate stimulus, ethylene and the jasmonic acid-mediated signaling pathway, and regulation of hormone levels. The top enriched GO categories based on the downregulated DEGs were the developmental process, phosphate metabolic process, protein modification process, defense response, growth, cell cycle, cell death, and abscisic

Fig. 3. Root nutrient uptake efficiency of Huanglongbing (HLB)-affected and healthy (HLY) sweet orange trees grown in hydroponic system with fertilizer (F) for 56 d. Different letters above each bar for each nutrient indicate significant difference between roots of HLB-affected and healthy trees based on Tukey's honestly significant difference test ($\alpha = 0.05$).

Table 2. Feeder root analysis of Huanglongbing (HLB)-affected and healthy (HLY) sweet orange trees grown with fertilizer (F) for 56 d.

Root characteristic	HLB-F	HLY-F	P value
Total feeder roots			
Root length (cm)	614.8 b ^z	1551.6 a	0.023
Average diameter (cm)	1.47 b	1.95 a	0.036
Root volume (cm ³)	2.49 b	6.02 a	0.036
New feeder roots			
Root length (cm)	47.4 b	139.5 a	0.037
Average diameter (cm)	0.73	0.65	NS
Root volume (cm ³)	0.23	0.47	NS
Old feeder roots			
Root length (cm)	567.3 b	1412.1 a	0.032
Average diameter (cm)	0.74 b	1.29 a	0.014
Root volume (cm ³)	2.27 b	5.55 a	0.045

^zMeans followed by different letters indicate statistically significant differences among different treatment combinations using Tukey's honestly significant difference test at $\alpha = 0.05$.

acid (ABA)-mediated signaling pathway. Five GO categories: metabolic process, response to stimulus, cellular nitrogen compound metabolic process, response to stress, and response to abiotic stress included both upregulated and downregulated DEGs. In all these GO groups, most of the genes were downregulated in HLB-F roots compared with HLY-F roots.

DEGS RELATED TO NUTRIENT TRANSPORT. A total of 65 DEGs related to transport were upregulated in HLB-F roots as compared with HLY-F roots; 20 of 65 DEGs were related to ion transport (GO:0006811) (Table 5). Similarly, the MapMan

analysis showed 5% of DEGs were related to transport. Most upregulated DEGs were involved in cation transport such as NH_4^+ , K, N, Zn, Fe, and Cu. Notably, Zn ion transport had the highest LFC (5.23) in this group. Upregulated DEGs that were involved in anion transport include PO_4^{3-} , B, Cl^- , and S transport. All 13 DEGs related to response in nutrient level (GO:0031667) were found to be upregulated in HLB-F roots compared with HLY-F (Table 5). In addition, a group of 115 DEGs belonging to the phosphate metabolic process (GO:0006796) were downregulated in HLB-F roots but not in HLY-F roots.

DEGS RELATED TO HORMONES. Based on the enriched GO categories and MapMan analysis, 33 DEGs were involved in hormone regulation and 2% DEGs in BIN 17 (hormone metabolism), respectively. Upregulated DEGs were related to hormonal regulation (GO:0010817), the jasmonic acid-mediated signaling pathway (GO:0009867), and the ethylene-mediated signaling pathway (GO:0009873). All the DEGs related to the ABA-mediated signaling pathway (GO:0009738) were downregulated in HLB-affected trees vs. healthy trees. These enriched GO category results agree with the outcome of MapMan: most DEGs involved in ABA (BIN 17.1), brassinosteroids (BIN 17.3), auxin (BIN 17.2), and salicylic acid (BIN 17.8) were downregulated (Fig. 6; Supplemental Tables 2 and 3). Whereas DEGs related to ethylene (BIN 17.5) and gibberellic acid (GA) (BIN 17.6) were upregulated in HLB-F roots compared with HLY-F roots (Fig. 7). Cytokinin (BIN 17.4) included one upregulated DEG and two downregulated DEGs.

DEGS RELATED TO STRESS, TREE-PATHOGEN/PEST INTERACTION, DEFENSE RESPONSE, AND SECONDARY METABOLISM. In HLB-F roots compared with HLY-F roots, 66 DEGs upregulated and 158 DEGs were downregulated, in response to stress (GO:0006950) (Fig. 6). Based on the results from the MapMan analysis, 5% of DEGs were related to BIN 20 (stress) (Fig. 5). In addition, tree-pathogen/pest interaction was highly enriched in HLB-F roots compared with HLY-F roots (Fig. 7; Supplemental Tables 3 and 4). The DEGs were related to ABA (BIN 17.1), auxin (BIN 17.2), brassinosteroids (BIN 17.3), glutathione S transferase (BIN 26.9), R-stress biotic receptors (BIN 20.1.2), peroxidases (BIN 26.12), WRKY transcription factors (BIN 27.3), cell wall biotic stress (BIN 10.5, 10.7 and 10.8), jasmonate (17.7.1), ethylene (17.5), pathogenesis-related (PR) proteins (BIN 20.1.7), salicylic acid (BIN 17.8.1), and MYB family protein (BIN 27.3.25) (Supplemental Table 3). Another highly affected category in tree-pathogen interactions was redox reactions. The associated DEGs were dismutase and catalase (BIN 21.6), redox thioredoxin (BIN 21.1), protein degradation (BIN 29.5), glutathione ascorbate (BIN 21.2.1), and glutaredoxin family proteins (BIN 21.4), suggesting that HLB-F trees were undergoing processes to mitigate oxidative stress. Fifty-five DEGs related to the GO term tree defense response (GO:0006952) were found to be downregulated in HLB-F roots compared with HLY-F roots. Fourteen DEGs related to the phenylpropanoid biosynthesis process were upregulated in HLB-F roots compared with HLY-F roots (Supplemental Fig. 2). An analysis via KEGG showed that the proteins encoded by 14 DEGs belonged to the phenylpropanoids process and lignin biosynthesis. Upregulated DEGs in the phenylpropanoid biosynthesis pathway were related to the genes that encoded phenylalanine ammonia lyase [PAL (EC 4.3.1.5)], hydroxycinnamoyl-coenzyme A shikimate:quinic acid hydroxycinnamoyl-transferase [HCT (EC 3.1.1)], caffeic acid/5-hydroxyferulic acid O-methyltransferase

Fig. 4. Longitudinal sections showing comparative anatomical changes in vascular bundles in feeder root of Huanglongbing (HLB)-affected and healthy (HLY) sweet orange trees at the start of experiment (day 0) and after 14 d after the addition of fertilizer (F) to hydroponic system. Before day 0 both HLB and HLY trees were grown in nutrient-deficient conditions for 6 months. Red arrow points at the difference in the vascular bundle diameter between days 0 and 14.

Table 3. Total number of differentially expressed genes (DEGs) among Huanglongbing (HLB)-affected and healthy (HLY) sweet orange trees grown in fertilizer solution (F) on days 0, 3, and 9 after the addition of Hoagland solution (fertilizer) to the hydroponic system. Before day 0 both HLB and HLY plants were grown in nutrient-deficient conditions for 6 months.

Comparison	DEGs	Upregulated DEGs	Downregulated DEGs
HLB-F vs. HLY-F day 0	9	9	0
HLB-F vs. HLY-F day 3	19	5	14
HLB-F vs. HLY-F day 9	2324	627	1697
HLB-F day 0 vs. d 3	8	8	0
HLB-F day 0 vs. d 9	23	7	16
HLB-F day 3 vs. d 9	57	31	26
HLY-F day 0 vs. d 3	1339	583	756
HLY-F day 0 vs. d 9	1659	773	886
HLY-F day 3 vs. d 9	2426	1241	1185

Table 4. List of nine differentially expressed genes in Huanglongbing (HLB)-affected sweet orange trees in comparison with healthy sweet orange trees on day 0 along with their orange gene IDs, log₂ fold change (LFC), and Arabidopsis homologs. Day 0 is the time point just before the addition of Hoagland solution (fertilizer) to the hydroponic system. Before day 0, both HLB and HLY plants were grown in nutrient-deficient conditions for 6 months.

Gene	LFC	Arabidopsis homologs	Description
orange1.lg014036m	2.07	AT3G51670.1	Phosphoglyceride transfer family protein
orange1.lg025435m	2.91	AT5G62720.1	Integral membrane HPP family protein
orange1.lg031858m	3.33	AT3G15680.1	Ran BP2/NZF zinc finger-like superfamily protein
orange1.lg030361m	3.57	AT1G69700.1	HVA22 homologue C
orange1.lg033664m	3.84	AT5G22580.1	Stress responsive A/B Barrel Domain
orange1.lg020451m	4.76	AT1G49570.1	Peroxidase superfamily protein
orange1.lg018585m	6.14	AT1G31260.1	Zinc transporter 10 precursor
orange1.lg046055m	6.44	AT1G75820.1	Leucine-rich receptor-like protein kinase family protein
orange1.lg032726m	7.76		Strictosidine synthase-related sub family/biosynthesis of terpenoid indole alkaloids present in meristematic tissue

[COMT (EC 2.1.1.68)], ferulate 5-hydroxylase (F5H), cinnamyl alcohol dehydrogenase [CAD (EC 1.1.1.195)], and peroxidases [POD (EC 1.11.1.7)].

DEGS RELATED TO DEVELOPMENT AND DEATH. This super GO group included four distinct GO terms: developmental process (GO:0032502), growth (GO:0040007), cell cycle (GO:0007049), and cell death (GO:0008219). The super group had 269 DEGs, which were all downregulated (Fig. 6) in HLB-F roots compared with HLY-F roots.

Relative gene expression

Gene expression and RNAseq results were found to be in agreement ($P = 0.015$, $r = 0.76$). A significant interaction effect between tree disease condition and days after treatment was found on the expression of *SAG20*, *ERF1*, *CAM8*, *OG-Fe*, *ABF3*, and *ZIP10* in HLB-F and HLY-F roots (Fig. 8). *SAG20* expression was significantly downregulated in HLB-F roots compared with HLY roots at day 0; on day 9 *SAG20* was upregulated in HLB-F roots resulting in similar expressions between HLB-F and HLY-F. On day 0, *ERF1* and *CAM8* in both HLB-F and HLY-F had similar expressions; however, on day 9 HLY-F roots showed significant downregulation of both genes but HLB-F remained the same. On the other hand, in *OG-Fe* and *ABF3*, both genes were equally expressed on day 0. On day 9 the expression in HLB-F roots was significantly downregulated for both genes. In *ZIP10*, no

the effect of HLB is more pronounced on roots as compared with the shoot system (Graham et al., 2013; Johnson et al., 2014).

During the experiment, the trees were grown with fertilizer for ≈ 2 months; the tree height and stem diameter were measured to estimate the growth of the trees at the end of experiment. No significant increases in tree height or stem diameter were observed among HLB (both F and NF) and HLY (both F and NF). Similarly, the tree biomass measured at the end of the experiment was not different for HLB-F and HLB-NF trees and HLY-F and HLY-NF trees, suggesting that the effect of fertilization on growth was not observed in the short term (Zekri and Koo, 1991). Often researchers do not see improvement in growth or yield of HLB-affected trees in only 1 to 2 years of HLB-nutrition research (Gottwald et al., 2012; Rouse et al., 2017; Vashisth, 2020). This suggests that HLB-nutrition research, like healthy citrus research, should be conducted for longer durations to appropriately evaluate the treatments, particularly with large, mature trees.

HLB-affected trees are well documented to have a low leaf chlorophyll content compared with healthy trees due to massive starch accumulation (Schneider, 1968) that occurs in chloroplasts and physically disrupts thylakoid structure (Sasek et al., 1985; Yelle et al., 1989). Both HLY and HLB trees in this study were grown in nutrient-deprived conditions for 6 months before the hydroponic and fertilizer treatments.

At day 0 the SPAD value of HLB-affected (F and NF) trees were significantly lower than HLY (F and NF) trees. However, when the HLB-F trees were given a nutrient solution, the leaf chlorophyll content significantly increased; at the end of the experiment, HLB-F and HLY-F trees had a similar leaf chlorophyll index. These observations suggest that good fertilization could improve the leaf chlorophyll index which then improves the photosynthetic capacity (Krause and Weis, 1991) of HLB-affected trees.

At the end of the experiment, all nutrients except N, were at similar levels among healthy trees (F and NF). Nitrogen was the only nutrient that was affected by the fertilizer condition of both HLB and HLY trees. On the other hand, when HLB-NF trees were compared with HLY-NF, the leaf nutrient content of Ca, Mg, S, K, Mn, Fe, and Zn were lower in HLB-NF trees, suggesting that HLB-NF trees were metabolizing these nutrients at a higher rate than HLY-NF trees. Manganese, Zn, and Fe are well known to be involved in the enzymatic process of reactive oxygen species (ROS) control and the sequestration

Fig. 5. Functional categories (BINs) of differentially expressed genes (DEGs) using MapMan enrichment analysis (Thimm et al., 2004; Usadel et al., 2005) in Huanglongbing (HLB)-affected and healthy (HLY) sweet orange trees at day 9 since the addition of fertilizer to the hydroponic system. Before day 0 both HLB and HLY trees were grown in nutrient-deprived conditions for 6 months.

expression was detected in HLY-F and HLB-F roots on day 0 and the expression increased on day 9. The increase in expression of *ZIP10* was significantly higher in HLB-F roots as compared with HLY-F roots.

Discussion

In this study, HLB-affected trees had lower biomass for root, shoot, and leaf compared with healthy trees. HLB-affected trees (F and NF) notably had $\approx 40\%$ to 50% less root biomass compared with HLY trees (F and NF); the reduction in shoot and leaf biomass was not as prominent in HLB-affected trees. Feeder root length, diameter, and volume were higher in HLY-F trees than HLB-F trees, depicting higher root decline and mortality in HLB-affected trees (Johnson et al., 2014). The root microscopy analysis of HLB-F trees showed that vascular bundle diameter was less when trees were deprived of fertilizer, suggesting severe implications for survival on roots. Overall,

Day 9 HLB vs. HLY

Fig. 6. Gene ontology (GO) classification of upregulated and downregulated differentially expressed genes (DEGs) in Huanglongbing (HLB)-affected and healthy (HLY) sweet orange trees at day 9 since the addition of fertilizer to the hydroponic system. Before day 0 both HLB and HLY trees were grown in nutrient-deficient conditions for 6 months. Blue and orange colors represent the number of upregulated and downregulated DEGs, respectively, in roots of HLB-affected compared with healthy trees.

process, thereby mitigating the deleterious effects of the disease (Romheld and Marschner, 1991). HLB-affected trees go through excessive oxidative stress as a result of infection. The tree then undergoes a significant number of processes to suppress or sequester ROS and counteract the effects of oxidative stress (Martinelli et al., 2012; Tang and Vashisth, 2020). Hence, it is likely that in these reactions micronutrients are used at a higher rate to mitigate disease effects; this implies that the fertilization practices should change for HLB-affected trees compared with healthy trees. This is supported by reports of Morgan et al. (2016), Vashisth (2020), and Zambon et al. (2019), in which higher than the recommended rates of micronutrients were beneficial for HLB-affected trees. Another noticeable finding is that when nutrients were available HLB-F trees accumulated the same amount of nutrients as HLY-F trees except for the secondary macronutrients Ca, Mg, and S, which were lower in HLB-affected (F and NF) trees than in HLY (F and NF). This observation sheds light on two points: 1) Mg, Ca, and S concentrations are affected by disease conditions. These nutrients are possibly metabolized at a higher rate in HLB-affected trees. It has been reported that S is involved in tree defense responses (Capaldi et al., 2015) and imparts antifungal and antibacterial properties to the tree. Magnesium and Ca are also known to reduce oxidative damage and contribute to antioxidant properties (Cakmak and Marschner, 1992; Pandey et al., 2015). Thus, it is possible

that HLB-affected trees, which are in a constant state of elevated tree defense response and oxidative stress, require these nutrients at higher rates than healthy citrus. 2) Even though HLB-NF trees had significantly lower leaf nutrient concentrations for 8 of 10 nutrients, HLB-F trees were low in only three nutrients under nutrient-available conditions compared with HLY-F. This suggests that the performance of HLB-affected trees can be elevated on fertilization (Morgan et al., 2016) as the nutrients are taken up by the trees and are accumulated in the leaves.

The nutrient accumulation results are also supported by EC and the nutrient analysis of the hydroponic solution conducted in this study. All the nutrient concentrations and EC of the hydroponic solution were the same at the start of the experiment (addition of Hoagland solution) as well as at the end, suggesting that the nutrient uptake was the same in HLB-F and HLY-F trees. It is worth noting that HLB-affected trees have significantly lower root and leaf biomass compared with healthy trees. Therefore, when the nutrient uptake efficiency of all the nutrients was calculated, HLB-F trees had a nutrient uptake efficiency 2- to 3-fold higher than HLY-F trees. This indicates that the HLB-F root system has a better nutrient uptake capacity than the HLY-F root system. HLB-affected trees are known to have compromised root systems so many growers have switched to foliar fertilization practices (Giles, 2011), assuming that the compromised roots of HLB-affected trees are not

Table 5. Differentially expressed genes belonging to gene ontology (GO) term ion transport (GO:0006811) and response to nutrients levels (GO:0031667) with respective orange gene IDs, Arabidopsis homologs, and log₂ fold change (LFC) in Huanglongbing (HLB)-affected sweet orange trees in comparison with healthy sweet orange trees on day 9 since the addition of Hoagland solution (fertilizer).

GO term	Gene	Arabidopsis homologs	LFC
Ion transport (GO:0006811)			
GO:0015696 Ammonium transport	orange1.1g011335m	AT2G38290.1	2.65
	orange1.1g044248m	AT4G13510.1	2.64
GO:0006817 Phosphate ion transport	orange1.1g002570m	AT2G33770.1	1.80
	orange1.1g010190m	AT3G54700.1	1.82
GO:0006813 Potassium ion transport	orange1.1g012060m	AT3G09030.1	2.04
GO:0015079 Potassium ion transmembrane transporter activity	orange1.1g003815m	AT2G40540.2	1.62
GO:0006829 Zinc ion transport	orange1.1g018051m	AT3G12750.1	5.23
GO:0010039 Response to iron ion	orange1.1g021703m	AT2G40300.1	1.97
GO:0006825 Copper ion transport	orange1.1g002770m	AT5G21930.1	1.53
GO:0006821 Chloride transport	orange1.1g003801m	AT5G33280.1	1.46
GO:0006812 Cation transport and cellular potassium ion homeostasis	orange1.1g044080m	AT3G53720.1	2.14
GO:0005432 Calcium: sodium antiporter activity	orange1.1g040465m	AT5G17860.1	1.97
GO:0006812 Cation transport	orange1.1g046783m	AT2G04620.1	1.57
GO:0005432 Calcium: sodium antiporter activity	orange1.1g009779m	AT1G08960.1	1.52
GO:0006812 Cation transport			
GO:0046713 Borate transport	orange1.1g046511m	AT4G10380.1	1.48
GO:0005313 L-glutamate transmembrane transporter activity	orange1.1g007398m	AT4G21120.1	2.45
GO:0015700 Arsenite transport	orange1.1g023184m	AT4G18910.1	2.36
GO:0006855 Drug transmembrane transport	orange1.1g000838m	AT1G15520.1	1.69
GO:0015992 Proton transport	orange1.1g003697m	AT1G16780.1	1.49
Response to nutrient levels (GO:0031667)			
GO:0046713 Borate transport	orange1.1g046511m	AT4G10380.1	1.48
GO:0046715 Active borate transmembrane transporter activity			
GO:0051536 Iron-sulfur cluster binding	orange1.1g005965m	AT2G29630.1	1.48
GO:0080041 ADP-ribose pyrophosphohydrolase activity			
GO:0006995 Cellular response to nitrogen starvation	orange1.1g044386m	AT4G35090.1	2.08
GO:0009970 Cellular response to sulfate starvation			
GO:0043562 Cellular response to nitrogen levels	orange1.1g023664m	AT5G21170.1	1.82
GO:0042594 Response to starvation	orange1.1g018248m	AT3G62770.1	1.74
GO:0055062 Phosphate ion homeostasis	orange1.1g002570m	AT2G33770.1	1.81
GO:0016036 Cellular response to phosphate starvation			
GO:0006995 Cellular response to nitrogen starvation	orange1.1g004956m	AT5G45380.1	3.46
GO:0015204 Urea transmembrane transporter activity			
GO:0016036 Cellular response to phosphate starvation	orange1.1g026944m	AT2G02990.1	1.85

efficient in nutrient uptake. Presented findings suggest that HLB-affected root systems can take up nutrients efficiently but a small root system may limit the uptake of the total amount of nutrients. Due to the imbalance in the reduction of root-to-shoot ratio, the roots need to increase their uptake efficiency, possibly by going through a rapid turnover and reduced longevity of feeder roots (Johnson and Graham, 2015). It is also likely that the significant increase in the vascular bundle to root diameter ratio that was observed in the HLB-affected roots from day 0 to 14 improves the nutrient uptake efficiency of the HLB-affected roots.

The transcriptome analysis performed on HLB-affected and healthy trees on days 0, 3, and 9 supports the greenhouse data. Under no-fertilizer condition (day 0), only 9 DEGs were expressed in HLB-affected trees, all of which were upregulated. This is also consistent with gene expression analysis where all the genes except *SAG20* were expressed equally among HLB-F and HLY-F on day 0. Both HLB-affected trees and healthy trees were undergoing the same kind of nutrient-deficient stress; however, HLB-affected trees were undergoing additional biotic

stress due to the bacterial disease. When healthy and HLB-affected trees are compared, as is the case here, it is typically unlikely to find such a small number of DEGs (Martinelli et al., 2012); therefore, it is possible that both types of trees were probably undergoing similar metabolic processes to cope during the 6 months of the fertilizer deficient conditions. However, the nine upregulated DEGs found in the HLB-F trees possibly play a critical role in the tree's response to disease, especially under nutrient-deficient conditions. Among these genes, the LFC of *strictosidine synthase* (orange1.1g032726) was 7.74 in HLB-F roots compared with HLY-F roots. Strictosidine synthase is the key enzyme for the synthesis of camptothecin, which is an alkaloid known to be involved in biotic and abiotic stress response of plants (Isah, 2019; Kusari et al., 2012). Another DEG, *zinc transporters 10 precursor* (*ZIP10*, orange1.1g018585), was upregulated by 6.14 LFC in HLB-F roots. ZIP 10 is involved in transporting the cations Zn, Fe, Mn, and Cu (Lin et al., 2009). Notably, Zn transporter family proteins are linked with the defense against oxidative stress (Guerinot and Eide, 1999). Trees depend on Zn-regulated

Fig. 7. Visualization of functional categories (BINs) of differentially expressed genes (DEGs), using MapMan (Thimm et al., 2004; Usadel et al., 2005), relating to plant pathogen/pest interaction Huanglongbing (HLB)-affected and healthy (HLY) sweet orange trees at day 9 since the addition of fertilizer to the hydroponic system. Before day 0 both HLB and HLY trees were grown in nutrient-deficient conditions for 6 months. This particular process includes BINs related to hormones, redox reactions, defense genes, signaling, heat shock proteins, and biotic stress.

transporters, Fe-regulated transporter (IRT)-like protein (ZIP), and natural resistance-associated macrophage protein families to maintain ion homeostasis (Hall and Guerinot, 2006). In this study, HLB-NF trees were found to be deficient in Mn, Zn, and Fe, suggesting that HLB-affected trees under nutrition-deprived conditions metabolize these nutrients at a higher rate than healthy trees. Because of this, the roots were possibly expressing *ZIP10* actively to uptake these critical micronutrients for survival. Gene expression results showing that *ZIP10* was significantly upregulated in HLB-F roots on day 9 compared with HLY-F support this possibility as well. The other DEGs that were upregulated in HLB-F trees on day 0 were *leucine-rich repeat receptor-like kinases*, which have a role in tree growth, morphogenesis, tree defense, and disease resistance (Becraft, 2002; de Lorenzo et al., 2009; Greeff et al., 2012; Tör et al., 2009); *peroxidase superfamily protein* is involved in oxidative stress (Gaspar et al., 1992); *stress responsive a/b barrel domain* and *ran bp2*

Fig. 8. Relative gene expression (log₂ fold change) of senescence associated gene 20 (*SAG20*), ethylene response factor 1 (*ERF1*), calcium dependent calmodulin (*CAM8*), 2-oxoglutarate and Fe dependent oxygenase superfamily protein (*OG-Fe*), auxin-responsive family protein (*ABF3*), zinc transporter 10 precursor (*ZIP10*) in roots of Huanglongbing (HLB)-affected and healthy (HLY) sweet orange trees on day 0 and 9 since the addition of fertilizer to the hydroponic system. Before day 0, both HLB and HLY trees were grown in nutrient-deficient conditions for 6 months. Data are means $\pm$ SD of three biological replicates. Different letters in each panel indicate significant difference among each other based on Tukey's honestly significant difference test ($\alpha = 0.05$). NS = not significantly different.

nzf figure like super family protein are involved in salicylic acid pathways (Wiermer et al., 2005); *hva22* homologue is a unique stress-induced protein that acts as a negative regulator for GA-mediated programmed cell death; *phosphoglyceride transfer family protein* and *integral membrane hpp family protein* involved in nitrate response in *Arabidopsis thaliana*, which induces the genes in nitrate transport, nitrate reduction, ammonium assimilation, and generation of NADPH through oxidative phosphate pathway (Scheible et al., 2004). It should be noted that the greenhouse results showed that nitrogen was deficient in both HLB-NF and HLY-NF trees.

On day 9, 2324 DEGs were observed in HLB-F roots compared with HLY-F roots, of which 627 genes were upregulated and 1697 were downregulated. It is worth pointing out that the DEGs in HLB-F and HLY-F between days 0 (no nutrient) and 9 (growing for 9 d in the nutrient) increased by ≈ 2300 genes; however, the increase was gradual because on day 3 only 19 DEGs were present. On day 9 all the GO categories except five were exclusively downregulated or upregulated. The two most enriched categories were “response to stress” and “abiotic stress.” Both categories had a high number of downregulated genes in HLB-F roots; fewer than one-third of the genes were upregulated. The GO category response to abiotic stress has not been previously reported in HLB-affected trees. This GO category is well known to be altered in response to drought stress (Tian et al., 2015), phosphate deficiency (Park et al., 2012), ABA, and hydrogen peroxide stress (Kulik et al., 2011). Response to stress has previously been found to be a significant GO category in citrus varieties that are HLB tolerant (Wu et al., 2020). BIN 20 (stress) included DEGs related to biotic and abiotic stress. Within the biotic stress BIN, a high number of genes were related to PR-protein. PR-proteins are known to be upregulated in citrus when CLAs is present (Zhong et al., 2015). MapMan analysis also revealed that 381 DEGs were involved in tree-pathogen/pest interaction. This group mainly included DEGs related to hormone, redox reaction, MYB family, WRKY family, proteolysis, and cell wall biotic stress. Similarly, Martinelli et al. (2012) and Zhong et al. (2015) reported several DEGs from this category expressed in HLB-affected root and leaf compared with their healthy counterparts. Fourteen upregulated DEGs were related to phenylpropanoid metabolic process, which encompassed secondary metabolic processes and the lignin metabolic process; secondary metabolism is well known to increase in response to CLAs infection (Martinelli et al., 2012). Secondary metabolite production is a plant defense response to pathogen and oxidative stress (Isah, 2019). GO categories related to hormones, jasmonic acid-mediated signaling pathway, ethylene-mediated signaling pathway, and regulation of hormones were upregulated in HLB-F trees. An increase in jasmonic acid and ethylene as well as alteration in levels of major plant hormones has been widely reported in leaves upon CLAs infection (Killiny et al., 2017; Martinelli et al., 2012; Rosales and Burns, 2011), potentially contributing to enhanced plant defense responses. Based on all the DEGs mentioned previously, it can be concluded that HLB-affected trees were undergoing a significant tree defense response. It should be noted that all the discussed genes were only differentially expressed on nutrient availability. This underlines the importance of nutrition to the trees. Nutrients not only help with growth and development but are also critical for tree defense response (Spann and Schumann, 2012).

Several GO categories were related to the development and cell death in HLB-F roots. Altogether this super GO group included 296 DEGs that were all downregulated. The GO categories included growth developmental processes, anatomic structure development, growth, cell cycle, meristem development, apoptosis, and cell death suggesting that the HLB-affected roots were undergoing significant root growth, development, and senescence process. It is known that HLB-affected trees undergo significant feeder root loss even if the feeder root growth (new growth) is found to be similar between healthy and HLB-affected trees (Johnson and Graham 2015; Johnson et al., 2014). Similarly, in the present study, the DEGs of GO categories related to meristem development, growth, and developmental processes as well as cell death and apoptosis were downregulated at the same time, which suggests that such events happen in HLB-affected trees grown under nutrient-available conditions. It can also be hypothesized that DEGs related to this super category increased the vascular bundle diameter to potentially improve the nutrient uptake.

Two other important GO categories observed were ion transport and response to nutrient levels. All 33 genes within these two categories were upregulated in HLB-F roots as compared with HLY-F roots. Both ion and anion transport categories are related to nutrient uptake in trees (Bi et al., 2014; Li et al., 2017; Zhu et al., 2019). In this study, HLB-F roots had a 2- to 3-fold higher nutrient uptake efficiency than HLY-F trees, suggesting that these three categories possibly contributed to increased nutrient uptake in HLB-F trees as compared with HLY-F.

Altogether, the results of transcriptomics suggest that the regulation of genes related to transport, growth, and development potentially improves the nutrient uptake in HLB-affected trees, but the small root biomass is a major limitation for HLB-affected trees. The availability of nutrients is also critical for both healthy and HLB-affected trees to carry out normal tree function, growth, development, and tree defense response. In nutrient-deficient conditions, many metabolic processes are presumably suppressed to conserve energy, which reduces growth. Healthy trees showed a high number of DEGs on transition from nutrient-deficient conditions to nutrient-available conditions compared with HLB-affected trees. This observation also suggests that, except for a handful of biotic stress-related genes, healthy and HLB-affected trees behave similarly under nutrient-deficient conditions. This underlines the importance of mineral nutrition in HLB-disease management and warrants further research.

Conclusions

The present greenhouse study is the first evaluation of nutrient uptake and its regulation in HLB-affected trees. HLB-affected trees experience nutrient deficiency that healthy trees do not when grown under similar nutrient conditions. HLB-affected roots are also functional and efficient in nutrient uptake; however, reduced biomass limits the nutrient uptake capacity of trees as a whole. To compensate for the reduced root-to-shoot ratio, the existing roots undergo anatomic and transcriptomic changes to improve nutrient uptake efficiency. It is possible that higher input of energy in nutrient uptake possibly results in reducing the root longevity of HLB-affected trees. Therefore, it is suggested that HLB-affected trees should be supplied with constant nutrients at higher rates than what is

recommended for healthy trees. Such fertilization practices possibly mitigate the abiotic stress in HLB-affected trees. Good nutrition management practices are critical for the productivity of HLB-affected trees as the availability and uptake of nutrients allow HLB-affected trees to respond to abiotic and biotic stresses.

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Supplemental Fig. 1. Phosphorus content in the hydroponic solution of Huanglongbing (HLB)-affected and healthy sweet orange trees grown in hydroponic system with fertilizer solution for 56 d. Fertilizer solution was added on day 0 of the experiment. The P content in solution indicates the amount of P uptake in the plant, higher the P content in solution, lower is the uptake by plant. Different letters over each bar indicate significant difference based on Tukey's honestly significant difference test ($\alpha = 0.05$); NS = not significantly different.

Polyphenol biosynthesis process

Supplemental Fig. 2. Overview of the phenylpropanoid pathway-related differentially expressed genes in Huanglongbing (HLB)-affected compared with healthy sweet orange trees on day 9 since the addition of fertilizer solution. Red and blue indicating downregulated and upregulated genes in HLB-affected trees, respectively.

Supplemental Table 1. List of genes along with gene-specific primer sequences used for relative gene expression analysis in feeder roots of Huanglongbing-affected and healthy sweet orange trees.

Description (gene symbol)	Gene	GenBank accession no.	Forward and reverse primer sequences (5' to 3')	Amplicon size (base pairs)
<i>senescence-associated gene 20 (SAG20)</i>	orange1.1g041268m	XM_006486163.3	AAAGCCATCACCAGTTCTC GTTCTTGACGGAATAGCCTTA	117
<i>ethylene response factor 1 (ERF1)</i>	orange1.1g042174m	XM_006477063.3	ACG TAG CGC TTC TCA AAC CC GCC AAT GAC CAC GAT GTT GA	136
<i>2-oxoglutarate and Fe dependent oxygenase superfamily protein (2OG-Fe)</i>	orange1.1g039524m	XM_006490040.2	GGACTTGACGAACGTTGCTT ATCAAGCAGCATGAAGCTTTG	143
<i>auxin-responsive family protein (ABF3)</i>	orange1.1g041715m	XM_006487050.3	AGCGGTGCTCTGGAGAAAG CTCTGCCTGTTTCTCTCCAA	168
<i>zinc transporter 10 precursor (ZIP10)</i>	orange1.1g018585m	XM_006481314.3	CTATCGGGACTCTGATGATT CCCAACTTTCTCTTCATCTG	113
<i>calcium dependent calmodulin (CAM8)</i>	orange1.1g031903m	XM_006489415.3	TGACGCTGAGGAAGAGCTTAAA ATCACCGTCCAAATCAGCCTC	163
<i>actin-7 (ACT)</i>	n/a	XM_006464503.2	GTTGGTTGACATGGAGAAG ACGGTTGAGTACAGAAATAAG	121
<i>thioredoxin-like protein YLS8 (DIM1)</i>	n/a	XM_006484463.2	CGAAACCTGTATGCAGATGG ACGGTTGAGGGATCGTAAAG	138

Supplemental Table 2. Differentially expressed genes categorized in the functional categories (BINs) related to hormone metabolism and signaling according to MapMan analysis (Thimm et al., 2004; Usadel et al., 2005) with respective Arabidopsis homologs and log₂ fold change (LFC) in Huanglongbing (HLB)-affected sweet orange trees in comparison with healthy sweet orange trees on day 9 after the addition of Hoagland (fertilizer) solution.

BIN	Description	Arabidopsis homologs	LFC
Abscisic acid			
17.1.1	hormone metabolism.abscisic acid.synthesis-degradation	AT1G16540	-3.26
17.1.1.2.1	hormone metabolism.abscisic acid.synthesis-degradation.degradation.8-hydroxylase	AT4G19230	-3.19
17.1.2	hormone metabolism.abscisic acid.signal transduction	AT4G26080	-1.74
17.1.2	hormone metabolism.abscisic acid.signal transduction	AT5G57050	-2.07
17.1.2	hormone metabolism.abscisic acid.signal transduction	AT3G63210	-2.38
17.1.3	hormone metabolism.abscisic acid.induced-regulated-responsive-activated	AT1G45249	-1.85
Ethylene			
17.5.1.1	hormone metabolism.ethylene.synthesis-degradation.1-aminocyclopropane-1-carboxylate synthase	AT3G61510	2.09
17.5.2	hormone metabolism.ethylene.signal transduction	AT5G07580	2.76
17.5.2	hormone metabolism.ethylene.signal transduction	AT5G47230	2.27
17.5.2	hormone metabolism.ethylene.signal transduction	AT3G23240	1.76
17.5.2	hormone metabolism.ethylene.signal transduction	AT4G17500	2.40
17.5.2	hormone metabolism.ethylene.signal transduction	AT3G15210	1.47
17.5.2	hormone metabolism.ethylene.signal transduction	AT2G40940	1.46
17.5.3	hormone metabolism.ethylene.induced-regulated-responsive-activated	AT3G62550	3.18
Brassinosteroids			
17.3.1.1.5	hormone metabolism.brassinosteroid.synthesis-degradation.BRs.metabolic regulation	AT2G26710	-2.36
17.3.1.1.5	hormone metabolism.brassinosteroid.synthesis-degradation.BRs.metabolic regulation	AT1G17060	-4.14
17.3.1.1.99	hormone metabolism.brassinosteroid.synthesis-degradation.BRs.other	AT3G13730	-3.34
17.3.1.2.5	hormone metabolism.brassinosteroid.synthesis-degradation.sterols.HYD1	AT1G20050	-1.79
17.3.2.99	hormone metabolism.brassinosteroid.signal transduction.other	AT5G08130	-2.52
Cytokinins			
17.4.1	hormone metabolism.cytokinin.synthesis-degradation	AT5G21482	1.99
17.4.2	hormone metabolism.cytokinin.signal transduction	AT5G10720	-3.29
17.4.2	hormone metabolism.cytokinin.signal transduction	AT2G17820	-3.66
Salicylic acid			
17.8.1	hormone metabolism.salicylic acid.synthesis-degradation	AT1G68040	-4.24

Supplemental Table 3. Differentially expressed genes categorized in the functional categories (BINs) related to plant-pathogen/pest interaction according to MapMan analysis (Thimm et al., 2004; Usadel et al., 2005) with respective Arabidopsis homologs and log₂ fold change (LFC) in Huanglongbing (HLB)-affected sweet orange trees in comparison with healthy sweet orange trees on day 9 since the addition of Hoagland (fertilizer) solution.

BIN	Description	Arabidopsis homologs	LFC
Absciscic acid			
17.1.1.1.1	hormone metabolism.abscisic acid.synthesis-degradation.synthesis.zeaxanthin epoxidase	AT5G67030	-2.97
17.1.1.2.1	hormone metabolism.abscisic acid.synthesis-degradation.degradation.8-hydroxylase	AT4G19230	-3.68
17.1.2	hormone metabolism.abscisic acid.signal transduction	AT4G26080	-1.82
17.1.3	hormone metabolism.abscisic acid.induced-regulated-responsive-activated	AT5G50720	-3.05
17.1.3	hormone metabolism.abscisic acid.induced-regulated-responsive-activated	AT1G69700	-4.20
17.1.3	hormone metabolism.abscisic acid.induced-regulated-responsive-activated	AT3G56850	-2.01
Auxins			
17.2.1	hormone metabolism.auxin.synthesis-degradation	AT3G02875	2.84
17.2.2	hormone metabolism.auxin.signal transduction	AT2G26170	-5.07
17.2.2	hormone metabolism.auxin.signal transduction	AT1G73590	-2.45
17.2.3	hormone metabolism.auxin.induced-regulated-responsive-activated	AT1G60690	-2.81
17.2.3	hormone metabolism.auxin.induced-regulated-responsive-activated	AT5G20820	-3.32
17.2.3	hormone metabolism.auxin.induced-regulated-responsive-activated	AT5G50760	3.14
17.2.3	hormone metabolism.auxin.induced-regulated-responsive-activated	AT4G22620	1.83
17.2.3	hormone metabolism.auxin.induced-regulated-responsive-activated	AT4G27450	3.50
17.2.3	hormone metabolism.auxin.induced-regulated-responsive-activated	AT2G46370	1.87
17.2.3	hormone metabolism.auxin.induced-regulated-responsive-activated	AT5G65470	-1.55
17.2.3	hormone metabolism.auxin.induced-regulated-responsive-activated	AT2G33310	-2.09
17.2.3	hormone metabolism.auxin.induced-regulated-responsive-activated	AT3G12830	2.62
17.2.3	hormone metabolism.auxin.induced-regulated-responsive-activated	AT2G14960	3.24
17.2.3	hormone metabolism.auxin.induced-regulated-responsive-activated	AT2G04850	-5.59
Brassinosteroids			
17.3.1.1.1	hormone metabolism.brassinosteroid.synthesis-degradation.BRs.DET2	AT5G16010	-2.81
17.3.1.1.5	hormone metabolism.brassinosteroid.synthesis-degradation.BRs.metabolic regulation	AT2G26710	-2.32
17.3.1.2.2	hormone metabolism.brassinosteroid.synthesis-degradation.sterols.SMT2	AT5G13710	-2.72
17.3.1.2.5	hormone metabolism.brassinosteroid.synthesis-degradation.sterols.HYD1	AT1G20050	-3.59
17.3.1.2.99	hormone metabolism.brassinosteroid.synthesis-degradation.sterols.other	AT2G07050	-1.75
17.3.2.1	hormone metabolism.brassinosteroid.signal transduction.BRI	AT2G01950	-2.22
17.3.2.1	hormone metabolism.brassinosteroid.signal transduction.BRI	AT3G51740	-3.57
17.3.2.2	hormone metabolism.brassinosteroid.signal transduction.BZR	AT1G78700	1.57
Glutathione S transferase			
26.9	misc.glutathione S transferases	A1G10370	1.59
26.9	misc.glutathione S transferases	AT3G09270	1.52
26.9	misc.glutathione S transferases	AT1G14550	1.69
26.9	misc.glutathione S transferases	AT2G30860	2.07
26.9	misc.glutathione S transferases	AT1G17180	4.42
26.9	misc.glutathione S transferases	AT2G29420	2.44
26.9	misc.glutathione S transferases	AT2G02390	-1.52
R-Stress biotic receptors			
20.1.2	stress.biotic.receptors	AT3G50470	7.37
Peroxidases			
26.12	misc.peroxidases	AT5G05340	4.26
26.12	misc.peroxidases	AT4G37530	5.68
26.12	misc.peroxidases	AT5G51890	-5.04
26.12	misc.peroxidases	AT2G38380	-2.43
26.12	misc.peroxidases	AT4G33420	-3.65
26.12	misc.peroxidases	AT5G58400	2.48
26.12	misc.peroxidases	AT5G42180	-5.79
26.12	misc.peroxidases	AT1G71695	2.67
26.12	misc.peroxidases	AT2G39040	2.44
26.12	misc.peroxidases	AT2G41480	2.19
26.12	misc.peroxidases	AT5G06720	2.12

Continued next page

Supplemental Table 3. Continued.

BIN	Description	Arabidopsis homologs	LFC
WRKY			
27.3.32	RNA.regulation of transcription.WRKY domain transcription factor family	AT1G62300	1.95
27.3.32	RNA.regulation of transcription.WRKY domain transcription factor family	AT1G69310	-1.53
27.3.32	RNA.regulation of transcription.WRKY domain transcription factor family	AT5G49520	-1.90
27.3.32	RNA.regulation of transcription.WRKY domain transcription factor family	AT2G44745	-2.88
27.3.32	RNA.regulation of transcription.WRKY domain transcription factor family	AT4G01250	2.45
27.3.32	RNA.regulation of transcription.WRKY domain transcription factor family	AT2G23320	2.09
27.3.32	RNA.regulation of transcription.WRKY domain transcription factor family	AT3G56400	2.74
27.3.32	RNA.regulation of transcription.WRKY domain transcription factor family	AT4G18170	1.61
27.3.32	RNA.regulation of transcription.WRKY domain transcription factor family	AT5G13080	2.93
27.3.32	RNA.regulation of transcription.WRKY domain transcription factor family	AT1G29280	1.63
Cell wall biotic stress			
10.5.2	cell wall.cell wall proteins.proline rich proteins	AT2G21140	5.24
10.8.2	cell wall.pectin*esterases.acetyl esterase	AT4G19420	4.10
10.8.1	cell wall.pectin*esterases.PME	AT1G02810	3.71
10.8.1	cell wall.pectin*esterases.PME	AT2G26440	3.61
10.1.6	cell wall.precursor synthesis.GAE	AT4G00110	3.54
10.1.2	cell wall.precursor synthesis.UGE	AT1G12780	3.33
10.8.99	cell wall.pectin*esterases.misc	AT1G23200	3.19
10.7	cell wall.modification	AT4G17030	3.18
10.8.99	cell wall.pectin*esterases.misc	AT2G45220	3.06
10.6.2	cell wall.degradation.mannan-xylose-arabinose-fucose	AT1G67830	2.82
10.7	cell wall.modification	AT2G39700	2.73
10.7	cell wall.modification	AT3G45960	2.66
10.2.1	cell wall.cellulose synthesis.cellulose synthase	AT4G24000	2.54
10.6.2	cell wall.degradation.mannan-xylose-arabinose-fucose	AT2G28100	-5.84
10.6.2	cell wall.degradation.mannan-xylose-arabinose-fucose	AT4G33840	-6.69
10.6.3	cell wall.degradation.pectate lyases and polygalacturonases	AT1G09890	-6.92
Jasmonate			
17.7.1	hormone metabolism.jasmonate.synthesis-degradation	AT1G65910	-3.85
17.7.1.2	hormone metabolism.jasmonate.synthesis-degradation.lipoxygenase	AT1G55020	1.80
17.7.1.2	hormone metabolism.jasmonate.synthesis-degradation.lipoxygenase	AT3G22400	-4.15
17.7.1.2	hormone metabolism.jasmonate.synthesis-degradation.lipoxygenase	AT3G45140	-2.63
Ethylene			
17.5.1.1	hormone metabolism.ethylene.synthesis-degradation.1-aminocyclopropane-1-carboxylate synthase	AT3G61510	1.79
17.5.1.1	hormone metabolism.ethylene.synthesis-degradation.1-aminocyclopropane-1-carboxylate synthase	AT4G11280	-1.82
17.5.2	hormone metabolism.ethylene.signal transduction	AT5G07580	3.45
17.5.2	hormone metabolism.ethylene.signal transduction	AT5G47230	2.20
17.5.2	hormone metabolism.ethylene.signal transduction	AT3G23240	3.57
17.5.2	hormone metabolism.ethylene.signal transduction	AT4G17500	2.22
17.5.2	hormone metabolism.ethylene.signal transduction	AT2G44840	2.63
17.5.2	hormone metabolism.ethylene.signal transduction	AT5G44210	1.55
17.5.2	hormone metabolism.ethylene.signal transduction	AT3G15210	1.94
17.5.2	hormone metabolism.ethylene.signal transduction	AT5G25190	-3.04
17.5.2	hormone metabolism.ethylene.signal transduction	AT2G40940	1.82
17.5.2	hormone metabolism.ethylene.signal transduction	AT4G31980	2.30
17.5.3	hormone metabolism.ethylene.induced-regulated-responsive-activated	AT4G29100	-1.49
17.5.3	hormone metabolism.ethylene.induced-regulated-responsive-activated	AT3G62550	3.22
PR-proteins			
20.1.7	stress.biotic.PR-proteins	AT5G24090	5.08
20.1.7	stress.biotic.PR-proteins	AT2G34930	3.31
20.1.7	stress.biotic.PR-proteins	AT1G47890	3.12
20.1.7	stress.biotic.PR-proteins	AT5G49040	2.26
20.1.7	stress.biotic.PR-proteins	AT4G04220	1.95
20.1.7	stress.biotic.PR-proteins	AT5G63020	1.85
20.1.7	stress.biotic.PR-proteins	AT2G21100	1.56

Continued next page

Supplemental Table 3. Continued.

BIN	Description	Arabidopsis homologs	LFC
20.1.7	stress.biotic.PR-proteins	AT3G14460	1.54
20.1.7	stress.biotic.PR-proteins	AT5G23400	-2.06
20.1.7	stress.biotic.PR-proteins	AT1G58170	-2.31
20.1.7	stress.biotic.PR-proteins	AT5G36930	-2.49
20.1.7	stress.biotic.PR-proteins	AT1G69550	-3.95
20.1.7	stress.biotic.PR-proteins	AT5G17680	-4.07
20.1.7	stress.biotic.PR-proteins	AT4G12010	-4.76
20.1.7	stress.biotic.PR-proteins	AT3G14470	-5.24
20.1.7.6.1	stress.biotic.PR-proteins.proteinase inhibitors.trypsin inhibitor	AT1G17860	-9.99
Salicylic acid			
17.8.1	hormone metabolism.salicylic acid.synthesis-degradation	AT1G68040	-6.42
17.8.1	hormone metabolism.salicylic acid.synthesis-degradation	AT3G11480	-4.71
17.8.1	hormone metabolism.salicylic acid.synthesis-degradation	AT5G66430	2.19
MYB			
27.3.25	RNA.regulation of transcription.MYB domain transcription factor family	AT3G23250	2.20
27.3.25	RNA.regulation of transcription.MYB domain transcription factor family	AT4G09460	1.94
27.3.25	RNA.regulation of transcription.MYB domain transcription factor family	AT1G79180	2.05
27.3.25	RNA.regulation of transcription.MYB domain transcription factor family	AT3G46130	-4.59
27.3.25	RNA.regulation of transcription.MYB domain transcription factor family	AT5G52600	-4.04
27.3.25	RNA.regulation of transcription.MYB domain transcription factor family	AT5G42630	-3.27
27.3.25	RNA.regulation of transcription.MYB domain transcription factor family	AT2G02820	-3.12

Supplemental Table 4. Differentially expressed genes categorized in the functional categories (BINs) related to cell wall modification according to MapMan analysis (Thimm et al., 2004; Usadel et al., 2005) with Arabidopsis homologs, and log₂ fold change (LFC) in Huanglongbing-affected sweet orange trees in comparison with healthy sweet orange trees on day 9 since the addition of fertilizer solution.

BIN	Description	Arabidopsis homologs	LFC
10.1.6	cell wall.precursor synthesis.GAE	At4G00110	3.59
10.5.2	cell wall.cell wall proteins.proline rich proteins	AT2G21140	3.28
10.6.2	cell wall.degradation.mannan-xylose-arabinose-fucose	AT5G49360	3.20
10.6.2	cell wall.degradation.mannan-xylose-arabinose-fucose	AT1G67830	3.00
10.6.2	cell wall.degradation.mannan-xylose-arabinose-fucose	AT1G02640	2.66
10.1.2	cell wall.precursor synthesis.UGE	AT1G12780	2.45
10.2.1	cell wall.cellulose synthesis.cellulose synthase	AT4G24000	2.41
10.7	cell wall.modification	AT4G17030	2.13
10.6.3	cell wall.degradation.pectate lyases and polygalacturonases	AT3G07010	2.06
10.6.3	cell wall.degradation.pectate lyases and polygalacturonases	AT1G09910	-2.01
10.1.9	cell wall.precursor synthesis.MUR4	AT1G30620	-2.05
10.8.1	cell wall.pectin*esterases.PME	AT1G53840	-2.06
10.2.2	cell wall.cellulose synthesis.COBRA	AT5G60920	-2.10
10.3	cell wall.hemicellulose synthesis	AT3G45400	-2.12
10.3.1.1	cell wall.hemicellulose synthesis.xyloglucan.XXXG galactose Transferase	AT2G20370	-2.13
10.3	cell wall.hemicellulose synthesis	AT2G03220	-2.30
10.6.2	cell wall.degradation.mannan-xylose-arabinose-fucose	AT5G66460	-2.42
10.4	cell wall.pectin synthesis	AT3G25140	-2.49
10.8.99	cell wall.pectin*esterases.misc	AT2G45220	-2.55
10.6.3	cell wall.degradation.pectate lyases and polygalacturonases	AT1G09890	-2.99
10.6.3	cell wall.degradation.pectate lyases and polygalacturonases	AT4G33440	-3.13
10.6.3	cell wall.degradation.pectate lyases and polygalacturonases	AT5G14650	-4.46
10.5.1	cell wall.cell wall proteins.AGPs	AT5G06390	-4.89
10.8.99	cell wall.pectin*esterases.misc	AT4G02330	-7.28