

Research paper

Genotype-specific metabolic shifts and polysaccharide dynamics in Huanglongbing (HLB)-infected citrus: Multi-platform metabolomics, glycoarray profiling, and ex vivo α -amylase activity

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ABSTRACT

Citrus Huanglongbing (HLB), caused by *Candidatus Liberibacter asiaticus* (CLAs), is the most destructive citrus disease worldwide, characterised by phloem occlusion via callose (β -1,3-glucan) deposition and subsequent pathological starch accumulation. This study investigated genotype-specific metabolic shifts in *Citrus medica* L. (Citron), *C. reticulata* cv. Fremont (Fremont mandarin), and *C. reticulata* cv. Sai-Nam-Phueng (Tangerine) under asymptomatic and symptomatic HLB conditions and evaluated the potential of enzymatic hydrolysis to mitigate polysaccharide accumulation. Multi-platform metabolomics (LC-MS, GC-MS, and ^1H NMR) combined with glycoarray profiling revealed that CLAs infection triggers comprehensive biochemical restructuring. While symptomatic tissues universally exhibited high bacterial load and genotype-dependent antioxidant responses, metabolic reprogramming was genotype-dependent. Citron displayed the most profound disruption, characterised by the depletion of signalling lipids (α -linolenic acid) and defence-related coumarins, alongside the retention of osmoprotectants (proline betaine). Conversely, Fremont mandarin exhibited a highly stable metabolic profile, while Sai-Nam-Phueng showed significant callose accumulation and flavonoid induction. A conserved stress-response volatile marker was identified across all infected genotypes at GC-MS retention time 56.39 min (Rtx-5ms capillary column, 30 m $\times$ 0.25 mm ID, 0.25 μm). Glycoarray analysis indicated the dominance of β -1,3-glucans in symptomatic tissues, which correlated with the formation of massive starch aggregates (300–500 μm) visualised by scanning electron microscopy. Crucially, ex vivo application of commercial α -amylase partially hydrolysed up to 10% of these starch deposits under ex vivo conditions. These findings elucidate distinct tolerance mechanisms involving lipid signalling turnover and osmotic regulation and suggest that while amylase specifically targets starch rather than callose, reducing this secondary starch accumulation represents a preliminary proof-of-concept that warrants further in vivo investigation before therapeutic claims can be substantiated.

1. Introduction

Huanglongbing (HLB), commonly known as citrus greening disease,

stands as the most catastrophic and destructive threat currently confronting the global citrus industry, leading to devastating economic losses worldwide. The disease is widespread across major growing

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regions including Asia, Africa and America (Albrecht et al., 2020; Da Graça and Korsten, 2004). Mostly found in Asia, the causative agent is the fastidious, phloem-restricted bacterium *Candidatus Liberibacter asiaticus* (CLAs), which is primarily transmitted by insect vector Asian citrus psyllid (*Diaphorina citri*) (Albrecht et al., 2020; Grafton-Cardwell et al., 2013). A central challenge in managing HLB is the inability to isolate and culture CLAs, which necessitates the adoption of systems-level approaches to analyse the host plant's molecular response *in situ* (Merfa and De La Fuente, 2020). The host plant responds to infection by altering defence and core metabolic pathways; therefore, to capture these early, precise biochemical shifts and identify biomarkers of disease or tolerance, comprehensive molecular profiling is indispensable (Munir et al., 2020; Suh et al., 2021). Metabolomic studies reveal that the infected citrus undergoes significant metabolic shifts, often involving organic acids, flavones, amino acid derivatives, flavone C-glycosides, nucleotide derivatives, and flavonol. In HLB-tolerant cultivars, the primary strategy involves upregulating pathways like aspartate and glutamate metabolism (AGM), purine metabolism (PM), and biosynthesis of plant hormones (BPH), resulting in the accumulation of growth-promoting hormones (auxins and cytokinins) and nitrogen-supplying purine metabolites (like xanthine and uric acid) to promote continuous plant growth and phloem regeneration, while defence-related salicylic acid metabolites are decreased (Suh, Tang, et al., 2021). Conversely, pathogen management using endophytes like *Bacillus subtilis* L1–21 induces a defence response characterised by the up-regulation of amino acids (lysine and tyrosine), xanthine, leucic acid, and α -linolenic acid, signalling activation of defence and systemic acquired resistance pathways (Munir et al., 2020).

Despite the critical insights provided by omics, the severe symptoms of HLB have commonly been attributed to localised phloem pathology. CLAs infection has been reported to promote the deposition of callose (β -1,3-glucan) into the phloem sieve pores (Granato et al., 2019; Koh et al., 2012; Meikle et al., 1991; Wang et al., 2021). Callose accumulation at the sieve pores has been proposed to impede phloem transport, contributing to carbohydrate imbalance and pathological starch accumulation in leaves (Achor et al., 2020; Koh et al., 2012). However, whether callose deposition and sieve-pore occlusion precede, coincide with, or follow starch accumulation in planta has not been firmly established, and these processes may instead unfold in parallel as part of a broader phloem stress response. β -1,3-glucanase (BG) enzymes specifically catalyse the hydrolysis of β -1,3-glucan linkages, regulating the accumulation and turnover of callose by acting as the critical counterbalance to callose synthesis, which is mediated by callose synthases (CalS/GSLs) (German et al., 2023; Munir et al., 2020; Zavaliev et al., 2011). This finely tuned dynamic equilibrium is vital for numerous physiological processes, including the restoration of symplastic connectivity (such as breaking bud dormancy and cotton fibre elongation) and the precise regulation of intercellular transport, as callose accumulation restricts the channel aperture of plasmodesmata while BG-catalysed degradation promotes macromolecular trafficking (Chen and Kim, 2009; Zavaliev et al., 2011). Furthermore, BGs are classified as Pathogenesis-Related (PR) proteins (PR-2) and are induced during infection, playing complex roles in defence by, for instance, degrading fungal cell wall β -1,3-glucans; conversely, their activity can be exploited by certain viruses to remove callose barriers and facilitate viral spread (Hong and Meng, 2003; Zavaliev et al., 2011). Therefore, decreasing this accumulation using callose-digesting enzymes, such as β -1,3-glucanases, is proposed as a therapeutic approach to potentially alleviate HLB severity and improve phloem transport, a strategy supported by general research suggesting that enhanced BG activity would increase callose degradation. Understanding and managing this physical blockage is paramount to restoring host function. Amylase enzymes are chemically specialised glycoside hydrolases that catalyse the hydrolysis of starch by acting specifically on α -1,4-glycosidic bonds (Van Der Maarel et al., 2002), while callose, the β -1,3-glucan polymer causing phloem plugging in HLB, is primarily defined by β -1,3-glycosidic linkages; thus,

effective non-specific degradation of callose by amylase is theoretically highly unlikely. In other plant-pathogen systems, a regulatory link has been proposed between carbohydrate metabolism and defence signalling: abscisic acid (ABA)-dependent activation of starch-degrading β -amylase (BAM1) has been associated with enhanced callose deposition during fungal infection (Gamir et al., 2018; Wang et al., 2021). Whether an analogous starch-callose regulatory link operates during CLAs infection of citrus has not yet been tested, and this cross-system extrapolation should be treated with caution. Despite this chemical incompatibility and the enzyme's primary role in starch catabolism (where it is generally used to clear accumulated starch in HLB samples), amylases represent an easily accessible and cost-effective class of enzymes for potential mass application by agricultural producers. Importantly, prior reports and our own preliminary observations confirm that CLAs infection leads to the pathological accumulation of starch as unusually large aggregates (300–500 μ m) in symptomatic leaf tissues (Gonzalez et al., 2011), an additional, physically substantial contributor to phloem congestion that is structurally amenable to α -amylase-mediated hydrolysis through its specificity for α -1,4-glycosidic bonds. Given the current absence of empirical evidence validating this non-specific approach for callose degradation *in vivo*, a critical objective of this research is to rigorously test the hypothesis of non-specific callose digestion by amylase and determine its therapeutic value in mitigating vascular occlusion in CLAs-infected citrus.

It should be emphasised that the precise sequence of events linking CLAs infection to phloem dysfunction in HLB is not yet fully resolved, and the literature has proposed several, non-mutually exclusive, mechanisms rather than a single, validated pathway. One possible contributor to HLB phloem dysfunction is the manipulation of host phloem-associated processes by CLAs Sec-dependent effectors. Characterised effectors can target host defence proteins and vesicle-trafficking components, while the small size and observed mobility of some CLAs effectors suggest that they may move symplastically from sieve elements to adjacent cells through plasmodesmata. However, direct evidence that a specific CLAs effector alters pore-plasmodesma-unit gating or companion-cell-sieve-element macromolecular trafficking is still lacking; such effects should therefore be considered a plausible but unconfirmed mechanism (Clark et al., 2018; Pagliaccia et al., 2017; Huang et al., 2024). In addition, a recent mechanistic advance has substantially refined understanding of HLB pathophysiology at the phloem level. CLAs colonisation triggers a host NADPH-oxidase-driven reactive oxygen species (ROS) burst in phloem sieve elements and companion cells, initiating a programmed cell death cascade that drives callose deposition and secondary starch accumulation, a sequence that can be partially mitigated by antioxidant compounds or gibberellin treatment under field conditions (Ma et al., 2022). A third, complementary hypothesis is that CLAs infection disrupts carbohydrate allocation and sugar signalling, as reflected by altered expression or activity of sucrose-metabolism enzymes, sugar transporters, invertases, and starch-metabolism genes. These changes may reinforce foliar starch accumulation and source-sink imbalance alongside impaired phloem transport; however, their temporal and causal relationship to callose deposition and sieve-pore occlusion has not been resolved (Albrecht and Bowman, 2008; Kim et al., 2009; Martinelli et al., 2013). Callose deposition in HLB can also be interpreted as part of a general stress-associated phloem response rather than as a CLAs-specific output. Because callose is deposited at plasmodesmata and sieve pores following wounding, pathogen attack, and multiple abiotic stresses, its presence in infected citrus indicates phloem stress and altered symplastic regulation but does not, by itself, identify the upstream mechanism or establish HLB-specific immune activation (Pírselová and Matusíková, 2013; Zavaliev et al., 2011).

Critically, RNAi-mediated suppression of the citrus callose synthase gene CsCalS7 significantly reduces callose deposition and HLB symptom severity without altering CLAs titer, providing direct genetic evidence that callose-mediated sieve-pore occlusion, rather than bacterial load

per se., is the proximate driver of symptom expression (Granato et al., 2026). Complementing these molecular findings, grafting CLas-resistant Oceanian citrus genotypes as interstocks has been shown to substantially restrict long-distance CLas movement through constitutively smaller phloem sieve pore dimensions rather than callose-based responses (Darolt et al., 2026), underscoring that phloem wall architecture and secondary metabolite profiles jointly determine the capacity of a given genotype to withstand CLas colonisation. Together, these papers fundamentally reframe HLB pathophysiology and directly validate the therapeutic rationale of our study: if callose blockade is immune-mediated and CsCaLS7-driven, then enzymatic reduction of callose (and co-accumulated secondary starch) represents a mechanistically grounded intervention strategy.

In this study, we employed metabolite profiling, utilising non-targeted analysis of volatile and non-volatile secondary metabolites to identify precise biochemical shifts that serve as predictive indicators of disease progression or host resilience. To complement this, glycoarray profiling was utilised to specifically analyse the host-glycan profile, aiming to uncover alterations in cell wall components and defence signalling glycoconjugates that are integral to the infection response and the deposition of protective polymers. This research therefore pursues two equally critical and complementary objectives. The first is to rigorously test the hypothesis of non-specific callose digestion by α -amylase as a potential therapeutic strategy for phloem restoration. The second is to decode the complex biochemical responses of citrus hosts under CLas pressure, with particular emphasis on the onset of early biochemical changes, the molecular events that precede and accompany symptom development, which are fundamental to understanding how citrus mounts its defence against HLB. Critically, genotype plays a pivotal role in mediating these responses: commercially cultivated citrus varieties, selectively bred for productivity and fruit quality rather than pathogen resistance, are demonstrably more susceptible to CLas infection, rendering genotype-specific profiling an essential component of any viable management framework. By exploring the ex vivo efficacy of commercial α -amylase against accumulated starch, we present a preliminary proof-of-concept for enzymatic intervention as a potential supporting strategy for reducing secondary polysaccharide occlusion, pending further in vivo validation. This integrated approach represents a significant advancement toward developing sustainable green biotechnological solutions for the long-term, eco-friendly management of HLB in global citrus production.

2. Materials and methods

2.1. Plant materials

Citrus plant materials were selectively collected from commercial citrus cultivation areas in Chiang Mai, Thailand (Table 1). This study focused on three economically important citrus types: citron (*Citrus medica* L.), tangerine cv. 'Sai-Nam-Phueng' (*C. reticulata* Blanco cv. 'Sai-Nam-Phueng'), and Fremont mandarin (*C. reticulata* cv. 'Fremont'). Samples were categorised based on disease symptomatology observed in the fields:

- Asymptomatic (putatively healthy) material was collected from orchards with excellent maintenance practices, where the risk of infection was considered minimal.
- Symptomatic material was collected from branches displaying visual evidence of citrus greening disease (Huanglongbing, HLB) symptoms within known infected orchards.

The presence of the causal agent of CLas was confirmed in all collected samples using quantitative real-time polymerase chain reaction (qPCR) prior to further analysis (Sangta et al., 2026). CLas load was quantified using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001), normalising the CLas-specific target gene against the host cytochrome

oxidase (COX) housekeeping gene, the former and the latter gene are detected by FAM and HEX fluorescent, respectively. The asymptomatic sample exhibiting the highest Ct value, representing the lowest detectable CLas signal in the dataset, was designated as the biological calibrator, anchoring all relative fold-change calculations (Wang et al., 2006). Nuclease-free water was included in each reaction as a no-template control (NTC) to verify the absence of reagent contamination but was not used as a biological calibrator. All tissue samples were flash-frozen in liquid nitrogen (N_2) and stored at $-80^\circ C$ until required for downstream analysis (Suh, Tang, et al., 2021).

2.2. Sample preparation

Leaf tissue samples were prepared for extraction following methods adapted from previous studies (do Prado Aparecido et al., 2017; Hamade et al., 2024). Sub-samples of the collected leaf material were ground to powder using liquid nitrogen (N_2) and a high-speed grinder lyser (PG-ECO-1500, Spring green evolution, Bangkok, Thailand). Leaf tissue powder (100 mg) was weighed and mixed with 400 μL of 50% v/v aqueous methanol. This suspension was subjected to ultrasonication in an ultrasonic bath at a frequency of 35 kHz for 30 min. The mixture was then centrifuged at $12,000 \times g$ for 10 min at $4^\circ C$. The supernatant was carefully transferred to a fresh tube. The remaining plant residue was subjected to two additional rounds of extraction by re-suspending the pellet in 400 μL of the extraction solvent and repeating the sonication and centrifugation procedure. The supernatants from the three sequential extractions were pooled to yield a total extract volume of $\sim 1200 \mu L$. The combined extracts were concentrated by drying overnight at room temperature using a HyperVac concentrator (Hanil Scientific Inc., Gimpo, Korea). The resulting crude extract was stored at $-20^\circ C$ until analysis. A portion of this crude methanolic extract was used for the determination of non-volatile metabolites via liquid chromatography and Nuclear Magnetic Resonance (NMR), while the remainder was utilised for the determination of total phenolic content, total flavonoid content, and antioxidant activities.

2.3. Quantification of Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and in vitro antioxidant capacity assays

Total Phenolic Content (TPC) was determined using the Folin-Ciocalteu method, with gallic acid as the reference standard. Before analysis, the crude methanolic extract was diluted in 50% aqueous methanol (methanol/water, 50/50, v/v) to ensure analyte concentrations fell within the detectable range. A diluted sample extract (25 μL) was mixed with 150 μL of 10% Folin-Ciocalteu reagent in a 96-well plate. After a brief mixing (e.g., 5 min), 120 μL of a 6% sodium carbonate (Na_2CO_3) solution was added. The reaction mixture was incubated in the dark at room temperature for 60 min. Absorbance was subsequently measured at 765 nm using a spectrophotometer. The TPC was calculated from a gallic acid standard curve and expressed as milligrams of gallic acid equivalents per gram of dry sample (mg GAE/g DW sample) (Chaiwan et al., 2024).

The Total Flavonoids Content (TFC) was determined using a colorimetric method with catechin as the standard. A diluted sample extract (50 μL) was mixed with 125 μL of distilled water, followed by the addition of 7.5 μL of a 5% sodium nitrite ($NaNO_2$) solution. The mixture was incubated at room temperature for 5 min. Subsequently, 15 μL of 10% aluminum chloride hexahydrate ($AlCl_3 \cdot 6 H_2O$) solution was added, and the mixture was allowed to react for an additional 6 min. Finally, 50 μL of a 1 M sodium hydroxide ($NaOH$) solution and 27.5 μL of distilled water were added, and the absorbance of the final mixture was measured immediately at 510 nm using a spectrophotometer. The TFC was calculated from a catechin standard curve and expressed as milligrams of catechin equivalents per gram of dry sample (mg CE/g DW sample) (Bunyarit et al., 2025).

In vitro antioxidant capacity assays namely 2,2'-azino-bis (3-ethyl-

Table 1

Plant material Collection and disease status observations.

Sample names	Descriptions	Pictures	Location of the orchards	Ct values of HEX	Ct values of FAM
CTAS	Asymptomatic citron (<i>C. medica</i> L.)		GPS: 19°42'49"N 99°04'13"E	22.41	22.03
CTS	Symptomatic citron		GPS: 19°42'49"N 99°04'13"E	23.01	19.58
SPAS	Asymptomatic tangerine cv. Sai-Nam-Phueng (<i>C. reticulata</i> Blanco cv. Sai-Nam-Phueng)		GPS: 20°36'18"N 99°09'08"E	23.19	23.17
SPS	Symptomatic tangerine cv. Sai-Nam-Phueng		GPS: 20°36'18"N 99°09'08"E	23.93	20.61
FMAS	Asymptomatic Fremont orange (<i>C. reticulata</i> cv. Fremont)		GPS: 20°30'54"N 99°15'26"E	23.39	21.48
FMS	Symptomatic Fremont orange		GPS: 20°30'54"N 99°15'26"E	22.51	15.49

benzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity were determined using our standard procedures described previously (Bunyarit et al., 2025; Chaiwan et al., 2024). The ABTS radical cation (ABTS^{•+}) solution was prepared by reacting a 7 mM ABTS solution with 2.45 mM potassium persulfate solution, followed by incubation in the dark at room temperature for 12–16 h. Before use, the ABTS^{•+} working solution was diluted with ethanol to achieve an absorbance of 0.70

± 0.02 at 734 nm. A 10 µL aliquot of the sample extract (or standard) was combined with 90 µL of the ABTS^{•+} working solution and incubated in the dark for 30 min at room temperature. The absorbance was quantified at 734 nm. The ABTS scavenging ability was determined as the percentage of antiradical activity using the following Equation (1):

$$\text{Antiradical activity (\%)} = \frac{\text{Absorbance CTRL} - \text{Absorbance sample}}{\text{Absorbance CTRL}} \times 100$$

For the DPPH assay, a solution of 0.1 mM DPPH in ethanol was prepared. A 10 µL aliquot of the citrus leaf extract (or standard) was mixed with 90 µL (or appropriate volume) of the DPPH solution and incubated at room temperature in the dark for 30 min. The absorbance was evaluated at 517 nm to determine the scavenging capability of the sample. The scavenging activity was calculated using the following Equation (2):

$$\text{Scavenging Activity (\%)} = \frac{\text{Absorbance CTRL} - \text{Absorbance sample}}{\text{Absorbance CTRL}} \times 100$$

To determine the concentration required to inhibit 50% of the radical (IC₅₀), a series of sample dilutions (ranging from 10-fold to 100-fold dilution) was prepared for each extract. The IC₅₀ value was calculated by plotting the percentage of antiradical activity against the concentration of the extract.

2.4. Cell wall polysaccharide extraction and quantitative determination of callose

Cell wall polysaccharides were extracted and callose was quantitatively determined by spectrofluorometric measurement (Bernardini et al., 2024). Briefly, for chlorophyll clearing, the tissue sample powder (5 mg) was incubated in 100% ethanol at room temperature for seven days. The ethanol was changed multiple times daily until complete removal of chlorophyll was achieved. This step is critical as residual chlorophyll can cause significant autofluorescence, leading to aberrant readings in the subsequent fluorescence assay. Following the removal of chlorophyll, the ethanol-treated pellet was air-dried and subsequently resuspended in 1650 µL of 1 M NaOH solution. The sample was sonicated for 20 min to facilitate dispersion and then incubated at 85 °C for 90 min. Frequent shaking (every 2–3 min) during incubation was performed to maximise callose extraction efficiency. The resulting supernatant, containing the extracted cell wall polysaccharides and callose, was collected and stored at 4 °C for up to six months prior to quantitative analysis. To determine the morphology of the cell wall polysaccharides, the collected extract was vacuum filtered using cellulose nitrate membrane filter papers (0.2 µm, Whatman GmbH) and lyophilised. The dried extract was mounted on stubs and coated with gold/palladium using a smart coater (DII-29030SCTR, JEOL, USA). Samples were examined using the JCM-7000 bench scanning electron microscope (SEM) (JEOL, USA) (Gonzalez et al., 2011).

The callose content in the cell-wall polysaccharide extract was quantitatively determined using a spectrofluorometric assay based on the specific binding of aniline blue dye to β-1,3-glucans. Aliquots of the extracted supernatant (10 µL) were mixed with a loading mixture containing 80 µL of 0.01% w/v aniline blue dye, 42 µL of 1 M HCl and

118 µL of 1 M glycine/NaOH buffer pH 9.5 to measure total fluorescence. A parallel set of aliquots was prepared using a blank loading mixture (where water replaced the aniline blue) to determine the intrinsic autofluorescence of the sample. Autofluorescence correction is particularly crucial for citrus tissues due to the notable autofluorescence originating from xylem components. Samples were incubated at 50 °C for 20 min protected from light (under foil) to allow for complete dye binding and prevent photobleaching of the aniline blue. Fluorescence intensity was subsequently measured using a fluorospectrophotometer (The SpectraMax® iD3s, Molecular devices, USA) with an excitation wavelength of 360 nm and an emission wavelength of 460 nm. The net callose-specific fluorescence was calculated by subtracting the autofluorescence signal and a "point 0" net fluorescence value (background signal) from the total fluorescence reading. The final callose content was expressed as micrograms of callose equivalents per gram of dry sample (mg/g DW). This quantification was achieved by interpolating the net fluorescence values against a standard curve generated using known concentrations of commercial β-1,3-glucan (syn. paramylon from *Euglena gracilis*, Sigma Aldrich, Germany).

2.5. Microarray polymer profiling

The alcohol-insoluble fraction of citrus leaf powder was prepared following established methodology (Bakshani et al., 2023). Sequential extractions were performed to isolate distinct polysaccharide fractions: (1) deionised water (DI) extraction; (2) 50 mM cyclohexanediaminetetraacetic acid (CDTA) at pH 7.5 to chelate Ca²⁺ ions and solubilise calcium-crosslinked pectins; (3) 4 M NaOH containing 0.1% (w/v) sodium borohydride (NaBH₄) to disrupt hydrogen bonds between hemicellulose and cellulose while saponifying ester linkages between hemicellulose and lignin, thereby releasing hemicellulose; and (4) enzymatic digestion with cellulase (from *Bacillus* spp., 5 U/mg, NZY-Tech, Lisbon, Portugal) in 20 mM Tris buffer (pH 8.8), incubated at 45 °C for 16 h to degrade residual cellulose and extract cellulose-bound glycans. After each extraction, samples were centrifuged at 15,000 × g for 10 min. Supernatants were collected and stored at 4 °C until analysis. All extractions employed a solid-to-liquid ratio of 1:3 (w/v).

For microarray analysis, extracted glycan fractions and glycan standards (1 mg/mL) were dispensed into 384-well microtiter plates (Greiner Bio-One, Frickenhausen, Germany). Each sample was mixed 1:1 (v/v) with printing buffer (47% glycerol, 52.9% distilled water, 0.06% Triton X-100, 0.04% ProClin™ 200) to a final volume of 40 µL. Mixtures were printed onto nitrocellulose membranes using a microarray printing robot (Marathon Argus, Arrayjet, Roslin, UK). Arrays were probed with monoclonal antibodies, commercially available carbohydrate-binding modules, and in-house produced carbohydrate-binding modules from plasmid DNA (Amsbury et al., 2024) (full details provided in Supplementary Table S1). Following primary antibody incubation, arrays were treated with alkaline phosphatase-conjugated secondary antibodies (anti-rat or anti-mouse, as appropriate). Binding was detected via chromogenic reaction using nitro-blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine (BCIP). Developed arrays were scanned using a CanoScan 8800 F flatbed scanner (Canon, Tokyo, Japan), and binding intensities were quantified with Array-Pro Analyser 6.3 software (Media Cybernetics, Rockville, MD, USA). Mean spot signal intensities were calculated for each sample, with the highest intensity normalised to 100 and all other signals expressed relative to this maximum.

2.6. Cell wall polysaccharide digestion with amylase

The enzymatic hydrolysis properties of the cell wall polysaccharide were determined using commercial α-amylase (20,000 units/g, Union Science, Thailand) (Gonzalez et al., 2011). A substrate solution (cell wall polysaccharide) at 10 mg/mL was mixed with an equal volume of α-amylase solution (2.60 mg/mL in phosphate buffer, pH 6.8) and

incubated for 5 min at 37 °C (Yin et al., 2020). The extent of hydrolysis, which cleaves α -1,4-glucosidic linkages, was quantified by measuring the resulting reducing sugars (glucose equivalents) using the 3,5-dinitrosalicylic acid (DNS) method. Absorbance was measured at 510 nm and the hydrolysis ratio was calculated as the mass of glucose product (W_M) determined from a D-glucose standard curve (0–5 mg/mL, Volchem, Thailand) divided by the initial mass of substrate (W_T).

$$\text{Hydrolysis Ratio(\%)} = \frac{W_M}{W_T} \times 100$$

Partially hydrolysed cell wall polysaccharides were collected by centrifugation, washed three times with water, and lyophilised for SEM observation as described previously.

2.7. Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

The methanol extracts were diluted in 50% aqueous methanol to ensure that analyte concentrations remained within the detector's linear dynamic range. All samples were then filtered through 0.22 μ m polytetrafluoroethylene (PTFE) syringe filters to eliminate particulates and transferred to glass vials for injection (Chai et al., 2021; López-Gresa et al., 2010). A blank sample, consisting only of the pure extraction solvent, was also prepared and analysed to identify any background contaminants. All samples were analysed in a random run order to minimise the impact of systematic variation or instrument drift over the course of the analysis. The analysis of non-volatile metabolites was performed using Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS (6545 Q-TOF LC/MS, Agilent Technologies, CA, USA). Separation was achieved on a Poroshell 120 EC-C18, 2.7 μ m, 4.6 $\times$ 150 mm (Agilent Technologies, USA) maintained at a temperature of 40 °C. The flow rate was set at 0.35 mL/min, and the injection volume was 1 μ L (Hamade et al., 2024). Mobile phases were composed of Phase A (0.04% acetic acid in ultra-pure water) and Phase B (0.04% acetic acid in acetonitrile). The gradient program was as follows: 5% B and 95% A at 0 min, decreasing to 5% A, and 5% B increasing to 95% B between 0 and 10 min and maintained for 1 min. Thereafter, A was increased to 95%, and B was decreased to 5% and maintained at the level until 14 min. Detection was carried out using the ESI source in both positive and negative ionisation modes, and a photodiode array (PDA) detector was used for UV detection across the 190–500 nm range. The ESI source parameters for the Agilent 6545 Q-TOF were set as follows: capillary voltage at 3500 V, fragmentor voltage at 120 V, drying gas temperature at 325 °C with a flow rate of 8 L/min, nebulizer pressure at 35 psi, sheath gas temperature at 350 °C with a sheath gas flow rate of 11 L/min. Nitrogen (>99.5% purity) was utilised as the drying gas. Metabolites were provisionally annotated or identified by matching their accurate masses, retention times, against established mass spectral databases (MassHunter METLIN Metabolite PCD/PCD, Agilent Technologies, CA, USA).

2.8. NMR Analysis

The dried crude extract was subsequently re-suspended in 800 μ L of deuterated methanol (methanol-d₄, Cambridge Isotope Laboratories, MA, USA) (Hamade et al., 2024). Following re-suspension, the samples were thoroughly vortexed, briefly placed in an ultrasonic bath to ensure complete dissolution, and finally centrifuged. The resulting clear supernatant was carefully transferred into 5 mm NMR tubes for subsequent analysis. All ¹H NMR spectra were acquired on an Ascend 500 MHz spectrometer equipped with a 5 mm broadband probe (Bruker Avance, Karlsruhe, Germany). The experiment employed the composite pulse presaturation sequence to suppress the residual water signal. Each spectrum was acquired using 16 scans (NS) with 65 K time domain points (TD) (do Prado Aparecido et al., 2017). Raw spectral data were processed using TopSpin software (version 4.5, Bruker Avance, Karlsruhe, Germany). Prior to Fourier transformation, the free induction

decay (FID) was zero-filled to 65 K points (SI) and multiplied by an exponential function corresponding to a line broadening (LB) of 0.3 Hz. Automatic phase correction was initially executed using the apk microprogram.

2.9. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis for Volatile Organic Compounds

Volatile organic compounds (VOCs) were extracted from citrus leaf tissue powder following a modified protocol based on Liu et al. (2012); Zhang et al. (2017). Briefly, 100 mg of homogenised citrus leaf tissue powder was weighed into a 2 mL microcentrifuge tube. Extraction was performed by adding 500 μ L of ultrapure water and 500 μ L of methyl tert-butyl ether (MTBE). Methyl nonanoate (43.75 μ g/mL) was added as an internal standard. Samples were then subjected to an ultrasonic bath (PS-20A, China) extraction maintained at 4 °C in the refrigerator for 1 h. Following sonication, extracts were centrifuged at 12,000 $\times$ g for 10 min at a temperature below 4 °C. The supernatant was subsequently filtered through a 0.22- μ m membrane to remove particulate matter. The organic layer containing the VOCs was carefully collected and dried over anhydrous sodium sulfate (Na₂SO₄) to remove any residual water.

VOC analysis was performed using an 8890 GC System, Agilent Technologies, United States coupled to a JMS-T2000GC AccuTOF™ GC-Alpha (JEOL Ltd., Tokyo, Japan). Chromatographic separation was achieved on a Rtx-5ms GC Capillary Column, 30 m, 0.25 mm ID, 0.25 μ m (Restek Corporation, PA, USA). A 2 μ L aliquot of the extracted sample was injected in split mode with a split ratio of 50:1. The oven temperature program was initiated at 40 °C, held for 3 min, then ramped at 3 °C/min to 160 °C (held for 1 min). The temperature was further increased at 5 °C/min to 200 °C (held for 1 min) and finally ramped at 8 °C/min to 240 °C (held for 3 min). The mass spectrometer was operated in positive electron ionisation (EI) mode at 70 eV. The flow rate was 1 mL/min and helium was used as carrier gas. The ion source (EI/FD and FI ion source) temperature was set at 260 °C, and the MS transfer line temperature at 280 °C. The ionisation was as follows: EI + :70 eV, 300 μ A, FI + :10 kV, 10 mA. Mass spectra were acquired in full scan mode across a mass range of m/z 45–400. Volatile compounds were tentatively identified by comparing their mass spectra and retention indices (where applicable) to those stored in the NIST/EPA/NIH Mass Spectral Library (NIST 2008) and the Wiley Registry of Mass Spectral Data (8th ed.).

2.10. Statistical analysis

All physiological and quantitative data were expressed as means $\pm$ standard error (SE) of at least three biological replicates. To evaluate significant differences between means across citrus genotypes and infection statuses, a one-way Analysis of Variance (ANOVA) was performed, followed by Duncan's Multiple Range Test (DMRT) as a post-hoc analysis with statistical significance defined at $p < 0.05$. A Pearson correlation matrix was computed to assess the strength and direction of linear relationships between chemical constituents and antioxidant capacities. Multivariate statistical analyses, specifically Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), were employed to visualise variances in metabolite profiles (NMR and LC-MS) and glycoarray antibody binding patterns. The Jamovi software ver 2.7.12 was utilised for all statistical analyses. Furthermore, the differential abundance of volatile organic compounds was illustrated using the built-in application of the GC-MS software to generate Volcano Plots; these plots mapped the magnitude of change (Log₂ Fold Change) against statistical significance (-Log₁₀ p -value) to classify metabolites as unique to asymptomatic (A), symptomatic (B), or stable (A=B) conditions.

3. Results

3.1. Quantitative assessment of CLas in citrus samples

The presence and bacterial load of CLas in the citrus samples were confirmed using qPCR. Pathogen detection was initially based on the Cycle Threshold (Ct) value, with samples exhibiting a Ct < 30 classified as HLB-positive (Suh et al., 2021). To comparatively assess relative infection levels across samples, bacterial loads were estimated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), which analyses relative changes in gene expression by normalising the target CLas sequence against the host plant's housekeeping gene (COX) and a healthy control calibrator. The relative quantification results, illustrated in Fig. 1, reveal a wide spectrum of CLas infection levels across the tested cultivars and sample types. The biological calibrator, the asymptomatic sample with the highest Ct value, indicating the lowest detectable CLas load within the dataset, was assigned a relative fold change of 1.00. Nuclease-free water was included as a no-template control (NTC) to verify the absence of reagent contamination and is not represented in the relative quantification data. Asymptomatic (FMAS and CTAS) showed relatively lower bacterial titers, ranging from about 150–700-fold change. Symptomatic samples showed a drastic increase in bacterial load. Fremont (FMS) exhibited the highest pathogen accumulation with a relative fold change of 7858 followed by Citron (CTS) at 1992. SPS also showed high titers at 1,845-fold change, the latter two reaching comparable levels despite representing distinct citrus species. These results demonstrate that symptomatic tissues serve as the primary reservoirs for CLas, with bacterial loads increasing several thousand-fold compared to asymptomatic or healthy tissues. The high titers observed in the symptomatic samples highlight their role as critical subjects for the subsequent phytochemical and metabolic profiling in this study. Furthermore, FMS showed significantly higher than all samples indicating that the sample was highly susceptible for CLas infection. This comparison reflects pathogen accumulation at a single symptomatic time point rather than a dedicated resistance or tolerance assay, however, and should not be read as a definitive genotype resistance ranking on its own; it also sits in some tension with the comparatively limited metabolic disruption observed in symptomatic Fremont elsewhere in this study, underscoring that pathogen titer and metabolic tolerance do not necessarily track one another in this system.

3.2. Quantification of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), in-vitro antioxidant capacity and callose content in different citrus extracts

The methanolic extraction of commercial citrus species, Citron, Sai-Nam-Phueng Tangerine, and Fremont Orange, under both asymptomatic (A) and symptomatic (S) conditions revealed distinct patterns in yield efficiency (Table 2). By using 50% v/v aqueous methanol, symptomatic samples consistently demonstrated higher or comparable metabolite extraction yields relative to their asymptomatic counterparts across all genotypes examined. Symptomatic Citron (CTS) achieved a notably higher yield (10.78%) compared to Asymptomatic Citron (CTA, 8.67%), representing a 24.30% increase. Similarly, Symptomatic Sai-Nam-Phueng (SPS) exhibited the highest extraction yield among all samples at 17.31%, substantially exceeding its asymptomatic counterpart (SPA, 10.10%). Fremont Orange followed a comparable pattern, with symptomatic samples (FMS, 13.35%) approaching but not surpassing asymptomatic yields (FMA, 14.52%). Among all genotypes examined, SPS demonstrated the highest extraction efficiency (17.31%), while CTA recorded the lowest yield (8.67%).

TPC responses to CLas infection were genotype-dependent and did not follow a consistent pattern across all citrus varieties examined (Table 2). In Sai-Nam-Phueng, symptomatic samples (SPS, 17.23 mg GAE/g DW sample) showed significantly higher TPC than asymptomatic counterparts (SPA, 11.65 mg GAE/g DW sample), representing the only genotype where a statistically significant elevation was observed under symptomatic conditions. In Citron, symptomatic samples (CTS, 8.98 mg GAE/g DW sample) were numerically higher than asymptomatic samples (CTA, 6.40 mg GAE/g DW sample); however, both groups were not statistically significant. In Fremont Orange, the trend was reversed: asymptomatic samples (FMA, 16.77 mg GAE/g DW sample) exhibited higher TPC than symptomatic samples (FMS, 15.35 mg GAE/g DW sample), contradicting the notion of a universal stress-induced elevation. These results indicate that phenolic accumulation in response to CLas infection is genotype-specific, with a significant stress-induced biosynthesis of phenolic compounds observed only in Sai-Nam-Phueng, and absent or reversed in Citron and Fremont Orange, respectively.

The TFC analysis revealed patterns consistent with TPC measurements. Symptomatic samples generally accumulated higher flavonoid

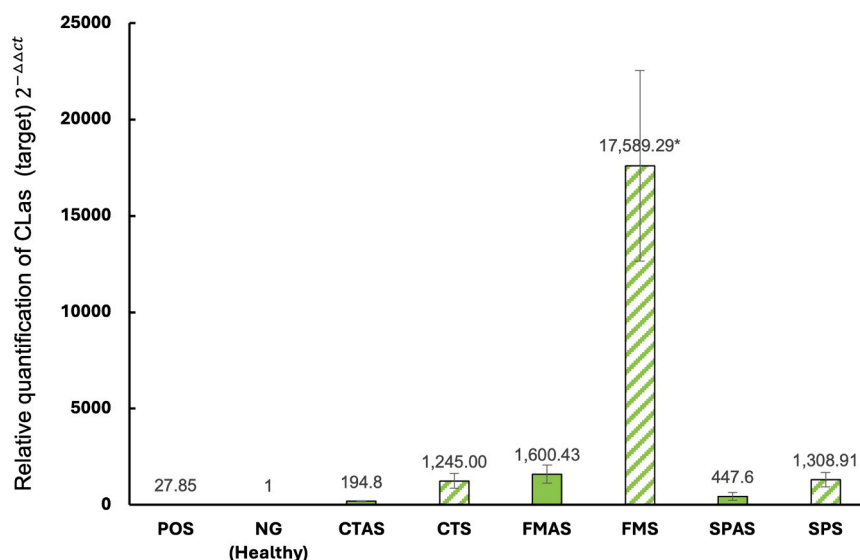


Fig. 1. CLas pathogen load across different citrus cultivars and symptom stages. Bars represent the relative fold change of CLas DNA quantified via qPCR using the $2^{-\Delta\Delta Ct}$ method. FM: Fremont; CT: Citron; SP: Sai-Nam-Phueng; AS: Asymptomatic; S: Symptomatic. Cycle Threshold (Ct) values are provided for borderline asymptomatic samples to indicate infection depth. Differences in CLas titer (ΔCt) among the six samples were evaluated by one-way ANOVA followed by Tukey's HSD. Statistical significance levels are indicated by asterisks: * ($p < 0.05$).

Table 2

Yield, Phenolic Content, Flavonoid Content, and Antioxidant Capacity of citrus leaf extracts.

Samples	Dried methanolic extract %	Total phenolic content mg GAE/g DW sample	Total flavonoid content mg CE/g DW sample	Antioxidant Capacity Assays		Callose content mg β -1,3- glucan equivalent/g DW sample
				DPPH (IC50)	ABTS (IC50)	
CTA	8.67 $\pm$ 0.22 ^a	6.40 $\pm$ 0.40 ^d	18.70 $\pm$ 0.25 ^d	10.97 $\pm$ 0.47 ^a	1.48 $\pm$ 0.03 ^a	0.16 $\pm$ 0.045 ^d
CTS	10.78 $\pm$ 0.59 ^c	8.98 $\pm$ 0.91 ^{cd}	21.59 $\pm$ 0.63 ^c	17.76 $\pm$ 0.48 ^b	1.33 $\pm$ 0.01 ^b	0.46 $\pm$ 0.006 ^c
FMA	14.52 $\pm$ 0.47 ^b	16.77 $\pm$ 1.34 ^a	20.29 $\pm$ 0.72 ^c	8.85 $\pm$ 0.05 ^b	0.50 $\pm$ 0.01 ^e	1.01 $\pm$ 0.04 ^b
FMS	13.35 $\pm$ 0.85 ^b	15.35 $\pm$ 1.43 ^{ab}	20.58 $\pm$ 0.29 ^c	13.49 $\pm$ 0.82 ^b	0.64 $\pm$ 0.02 ^d	1.16 $\pm$ 0.14 ^b
SPA	10.10 $\pm$ 0.59 ^c	11.65 $\pm$ 1.10 ^{bc}	23.77 $\pm$ 0.52 ^b	45.40 $\pm$ 0.67 ^c	0.98 $\pm$ 0.02 ^c	0.08 $\pm$ 0.002 ^e
SPS	17.31 $\pm$ 0.20 ^a	17.23 $\pm$ 2.15 ^a	29.42 $\pm$ 1.26 ^a	9.37 $\pm$ 0.67 ^a	0.46 $\pm$ 0.01 ^e	2.58 $\pm$ 1.49 ^a

Values are means (n = 3) $\pm$ standard error, and values with different superscript letters are significantly different in each column according to Duncan's multiple range test ($p < 0.05$). GAE = gallic acid equivalent, CE = catechin equivalent. abbreviations: Citron (CT), Fremont Orange (FM), Sai-Nam-Phueng Tangerine (SP) with asymptomatic (A) and symptomatic (S) conditions

concentrations compared to their asymptomatic equivalents. Symptomatic Citron (CTS, 21.59 mg CE/g DW sample) exceeded Asymptomatic Citron (CTA, 18.70 mg CE/g DW sample) by 15.50%. Symptomatic Sai-Nam-Phueng demonstrated the most pronounced difference, with SPS (29.42 mg CE/g DW sample) showing 23.80% higher TFC than SPA (23.77 mg CE/g DW sample). Fremont Orange exhibited minimal variation between conditions: FMA (20.29 mg CE/g DW sample) and FMS (20.58 mg CE/g DW sample) differed by less than 1.50%, indicating relative stability in flavonoid content regardless of physiological state. Notably, Sai-Nam-Phueng Tangerine consistently exhibited the highest TFC values among all samples examined, regardless of symptomatic status, suggesting inherently superior flavonoid biosynthetic capacity in this genotype.

DPPH assay results, where lower IC50 values indicate stronger antioxidant activity, revealed genotype-dependent patterns that do not uniformly support enhanced antioxidant capacity in symptomatic samples (Table 2). In Citron, asymptomatic samples (CTA, 10.97 μ g/mL) showed significantly stronger DPPH radical scavenging than symptomatic samples (CTS, 17.76 μ g/mL), representing a 38.20% reduction in antioxidant efficacy under symptomatic conditions, directly contradicting a stress-enhancement hypothesis for this genotype. In Fremont Orange, asymptomatic samples (FMA, 8.85 μ g/mL) also demonstrated numerically stronger scavenging than symptomatic samples (FMS, 13.49 μ g/mL) by 34.40%; however, this difference was not statistically significant. In Sai-Nam-Phueng, symptomatic samples (SPS, 9.37 μ g/mL) showed significantly stronger DPPH activity than asymptomatic samples (SPA, 45.40 μ g/mL), achieving a 79.30% lower IC50. Thus, significantly enhanced DPPH antioxidant capacity under symptomatic conditions was observed only in Sai-Nam-Phueng; Citron showed the opposite (asymptomatic significantly stronger), and Fremont Orange showed no statistically significant difference between conditions. The strongest DPPH radical scavenging activities were recorded in Fremont Orange asymptomatic samples (FMA, 8.85 μ g/mL) and Symptomatic Sai-Nam-Phueng (SPS, 9.37 μ g/mL), both below 10.00 μ g/mL. ABTS assay results were similarly genotype-dependent. In Citron, symptomatic samples (CTS, 1.33 μ g/mL) showed significantly stronger ABTS activity than asymptomatic samples (CTA, 1.48 μ g/mL), representing a 10.20% improvement. In Fremont Orange, however, the pattern was reversed: asymptomatic samples (FMA, 0.50 μ g/mL) demonstrated significantly stronger activity than symptomatic samples (FMS, 0.64 μ g/mL), indicating that CLas infection did not enhance ABTS antioxidant capacity in this genotype. In Sai-Nam-Phueng, symptomatic samples (SPS, 0.46 μ g/mL) showed markedly superior performance compared to asymptomatic samples (SPA, 0.98 μ g/mL), achieving 53.40% lower IC50. Overall, ABTS results do not consistently support an universal

stress-induced enhancement: symptomatic samples were significantly stronger in Citron and Sai-Nam-Phueng, while asymptomatic samples were significantly stronger in Fremont Orange. The most potent ABTS radical scavenging was observed in Symptomatic Sai-Nam-Phueng (SPS, 0.46 μ g/mL) and Fremont Orange asymptomatic samples (FMA, 0.50 μ g/mL), both below 0.65 μ g/mL.

3.3. Callose content and correlation analysis

The callose content, measured as β -1,3-glucan equivalent/g DW sample, showed a substantial variation across the six samples. SPS clearly exhibited the highest callose concentration (2.58 mg β -1,3-glucan equivalent/g DW sample) and was statistically distinct from all other samples, while the next highest levels were found in FMS (1.16 mg β -1,3-glucan equivalent/g DW sample) and FMA (1.00 mg β -1,3-glucan equivalent/g DW sample). Conversely, SPA had the lowest callose content (0.08 mg β -1,3-glucan equivalent/g DW sample), with CTA (0.17 mg β -1,3-glucan equivalent/g DW sample) and CTS (0.46 mg β -1,3-glucan equivalent/g DW sample) containing comparatively low amounts. A Pearson correlation matrix was computed to examine the relationships between various measured properties, with particular focus on how the extracts' chemical composition relates to their antioxidant activity (Fig. 2). TPC exhibited a highly significant strong negative correlation with ABTS IC50 (Corr: -0.90), indicating that higher phenolic content is strongly associated with greater ABTS antioxidant activity. Similarly, TFC also showed a significant negative correlation with ABTS IC50 (Corr: -0.48) as well as a positive correlation with callose (Corr: 0.61), suggesting that higher flavonoid content is associated with higher ABTS activity and also with callose accumulation. Additionally, the correlation coefficients between callose content and ABTS IC50 was statistically significant, indicating a strong reliable, linear relationship between callose concentration and flavonoid compounds or antioxidant capacity in the tested samples.

The relationships among TPC, TFC, antioxidant capacity, callose content, and CLas reveal a coordinated but genotype-specific defence landscape. In Sai-Nam-Phueng tangerine, the symptomatic accession (SPS) exhibited both the highest CLas relative fold-change (Fig. 1) and the highest callose content (2.58 mg β -1,3-glucan equivalent/g DW), consistent with callose deposition being a direct phloem response to CLas colonisation (Koh et al., 2012; German et al., 2023). By contrast, Fremont mandarin maintained elevated callose in both symptomatic (FMS) and asymptomatic (FMA) tissues regardless of symptom status, suggesting constitutive callose priming as part of its tolerance strategy, a pattern corroborated by its comparably high TFC and antioxidant activity (Table 2). In Citron, however, despite confirmed CLas

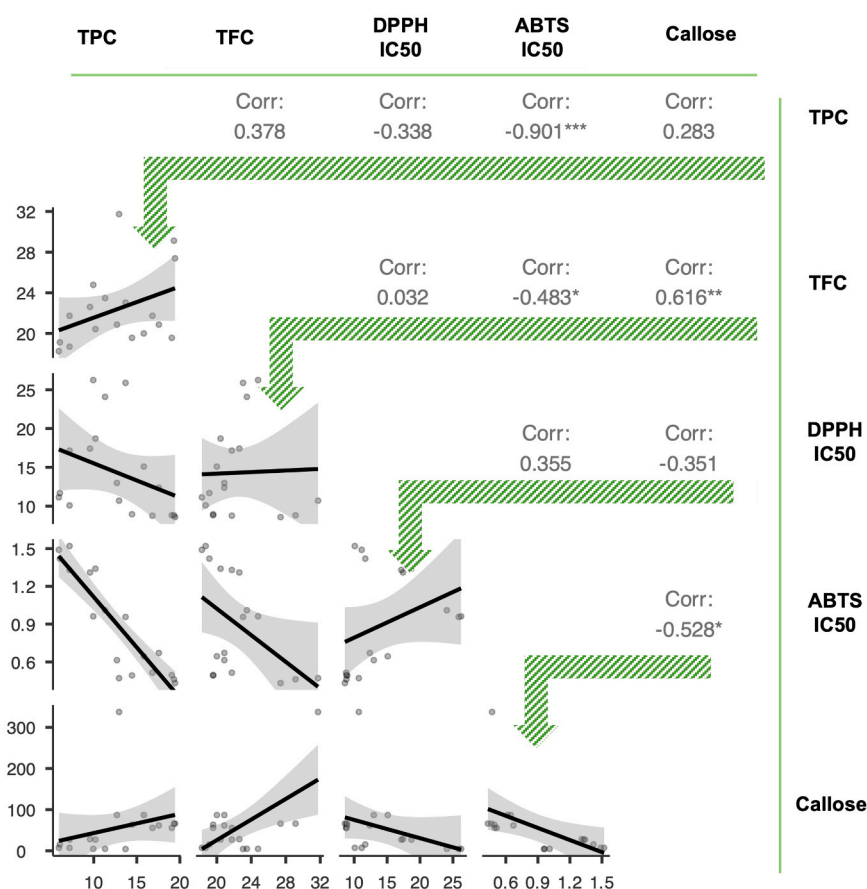


Fig. 2. Correlation Matrix of Chemical Constituents and Antioxidant Activity. Scatter plots and Pearson correlation coefficients (Corr) are shown for Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH IC₅₀, ABTS IC₅₀, and Callose content. Statistical significance levels are indicated by asterisks: * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

presence in symptomatic tissue (CTS), callose levels remained low and starch accumulation was more pronounced (Fig. 3), indicating a divergent metabolic response that prioritises carbohydrate sequestration over β -1,3-glucan deposition. The significant positive correlation between TFC and callose (Corr: 0.61, Fig. 2) is consistent with reports that

flavonoid-enriched environments can upregulate callose synthase activity, potentially linking secondary metabolite accumulation to structural reinforcement of phloem cell walls (Wang et al., 2021; Granato et al., 2019). Furthermore, the strong negative correlation between TPC and ABTS IC₅₀ (Corr: -0.90 , Fig. 2) indicates that genotypes with high

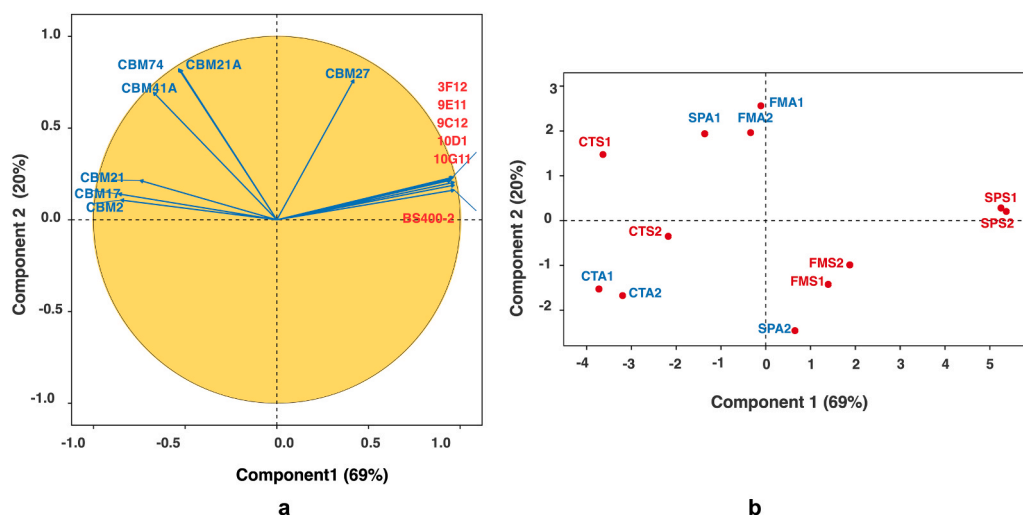


Fig. 3. Principal component analysis (PCA) of antibody binding specificity to polysaccharides in commercial citrus species. (A) PCA loadings plot showing antibodies that bind specifically with α -1,4-glucans (CBM2, CBM27, CBM17, CBM21, CBM41A, CBM21A, and CBM74) and β -1,3-glucans (3F12, 10G11, 9C12, 10D1, BS-400-2, and 9E11). (B) Scores plot showing the distribution of symptomatic and asymptomatic samples from Citron, Sai-Nam-Phueng tangerine, and Fremont mandarin based on their polysaccharide composition profiles.

phenolic content also mount the most effective antioxidant responses, a capacity likely important for mitigating reactive oxygen species generated during CLAs-induced phloem stress. Taken together, these data suggest that genotypes with high TPC and TFC constrain CLAs proliferation through both direct antimicrobial pressure and indirect structural reinforcement of phloem cell walls via callose deposition, whereas genotypes with lower secondary metabolite levels compensate through

alternative metabolic rerouting such as starch accumulation.

3.4. Microarray polymer profiling

Hierarchical cluster analysis of 53 antibodies screened against glycan extracts from various citrus and related Rutaceae species revealed distinct clustering patterns based on polysaccharide specificity.

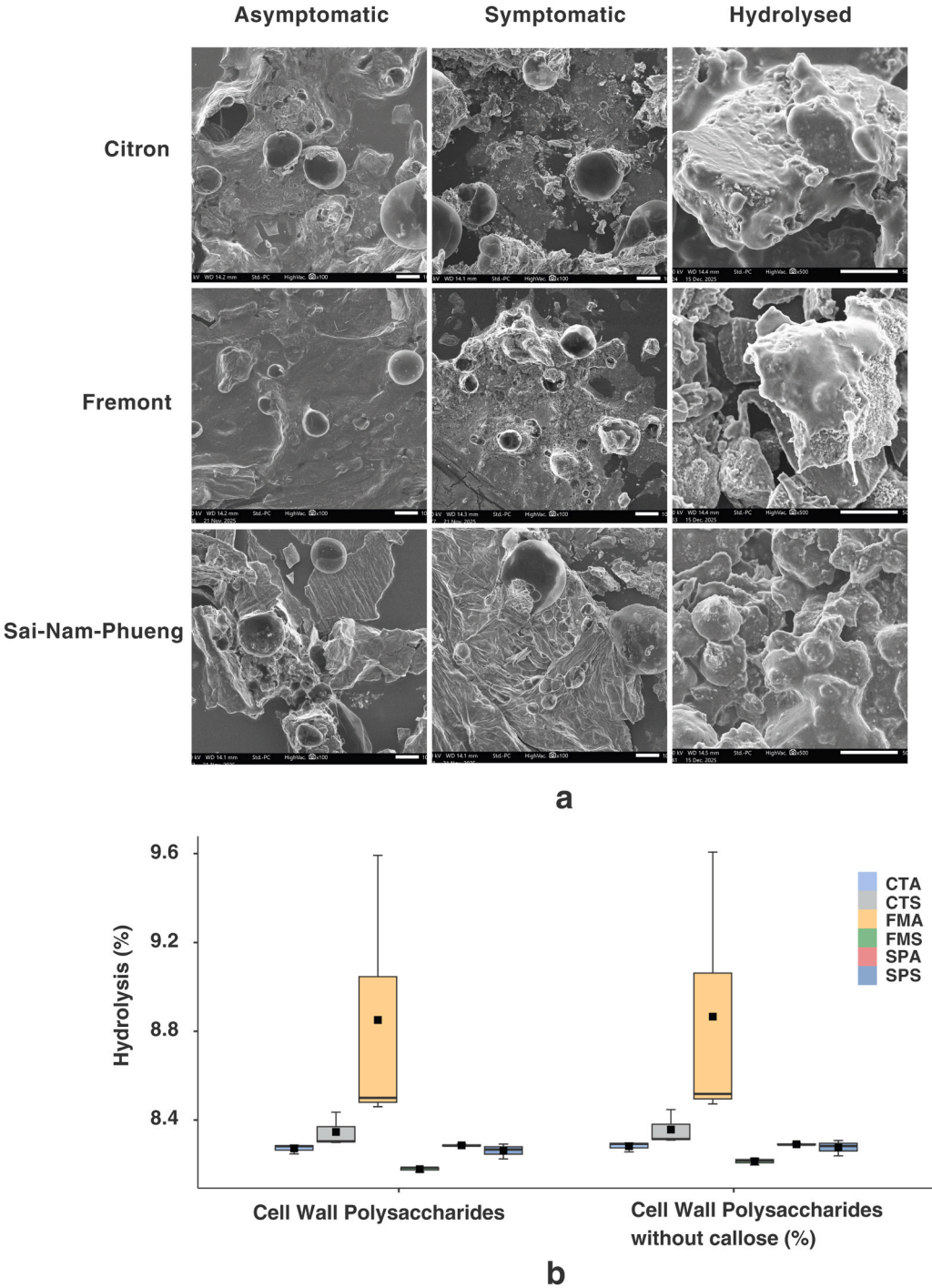


Fig. 4. (A) SEM Analysis of polysaccharide deposits in Citrus Leaf Tissue. Asymptomatic leaf samples Citron, Sai-Nam-Phueng tangerine, and Fremont mandarin with comparatively smaller, dispersed polysaccharide deposits, consistent with efficient carbohydrate export. Symptomatic leaf samples displaying larger, accumulated polysaccharide deposits (consistent with starch-type polysaccharides based on extraction procedure, glycoarray profiling, and α -amylase hydrolysis), often ranging from 300 to 500 μ m in diameter. Hydrolysis of Cell Wall Polysaccharides with commercial amylase successfully digested starch deposits. (B) The hydrolysis activity of cell-wall polysaccharides derived from the samples. Asymptomatic Citron (CTA), Symptomatic Citron (CTS), Asymptomatic Sai-Nam-Phueng (SPA), Symptomatic Sai-Nam-Phueng (SPS), Asymptomatic Fremont Orange (FMA), and Symptomatic Fremont Orange (FMS).

Supplementary Figure S1 demonstrates that antibodies 3F12, 10G11, 9C12, 10D1, and BS-400-2, which bind β -1,3-glucans as defined by Meikle et al. (1991), clustered together, indicating a common detection pattern linked to callose formation. Carbohydrate binding modules (CBMs) derived from different classes of polysaccharide-processing enzymes were examined following the approaches of Boraston et al. (2003), Najmudin et al. (2006), and Venditto et al. (2016), and were distributed across multiple clusters. CBM2, CBM27, CBM17, CBM21, CBM41A, CBM21A, and CBM74 were selected for further analysis as they clustered near the callose-binding antibodies. Principal component analysis (PCA) of glycoarray data accounting for more than 80% of the variance (PC1, 69%, PC2, 20%) revealed two distinct antibody groups binding specifically with amylose and callose polysaccharides. Fig. 3B shows that the scores plot revealed a clustering pattern driven primarily by polysaccharide composition rather than symptom status alone.

Sai-Nam-Phueng symptomatic samples (SPS) occupied strongly positive PC1 coordinates, consistent with a dominant β -1,3-glucan (callose) signal. Table 2 shows that SPS samples contained the highest recorded callose content (2.58 mg β -1,3-glucan equivalent/g DW sample). Fremont mandarin symptomatic (FMS) and asymptomatic (FMA) samples also clustered in the positive PC1 region, with Table 2 indicating comparably elevated callose levels (FMS, 1.16 and FMA, 1.01 mg β -1,3-glucan equivalent/g DW sample). These samples demonstrated elevated callose regardless of visible symptom status. In contrast, Table 2 shows that Citron symptomatic samples possessed relatively low callose content (CTS, 0.46 mg/g DW sample), with clustering in negative PC1 coordinates alongside α -1,4-glucan (starch)-binding probes, indicating that starch rather than callose predominates in this genotype under infection. Asymptomatic Sai-Nam-Phueng (SPA) and Citron (CTA) samples, both with the lowest callose values (SPA, 0.08 and CTA, 0.16 mg/g DW sample), similarly clustered in the starch-associated negative PC1 region.

3.5. Cell wall-polysaccharide hydrolysis

SEM images of cell wall polysaccharide extracts from citrus leaf tissues revealed distinct differences in the size of polysaccharide deposits related to the symptomatic status of the samples (Fig. 4a). Asymptomatic samples exhibited comparatively smaller deposits of cell wall polysaccharides, consistent with normal metabolic function involving the efficient partitioning and transport of carbohydrates from source leaves. In contrast, symptomatic samples showed a pronounced tendency toward larger polysaccharide deposit sizes. This accumulation of large polysaccharide deposits aligns with pathological changes characteristic of phloem-disrupting diseases such as HLB. In symptomatic leaves, pathogen infection induces the production and deposition of callose (β -1,3-glucan) within the phloem sieve plates, leading to phloem blockage. This callose buildup severely restricts the transport of photoassimilates, primarily sucrose, from the source leaf to the sink organs. The resulting inability to export sucrose forces the plant to convert the excess sugar into a storage polysaccharide, primarily starch, which then accumulates in large particles within the chloroplasts. While morphological evidence alone is acknowledged to be insufficient for definitive polysaccharide identification, the assignment of these large deposits as predominantly starch-type (α -1,4-glucan) aggregates is supported by three complementary lines of evidence. First, the cell wall polysaccharide extract used for SEM analysis was prepared by sequential alkaline (1 M NaOH) extraction, a procedure that selectively enriches for callose (β -1,3-glucan) and starch-type α -1,4-glucan polysaccharides while removing soluble sugars and lipids. Second, glycoarray profiling of extracts from the same samples indicated the co-presence of callose (β -1,3-glucan epitopes recognised by mAbs BS-400-2, 3F12, and 9E11) and starch-type α -1,4-glucan residues (bound by CBM21, CBM27, and CBM74), establishing the polysaccharide composition of the extracted material independent of morphology. Third, enzymatic hydrolysis with commercial α -amylase, which specifically cleaves α -1,4-glycosidic

bonds, resulted in partial degradation of the extracted material, providing biochemical evidence consistent with the presence of starch rather than callose or cellulose.

Large polysaccharide particles ranging from 300 to 500 μ m in diameter were observed in symptomatic leaf extracts under SEM. Although morphological evidence alone is insufficient for definitive polysaccharide identity assignment, these structures are consistent with starch granule aggregates on the basis of the extraction procedure, glycoarray profiling results, and enzymatic hydrolysis data described above. It should also be noted that this size likely represents large aggregations or clusters of granules within the cells, given typical individual starch granule dimensions, which partially hydrolysed up to 10% of the starch substrate within 5 min under ex vivo conditions (Fig. 4a). It is worth highlighting that the FM genotype exhibited a distinctive hydrolysis pattern, with the asymptomatic group illustrating higher levels of hydrolysis (Fig. 4b). Notably, hydrolysis was observed across both symptomatic and asymptomatic samples and across all tested citrus varieties, suggesting that the accumulated material contains α -1,4-glucan-linked starch regardless of symptom status, although further biochemical confirmation would strengthen this interpretation.

3.6. LC-MS Analysis

Metabolomic analysis revealed highly distinct signatures across the three tested germplasms. A total of 52 unique metabolic features were identified, with significant inter-germplasm variability in metabolite composition (Supplementary Table S2). Sai Nam Phueng (SPA/SPS) exhibited the greatest metabolic diversity with 34 unique features, followed by Citron (CTA/CTS) with 22 features and Fremont Orange (FMA/FMS) with 20 features. Limited metabolite sharing among germplasms reflected substantial genetic and biochemical divergence. The distribution and abundance of liquid chromatography-mass spectrometry (LC-MS) features across the six sample groups (SPS, SPA, FMS, CTS, CTA, FMA) were visualised using a ComplexUpset diagram (Fig. 5a). The number of unique LC-MS features per group ranged from 5 to 11. FMA contained the largest feature set ($n = 11$), while FMS contained the smallest ($n = 5$). SPS and CTS contained 8 and 7 features, respectively. Intersection analysis quantified feature sharing between group combinations. The most abundant unique feature sets were found exclusively in FMA and SPS ($n = 8$ each), representing highly distinctive molecular characteristics. SPA and CTA also showed high uniqueness ($n = 6$ each). Notably, only one feature was common to all six groups, indicating minimal complete overlap across the dataset. The CTA-FMA intersection contained 4 shared features, while the three-way intersection of CTS-CTA-FMA contained 2 features. Complex multi-group intersections (e.g., SPA-FMS-CTS-CTA and FMS-CTS-CTA) each contained 2 features.

Principal component analysis (PCA) (Fig. 5b) revealed relationships between sample groups based on metabolite profiles. The first two components explained 27.30% and 19.50% of total variance, respectively. Vector analysis indicated that SPA and FMA metabolite profiles were highly similar. Symptomatic samples from all three genotypes clustered in the same quadrant, with CTS and SPS vectors pointing toward the upper right. In contrast, the CTA vector pointed toward the lower left, nearly opposite the main cluster, indicating chemical distinctiveness and negative correlation with features driving other groups.

Only one feature, sphinganine (C16), was the baseline for all three germplasms despite symptom appearance. This foundational sphingoid base, along with N-(octanoyl) sphinganine (C8-dihydroceramide), 3-oxohexacosanoic acid, and N-(2-hydroxyhexadecanoyl) sphinganine (detected in FM and SP), represents essential building blocks for cell membranes, including sphingolipids and fatty acids required universally for structural membrane and signaling molecule biosynthesis. The 1,1-cyclopropanedicarboxylic acid and its synonyms (mesaconic acid, methylfumalic acid, glutaconic acid, citraconic acid, and itaconic acid)

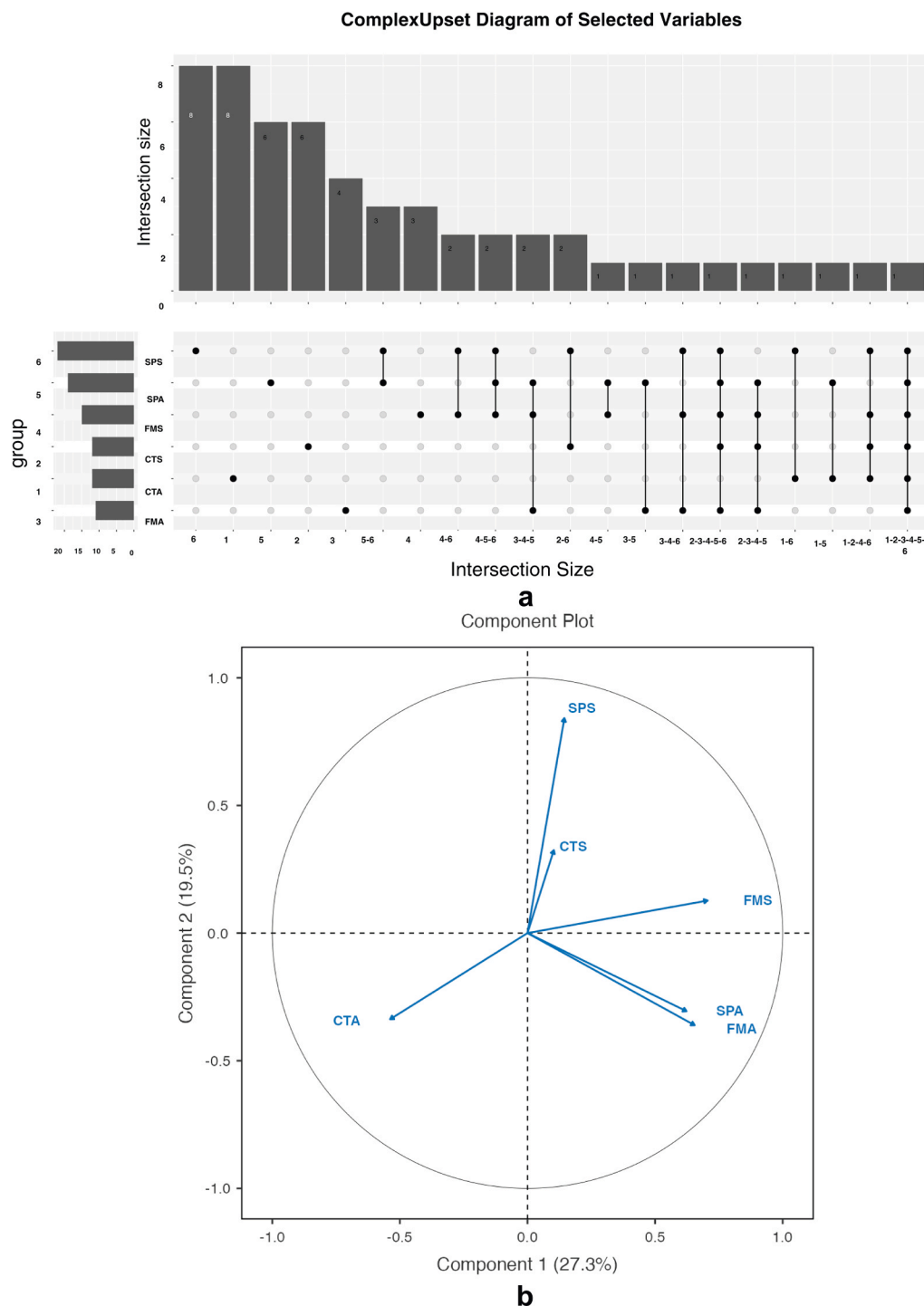


Fig. 5. (A) ComplexUpset Diagram of Selected Variables illustrating the distribution of LC-MS features across six sample groups [CTA (1), CTS (2), FMA (3), FMS (4), SPA (5), SPS (6)]. The bottom-left bar chart displays the total number of features (Set size) for each group. The top bar chart displays the size (count) of the specific feature intersections. The matrix below shows which groups contribute to each intersection (black dots indicate inclusion). (B) Component plot of metabolite profiles across six experimental groups. Principal Component Analysis (PCA) loading plot visualising the variation between groups (CTA, CTS, FMA, FMS, SPA, SPS). Asymptomatic Citron (CTA), Symptomatic Citron (CTS), Asymptomatic Sai-Nam-Phueng (SPA), Symptomatic Sai-Nam-Phueng (SPS), Asymptomatic Fremont Orange (FMA), and Symptomatic Fremont Orange (FMS).

were detected exclusively in CT samples and symptomatic FM and SP samples, representing tricarboxylic acid (TCA) cycle intermediates, as documented by Al Kadhi et al. (2017) and Fiori et al. (2018), and indicating conserved central carbon metabolism. Additional TCA-related compounds, including (*E*)-prop-1-ene-1,2,3-tricarboxylic acid and malic acid, were detected only in CTS and SPS, respectively. The asymptomatic state was characterised by exclusive metabolic

features reflecting normal metabolic homeostasis. This baseline profile included sugar acid derivatives (2,5-didehydro-D-gluconate and ascorbalamic acid), indicating active carbohydrate metabolism and ascorbate-related pathways. Fatty acid-derived esters (5Z-octenyl acetate and methyl (ξ)-3-nonenote) suggested intact lipid metabolism and volatile compound production typical of healthy citrus tissue. Alkaloids (clavipictine B and deltaline) and a triterpenoid (ganoderic acid F)

indicated constitutive expression of specialised metabolic pathways involved in baseline defence or signalling, as described by Li et al. (2024). Additional features (butyl ethylmalonate, glu-P-2, and 6-ladderane-1-hexanol with related isomers) collectively defined the healthy metabolic baseline that becomes disrupted during symptom development.

The symptomatic state displayed distinct exclusive features representing either de novo stress-induced biosynthesis or catabolic breakdown products from cellular perturbation. Defence-associated secondary metabolites included trans,trans-farnesol (a sesquiterpene alcohol involved in antimicrobial defence and stress signalling) and pelargonidin 3-lathyruside (an anthocyanin induced during pathogen challenge and oxidative stress), as documented by Li et al. (2024). Phenolic compounds included isopropyl 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoate, apigenin 7-O-(3'-acetyl-6"-E-p-coumaroyl- β -D-glucopyranoside), tambulin 3,5-diacetate, catechin 3-O-(1-hydroxy-6-oxocyclohex-2-ene-1-carboxylate), and propan-2-yl 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoate, representing highly specialised products of secondary metabolic pathways, as described by Rao et al. (2023).

Organic acids included trans-aconitate (a TCA cycle intermediate often accumulated under metabolic stress), documented by Zhang et al. (2025), and substituted derivatives such as 2,2'-(3-Methylcyclohexane-1,1-diyl) diacetic acid and 2-O-caffeoylglucaric acid, indicating altered carbon flux and enhanced phenylpropanoid metabolism, as reported by He et al. (2024) and Teutschbein et al. (2010). Lipid-derived compounds included 13E-docosenamide and related fatty acid amides, potentially functioning in lipid signalling or representing membrane degradation products, as noted by He et al. (2019) and Zhou et al. (2017). The detection of sodium tetradecyl sulfate, a surfactant-like compound, possibly represents a novel defence metabolite or detoxification product. Additional symptomatic features included anapheline, diglycidyl resorcinol ether, and 3-(4-methoxyphenyl)-2-methyl-2-propenal with isomers.

It is important to note the detection of several artifacts, comprising both naturally occurring and synthetic contaminants. These findings likely result from instrumental spectral mismatching or the introduction of impurities during sample processing and extraction (Supplementary Table S1).

3.7. NMR Analysis

NMR database analysis of methanolic extracts from the samples identified 17 primary metabolites, including amino acids, sugars, and related compounds using a reference presented in Supplementary Table S3. The metabolites were characterised by their distinctive ^1H chemical shifts, providing a comprehensive metabolic fingerprint of the citrus leaf tissue.

The analysis revealed a diverse array of amino acids and their derivatives. Alanine was detected at δ 1.48 and 3.77 ppm, while leucine showed characteristic peaks at δ 0.96 and 1.73/1.71 ppm. Arginine exhibited multiple resonances at δ 3.24, 3.30, and 3.78 ppm, and asparagine was identified at δ 2.86, 2.86, and 4.01 ppm. The branched-chain amino acid valine displayed peaks at δ 1.04 and 2.27 ppm. Proline demonstrated chemical shifts at δ 2.01, 2.08, 2.33, and 4.13 ppm, while its derivative proline betaine (N,N-dimethyl-proline) was detected at δ 3.11 and 3.30 ppm. Tyrosine showed aromatic and aliphatic proton signals at δ 6.95/6.94 and 7.20 ppm. Tryptophan, another aromatic amino acid, exhibited multiple peaks at δ 7.34, 7.56, and 7.73/7.71 ppm. Modified amino acids were also present, including γ -aminobutyrate (GABA) at δ 1.91 and 3.02/3.01 ppm, choline at δ 3.20 and 3.51 ppm, and dimethylamine at δ 2.78 ppm. Additional nitrogen-containing metabolites included putrescine at δ 1.76 and 3.06 ppm, threonine at δ 1.33, 3.60, and 4.25 ppm, glutamine at δ 2.18, 2.45, and 3.79 ppm, and glycine betaine, an important osmoprotectant, at δ 3.20 and 3.90 ppm.

Several organic acids were identified in the extracts. Fumaric acid, a tricarboxylic acid cycle intermediate, showed a characteristic singlet at δ 6.53 ppm. Malate, another TCA cycle component, displayed peaks at δ 2.44, 2.70, and 4.31–4.33 ppm, indicating the presence of both CH_2 and CH protons. Sugar analysis revealed both α - and β -anomers of glucose, with chemical shifts at δ 4.65 and 5.23/5.24 ppm for the anomeric protons. Sucrose, the primary transport sugar in plants, was detected at δ 4.22 and 5.42 ppm, corresponding to its distinctive anomeric and ring proton resonances.

The NMR metabolomic data were subjected to PCA to assess inherent metabolic variation and group separation among the samples (Fig. 6a). The initial PCA indicated the high quality of the metabolic data, with the first two principal components (PC1 and PC2) accounting for > 99% of the total variance in the dataset. The score plot illustrated a clear metabolic distinction where citron samples segregated from the other citrus varieties (Fremont orange and tangerine). The loading plots (Fig. 6b) identified choline, proline betaine, and asparagine as the strongest discriminating metabolites along PC1. Cluster analysis also revealed that the samples clustered into three distinct metabolite groups (Figs. 6b–1). Proline betaine was found to be particularly dominant in the citron tissue, indicating a potential metabolic feature unique to this variety. Notably, the PCA was unable to distinguish between symptomatic and asymptomatic tissues based on the overall primary metabolite profile, suggesting that the primary metabolite concentration changes are driven by variety (genotype) rather than the disease state in this study.

3.8. Volatile compound profiles of citrus extracts

The GC-MS data revealed distinct metabolic shifts in response to CLAs infection. Compounds were categorised based on their presence in asymptomatic (A) and symptomatic (B) samples to identify potential biomarkers of infection and resistance. The volcano plots (Fig. 7) illustrate the differential metabolomic profiles between asymptomatic and symptomatic genotypes by plotting the magnitude of change against statistical significance. Each metabolite is represented as a single point, with its position on the x-axis determined by the fold change. Negative x-axis values identify metabolites dominant in asymptomatic samples, while positive values indicate those more abundant in symptomatic samples; extreme values beyond the axis denote compounds unique to a single condition ("A Only" or "B Only"), where A represents "Asymptomatic" and B represents "Symptomatic" samples. The y-axis represents statistical significance based on p-values, where higher vertical positioning indicates a more robust statistical difference.

This comparative volatile metabolite analysis revealed significant chemical divergence between the samples across the three citrus genotypes. As shown in Table 3, volatiles were classified based on their unique presence in specific conditions ("A Only" or "B Only") or their stability across both states ($A = B$). Across most cultivars, a subset of compounds remained stable ($A = B$) regardless of infection status, suggesting they are constitutive metabolites not significantly altered by CLAs pathogenesis. These include propionic acid derivatives, methyl nonanoate, and hexadecanamide. These stable profiles serve as a metabolic baseline for the genus across varying infection pressures, as documented by González-Mas et al. (2011) and Pereira-Caro et al. (2015).

For specific germplasm, the Citron group exhibited the most profound metabolic disruption. The symptomatic samples (B) were characterised by the significant disappearance of several bioactive secondary metabolites present in healthy tissues, suggesting that CLAs infection either suppresses the biosynthetic pathways of these compounds or leads to their rapid exhaustion during the host's physiological defence response. Notably, 3-chloro-6-piperidin-4-ylxopyridazine, 5,7-dimethoxychromen-2-one, 6-methoxyfuro[2,3-*h*]chromen-2-one, 10-O-(2,5-dimethylphenyl) 1-O-ethyl decanedioate, 4-hydrazino-6-(2-pyridinyl)-1,3,5-triazin-2-ylamine, and nonanamide were absent in symptomatic

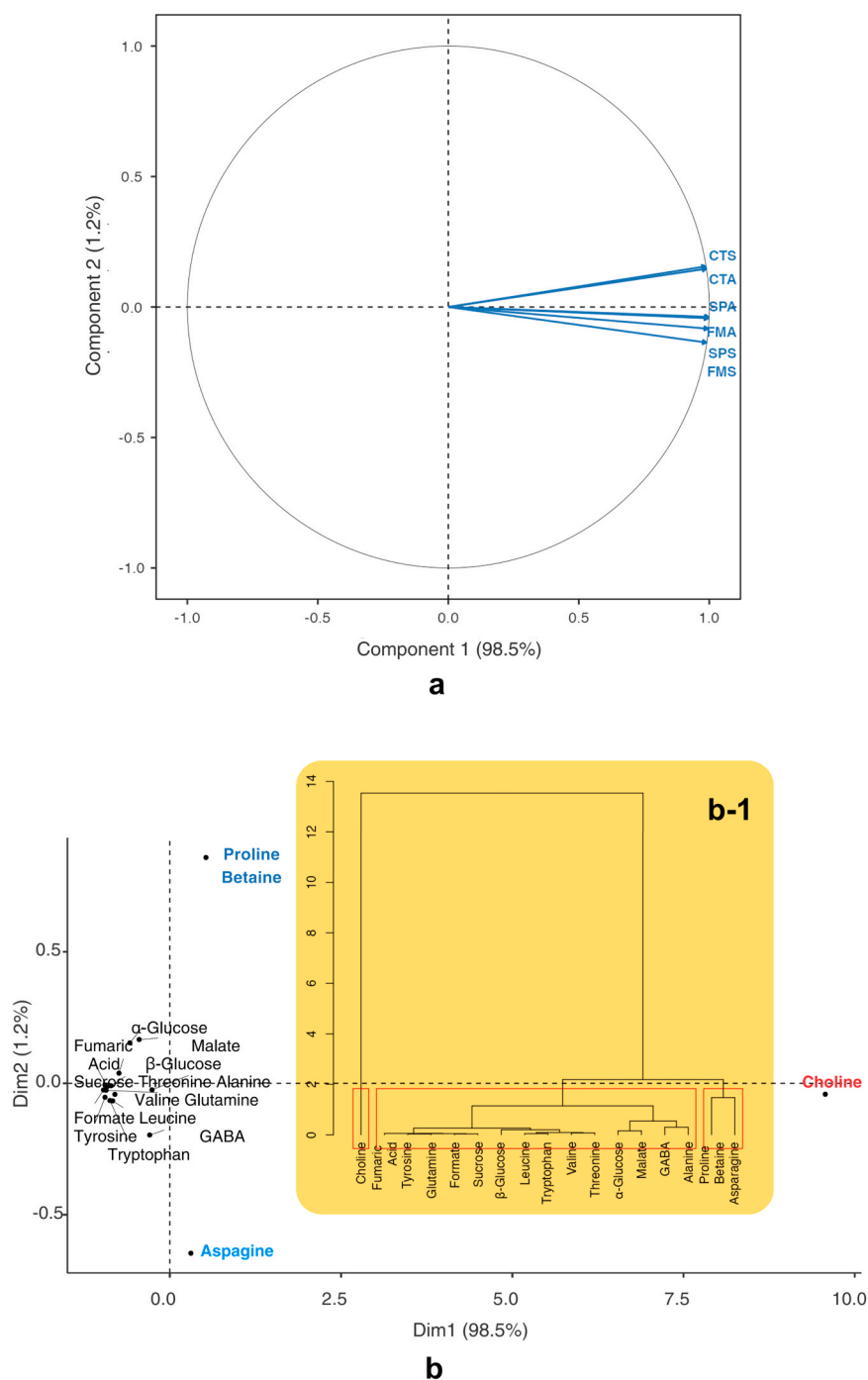


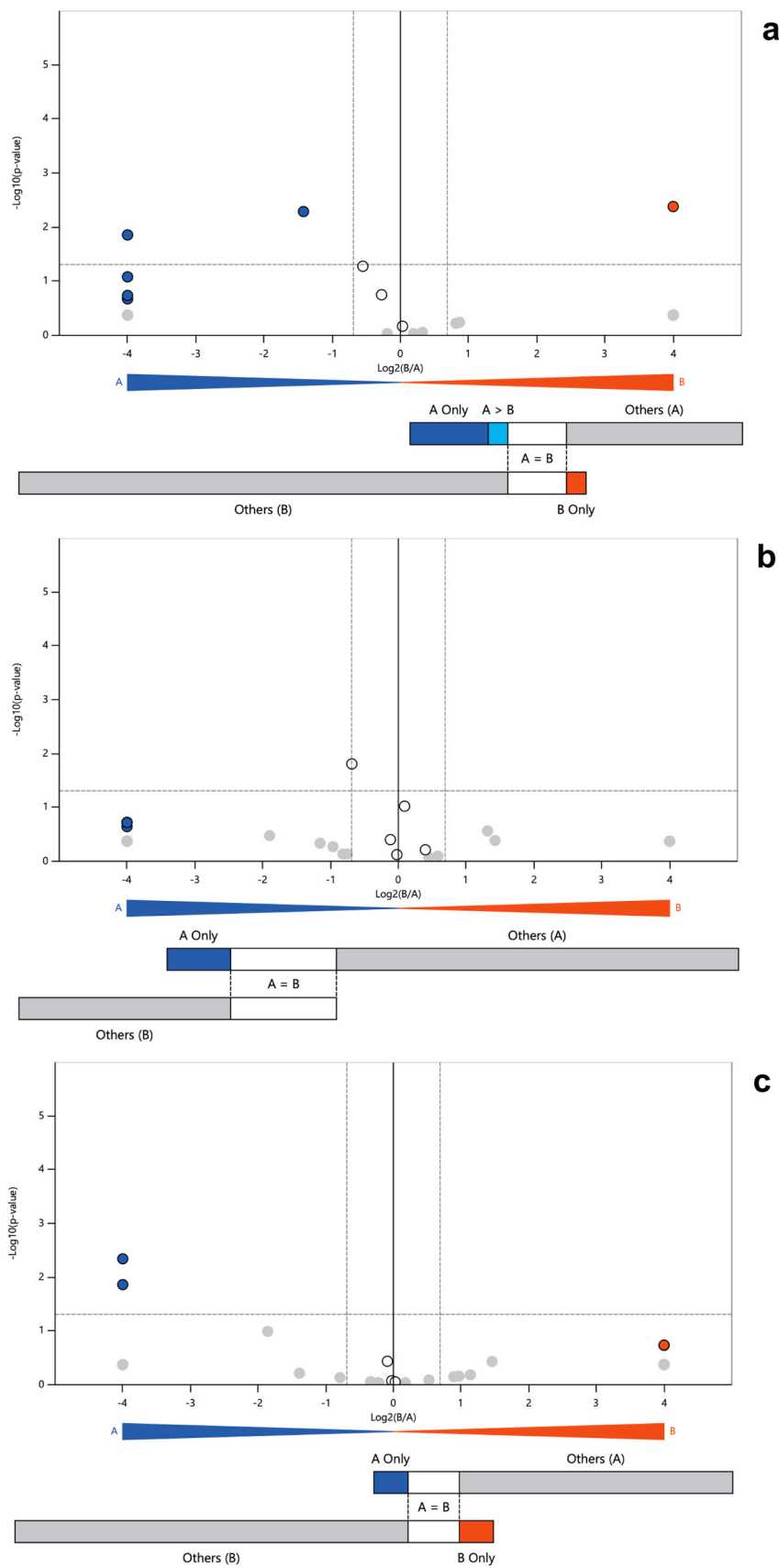
Fig. 6. (A) Chemometric analysis of metabolites detected using NMR illustrating sample loadings and (B) variable loading and hierarchical Cluster Analysis (HCA) performed on the non-volatile metabolite profiles of six citrus leaf extracts: Asymptomatic Citron (CTA), Symptomatic Citron (CTS), Asymptomatic Sai-Nam-Phueng (SPA), Symptomatic Sai-Nam-Phueng (SPS), Asymptomatic Fremont Orange (FMA), and Symptomatic Fremont Orange (FMS).

tissues. Furthermore, primary lipids such as α -linolenic acid (9,12,15-octadecatrienoic acid) and 1,5-cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl)-, [S-(Z,E)]- were significantly higher in healthy samples, whereas (9E,11E)-octadecadienoic acid was identified as an exclusive marker for CTS samples.

The SP group demonstrated a unique metabolic signature distinct from CT and FM. Unlike the other cultivars, where palmitic acid (n-hexadecanoic acid) remained stable, SP showed a significant reduction of this fatty acid in infected tissues. Similarly, α -linolenic acid was more abundant in the asymptomatic Class A. Both CTA/CTS and SPA/SPS showed that α -linolenic acid is significantly higher in healthy plants (A

> B), indicating that HLB infection may negatively impact the biosynthesis or preservation of this essential fatty acid.

Variety-specific markers included pyrrolo[3,2-c]pyridine, 4,5,6,7-tetrahydro-5-benzyl- and 1-hexadecen-3-ol, 3,5,11,15-tetramethyl-, found only in asymptomatic samples, while 1H-indene, octahydro-, cis-appeared exclusively as a post-infection marker. In contrast to the disruption seen in Citron, the Fremont group displayed the highest degree of metabolic stability. Nearly all identified compounds followed an A = B pattern, indicating a less reactive metabolic profile under the observed infection levels. The sole variance was the lactone 3a-methyl-cis-hexahydrophthalide, which emerged as a unique metabolite only in



(caption on next page)

Fig. 7. The volcano plots visualise the differential abundance of metabolites based on statistical significance (Y-axis) and magnitude of change (Log2 Fold Change B/A on the X-axis). The colour coding distinguishes the origin and abundance of the identified compounds. Blue (Left / Negative Log2): Represents metabolites that are unique to or significantly higher in the asymptomatic samples [CTA (a), FMA (b) and SPA (c)]. Red (Right / Positive Log2): Represents metabolites that are unique to or significantly higher in the symptomatic samples (CTS, FMS, SPS). Grey/White (Centre): Represents metabolites with no statistically significant difference between the two conditions, classified as "A = B". Compounds were classified as significant based on a fold change threshold of ≥ 2 and a p-value ≤ 0.05 .

Table 3

Comparative volatile metabolomic profiles across genotypes and stages Citron (CT), Sai-Nam-Phueng (SP) and Fremont Orange (FM).

Sample	RT (min)	Chemical Name	IUPAC	Formula	MW	Match Factor	Class (A vs B)
FM	8.85	Propionic acid, 2-tert-butoxycarbonylamino-3,3,3-trifluoro-2-hydroxy-, methyl ester	Methyl 3,3,3-trifluoro-2-hydroxy-2-[(2-methylpropan-2-yl)oxycarbonylamino]propanoate	C ₉ H ₁₄ F ₃ NO ₅	273	619	A = B
	25.67	Nonanoic acid, methyl ester	Methyl nonanoate	C ₁₀ H ₂₀ O ₂	172	828	A = B
	33.09	Bicyclo[6.1.0]non-1-ene	Bicyclo[6.1.0]non-1-ene	C ₉ H ₁₄	122	595	A = B
	52.63	n-hexadecanoic acid	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	441	A = B
	55.36	5-decene, 1-bromo-, (E)-	(E)-1-bromodec-5-ene	C ₁₀ H ₁₉ Br	218	469	A = B
	56.39	3a-methyl-cis-hexahydrophthalide	3-methyl-3a,4,5,6,7,7a-hexahydro-3H-2-benzofuran-1-one	C ₉ H ₁₄ O ₂	154	619	B Only
	56.53	9,12,15-octadecatrienoic acid, (Z,Z,Z)-	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	C ₁₈ H ₃₀ O ₂	278	823	A = B
	56.79	Nonanamide	Nonanamide	C ₉ H ₁₉ NO	157	603	A = B
	57.20	Hexadecanamide	Hexadecanamide	C ₁₆ H ₃₃ NO	255	848	A = B
	58.87	Undecanamide, 11-bromo-	11-bromoundecanamide	C ₁₁ H ₂₂ BrNO	263	498	A = B
CT	59.17	4-methoxy-1-pentene	4-methoxypent-1-ene	C ₆ H ₁₂ O	100	473	A = B
	8.85	Propionic acid, 2-tert-butoxycarbonylamino-3,3,3-trifluoro-2-hydroxy-, methyl ester	Methyl 3,3,3-trifluoro-2-hydroxy-2-[(2-methylpropan-2-yl)oxycarbonylamino]propanoate	C ₉ H ₁₄ F ₃ NO ₅	273	594	A = B
	25.67	Nonanoic acid, methyl ester	Methyl nonanoate	C ₁₀ H ₂₀ O ₂	172	832	A = B
	33.07	1,5-cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl)-, [S-(Z,E)]-	(1E,5Z,8S)-1,5-dimethyl-8-prop-1-en-2-ylcyclodeca-1,5-diene	C ₁₅ H ₂₄	204	567	A > B
	49.56	3-chloro-6-(4-Piperidyl)pyridazine	3-chloro-6-piperidin-4-ylpyridazine	C ₉ H ₁₂ ClN ₃ O	213	548	A Only
	52.62	n-hexadecanoic acid	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	501	A = B
	53.02	2H-1-benzopyran-2-one, 5,7-dimethoxy-	5,7-dimethoxychromen-2-one	C ₁₁ H ₁₀ O ₄	206	740	A Only
	54.82	6-methoxyfuro[2,3-h]chromen-2-one	6-methoxyfuro[2,3-h]chromen-2-one	C ₁₂ H ₈ O ₄	216	765	A Only
	55.35	9-octadecenamide	Octadec-9-enamide	C ₁₈ H ₃₅ NO	281	548	A = B
	55.53	Sebacic acid, 2,5-dimethylphenyl ethyl ester	10-O-(2,5-dimethylphenyl) 1-O-ethyl decanedioate	C ₂₀ H ₃₀ O ₄	334	427	A Only
SP	55.98	4-hydrazino-6-pyridin-2-yl-triazin-2-ylamine	4-hydrazino-6-(2-pyridinyl)-1,3,5-triazin-2-ylamine	C ₈ H ₉ N ₇	203	557	A Only
	56.39	(9E,11E)-octadecadienoic acid	(9E,11E)-octadeca-9,11-dienoic acid	C ₁₈ H ₃₂ O ₂	280	629	B Only
	56.52	9,12,15-octadecatrienoic acid, (Z,Z,Z)-	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	C ₁₈ H ₃₀ O ₂	278	816	A > B
	56.78	Nonanamide	Nonanamide	C ₉ H ₁₉ NO	157	602	A = B
	57.19	Hexadecanamide	Hexadecanamide	C ₁₆ H ₃₃ NO	255	840	A = B
	59.09	Nonanamide	Nonanamide	C ₉ H ₁₉ NO	157	610	A Only
	8.85	Propionic acid, 2-tert-butoxycarbonylamino-3,3,3-trifluoro-2-hydroxy-, methyl ester	Methyl 3,3,3-trifluoro-2-hydroxy-2-[(2-methylpropan-2-yl)oxycarbonylamino]propanoate	C ₉ H ₁₄ F ₃ NO ₅	273	610	A = B
	13.56	Pyrrolo[3,2-c]pyridine, 4,5,6,7-tetrahydro-5-benzyl-	5-benzyl-1,4,6,7-tetrahydropyrrolo[3,2-c]pyridine	C ₁₄ H ₁₆ N ₂	212	593	A Only
	19.70	Lavandulyl butyrate	(5-methyl-2-prop-1-en-2-ylhex-4-enyl) butanoate	C ₁₄ H ₂₄ O ₂	224	449	A = B
	25.67	Nonanoic acid, methyl ester	Methyl nonanoate	C ₁₀ H ₂₀ O ₂	172	828	A = B
SP	33.09	1,5-cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl)-, [S-(Z,E)]-	(1E,5Z,8S)-1,5-dimethyl-8-prop-1-en-2-ylcyclodeca-1,5-diene	C ₁₅ H ₂₄	204	780	A = B
	49.56	Methanone, (3,3-dimethylcyclopentyl)-1H-imidazol-4-yl-	(3,3-dimethylcyclopentyl)-(1H-imidazol-5-yl) methanone	C ₁₁ H ₁₆ N ₂ O	192	540	A Only
	52.63	n-hexadecanoic acid	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	593	A > B
	55.36	Decane-1-sulfonic acid (4-methoxy-phenyl)-amide	N-(4-methoxyphenyl)decane-1-sulfonamide	C ₁₇ H ₂₉ NO ₃ S	327	478	A = B
	56.00	1-hexadecen-3-ol, 3,5,11,15-tetramethyl-	3,5,11,15-tetramethylhexadec-1-en-3-ol	C ₂₀ H ₄₀ O	296	541	A Only
	56.39	1H-indene, octahydro-, cis-	(3aR,7aS)-2,3,3a,4,5,6,7,7a-octahydro-1H-indene	C ₉ H ₁₆	124	663	B Only
	56.53	9,12,15-octadecatrienoic acid, (Z,Z,Z)-	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	C ₁₈ H ₃₀ O ₂	278	795	A > B
	56.79	DL-lactamide, N,N-dimethyl-, methyl ether	2-methoxy-N,N-dimethylpropanamide	C ₆ H ₁₃ NO ₂	131	623	A = B
	57.20	Hexadecanamide	Hexadecanamide	C ₁₆ H ₃₃ NO	255	832	A = B
	58.89	Tridecanamide	Tridecanamide	C ₁₃ H ₂₇ NO	213	526	A = B
SP	59.21	Hydroxymethyl 2-hydroxy-2-methylpropionate	2-hydroxyethyl 2-hydroxy-2-methylpropanoate	C ₆ H ₁₂ O ₄	148	820	A = B

Note: A = B: No significant variance; the compound is present in both samples at comparable levels. A > B: The compound is significantly more abundant in Sample A (Asymptomatic). A Only: The compound was only detected in Sample A (Asymptomatic). B Only: The compound was only detected in Sample B (Symptomatic).

the symptomatic FM samples.

Across all three groups, a specific compound appeared exclusively in the Infected (B Only) samples at roughly the same retention time (~56.39 min). However, the library identified these as different compounds for each group: 3a-Methyl-cis-hexahydrophthalide (FMS), (9E,11E)-octadecadienoic acid (CTS), and 1H-Indene, octahydro-, cis- (SPS). This suggests a common metabolic stress response pathway activating at this elution point.

4. Discussion

The aromatic amino acids tyrosine and tryptophan serve as essential precursors for defensive phenolics and alkaloids, enabling rapid de novo biosynthesis of protective compounds upon pathogen invasion, as documented by Rao et al. (2018) and Rao et al. (2021). HLB-tolerant citrus varieties generally possess higher concentrations of these precursor amino acids. In citrus canker-infected plants, tryptophan and tyrosine increase significantly during both early and late infection

stages, suggesting their mobilisation for defence compound synthesis (do Prado Aparecido et al., 2017). Proline accumulates in response to both biotic and abiotic stresses, acting as a compatible osmolyte and a Reactive Oxygen Species (ROS) scavenger that stabilises cellular membranes. The presence of proline betaine further suggests an enhanced osmotic stress response mechanism, as this derivative is known for its osmoprotectant properties (do Prado Aparecido et al., 2017). The dominance of proline betaine in citron tissue points to a genotype-specific metabolic strategy for osmotic stress management, potentially reflecting the adaptation of this variety to maintain cellular homeostasis under CLAs-induced metabolic stress. Glutamine and asparagine are involved in nitrogen assimilation to detoxify ammonia produced during stress conditions, increasing significantly during pathogen infection for ammonium scavenging, indicating a combined defence effort against both Reactive Nitrogen Species (RNS) and ROS. The branched-chain amino acids leucine and valine are also present at higher levels in HLB-tolerant varieties, contributing to overall stress tolerance. Putrescine accumulation functions as a signal of biotic stress and serves as a precursor for secondary metabolites such as alkaloids. Glycine betaine is an important osmoprotectant that accumulates under stress conditions to maintain cellular osmotic balance and protect cellular structures from stress-induced damage, while GABA is known to accumulate under various stress conditions and may play a role in stress signalling and metabolic regulation (Rao et al., 2018).

Several organic acids identified in this study reflect the plant's physiological response to infection and energy metabolism alterations (Freitas et al., 2015). Symptomatic HLB leaves typically show a significant decrease in malate concentration, suggesting rapid consumption to sustain normal citric acid cycle functions and to supply carbon skeletons for amino acid biosynthesis. Interestingly, fumaric acid is reported to be higher in HLB-tolerant varieties, potentially indicating enhanced TCA cycle efficiency or alternative metabolic routing in resistant genotypes (Freitas et al., 2015; Rao et al., 2018). These genotype-specific variations in amino acids and organic acids establish the primary metabolic foundation that either supports or constrains secondary metabolite biosynthesis under pathogenic stress.

The polysaccharide profiling data reveal that CLAs-induced cell wall remodelling follows a genotype-dependent rather than symptom-dependent pattern. The PCA scores distribution corresponds to the quantified callose content in Table 2, wherein samples with high callose (SPS >> FMS $\approx$ FMA) group in the callose zone, while samples with low callose (SPA < CTA < CTS) group in the starch zone regardless of their symptom status. This genotype-specific polysaccharide profile indicates that callose accumulation at sieve plate pores is not a universal consequence of CLAs colonisation, but rather a host-determined response that segregates into two distinct biochemical strategies; callose-dominant (Fremont and symptomatic Sai-Nam-Phueng) and starch-dominant (Citron and asymptomatic Sai-Nam-Phueng) phenotypes. The significance of these polysaccharide signatures extends beyond generic cell wall remodelling. According to Torode et al. (2018), the sieve element wall possesses a biochemically distinct primary cell wall built on a cellulose scaffold embedded in pectic polysaccharides and hemicelluloses, with an inner sieve-element-specific layer enriched in branched pectic galactans, specifically a β -1,6-galactosyl-substituted 1,4-galactan (RG-I side chain) that confers the mechanical elasticity required for turgor-driven phloem mass flow. This galactan-rich inner wall layer is absent from adjacent companion cells and parenchyma, and as noted by Blake et al. (2008), this creates a chemically unique microenvironment that CLAs must adapt to during colonisation.

Although CLAs is widely recognised as a phloem-limited bacterium, the pathogen's interaction with this specialised cell wall chemistry has profound implications for bacterial translocation and persistence. Following Achor et al. (2020), ultrastructural analyses demonstrate that CLAs adheres closely to the sieve element plasma membrane adjacent to sieve plate pores in its elongated bacilliform form; concurrently, CLAs infection triggers progressive callose deposition that constricts pore

aperture from approximately 265 nm in healthy tissue to ≤ 105 nm in symptomatic tissue. This physical restriction constrains both bacterial movement and phloem mass flow. However, the quantitative differences in callose deposition among genotypes (SPS, 2.58 mg/g versus CTS, 0.46 mg/g) suggest that callose-mediated pore occlusion is not the sole determinant of bacterial establishment or symptom expression across all citrus genotypes. The starch accumulation observed in low-callose genotypes (particularly Citron) represents an alternative host strategy, rather than deploying resources toward physical barrier formation, these varieties prioritise the conversion and storage of accumulated sugars, reflecting the primary metabolite pools available for energy and biosynthesis during stress.

The elevated TPC and TFC observed in certain symptomatic genotypes may reflect an active antimicrobial defence mechanism rather than passive accumulation. Phenolic acids and flavonoids are well-established antibacterial metabolites; in vitro and ex vivo evidence demonstrates that compounds such as ferulic acid, hesperidin, quercetin, and naringenin inhibit CLAs and its culturable surrogate *L. crescens* at concentrations comparable to oxytetracycline (Rao et al., 2018; Aksenov et al., 2024). Critically, HLB-tolerant citrus germplasm generally accumulates higher concentrations of these secondary metabolites than susceptible cultivars, and CLAs titers in the field are inversely associated with such metabolite pools (Stover and McCollum, 2011). Recent spatial metabolomics data indicate that CLAs actively disrupts flavonoid biosynthesis in infected tissue, diverting ferulic acid into feruloylputrescine, a conjugated, non-bactericidal polyamine, as a self-defence strategy against these antimicrobial host compounds (Aksenov et al., 2024). This disruption is compounded by the known suppression of the shikimate–phenylpropanoid pathway during CLAs colonisation of phloem tissue (Rao et al., 2018). Additionally, Sivager et al. (2021) establish a mechanistic link between antioxidant enzyme activity and citrus HLB tolerance. By comparing the diploid Mexican lime (*C. aurantiifolia*) with the triploid Persian lime (*C. latifolia*), the authors demonstrate that polyploidy-associated structural enlargement alone cannot explain tolerance; instead, superior antioxidant capacity emerges as the critical biochemical determinant. Together, these findings suggest that the genotype-dependent TPC and TFC patterns reported here do not simply reflect constitutive metabolic differences but are shaped by the differential capacity of each germplasm to sustain phenylpropanoid flux under pathogen pressure. Citrus genotypes that maintain high flavonoid and phenolic acid levels despite CLAs infection may therefore impose a bacteriostatic or bactericidal environment in phloem tissues, contributing to reduced disease severity observed in more tolerant genotypes (Stover and McCollum, 2011; Yang et al., 2018; Pandey et al., 2021).

The polysaccharide composition of the sieve element wall also influences the local distribution and binding capacity of antimicrobial phenolics and flavonoids within the phloem matrix. Pectic galactans and arabinogalactans can sequester or facilitate diffusion of defence compounds at the pathogen interface, thereby modulating the chemical environment surrounding CLAs cells. The clustering of callose-dominant genotypes (Fremont, symptomatic Sai-Nam-Phueng) with elevated CBMs and callose-binding antibodies suggests that these genotypes mount a more pronounced callose-based physical barrier response. In contrast, starch-dominant genotypes (Citron, asymptomatic Sai-Nam-Phueng) may allocate resources to alternative defence mechanisms or metabolic accommodation strategies that do not prioritise callose deposition. This differential allocation implies distinct biochemical strategies for managing CLAs colonisation, with implications for both bacterial survival kinetics and symptom development.

The distinct polysaccharide profiles observed in this study reflect fundamentally different metabolic responses to CLAs-induced phloem obstruction. In callose-dominant genotypes (Fremont, symptomatic Sai-Nam-Phueng), the accumulation of callose at sieve plate pores creates the characteristic physical blockage that impedes photoassimilate transport. By contrast, starch-dominant genotypes (Citron,

asymptomatic Sai-Nam-Phueng) prioritise the conversion and storage of accumulated sugars as starch within chloroplasts, representing a compensatory mechanism to manage energy metabolism when the normal sucrose export pathway is compromised. The genotype-specific allocation between callose-based physical barrier formation and starch-based metabolic storage suggests that different citrus varieties have evolved distinct tolerance mechanisms in response to CLas colonisation, with profound implications for understanding HLB symptom development and disease progression.

The finding that Fremont mandarin maintains constitutively elevated callose levels in both symptomatic and asymptomatic tissues (FMS, 1.16 and FMA, 1.01 mg/g DW) warrants further investigation. This phenotype suggests either that Fremont mandarin expresses a genotype-wide propensity for callose accumulation independent of CLas presence, or that early-stage CLas colonisation in asymptomatic tissue (below detection threshold) triggers callose deposition prior to symptom manifestation. If the latter interpretation holds, callose accumulation may serve as a more sensitive diagnostic marker for CLas colonisation than visible symptom expression in this genotype.

The complex metabolomic signatures revealed in this study demonstrate that symptom development triggers wholesale biochemical restructuring rather than incremental adjustment of pre-existing pathways. The data indicate that the causal agent responsible for symptom expression induces marked changes in host metabolite production, resulting in suppression or depletion of basal metabolites and concurrent establishment of a symptom-specific metabolomic signature optimised for stress response, pathogen defence, or accommodation of infection-induced cellular damage.

The conservation of sphinganine and other sphingoid bases across all germplasms underscores the universal requirement for membrane structural integrity despite CLas colonisation. However, the dramatic divergence in stress-associated metabolites, including the accumulation of trans-aconitate, phenolic defence compounds, and sesquiterpene alcohols in symptomatic tissues, reflects genotype-specific metabolic coping strategies. The detection of trans,trans-farnesol and pelargonidin 3-lathyroside in symptomatic samples indicates activation of secondary metabolic pathways dedicated to oxidative stress mitigation and antimicrobial defence, providing a biochemical correlate to the observable macroscopic symptoms and phloem obstruction documented in the polysaccharide analysis.

The asymptomatic baseline, characterised by alkaloids, triterpenoids, and intact volatile compound production, represents a metabolomic state of homeostasis in which the plant maintains normal carbohydrate and lipid metabolism despite CLas presence. The divergence between this baseline and the symptomatic signature suggests that symptom manifestation is not merely a consequence of bacterial biomass or vascular obstruction alone, but rather reflects a threshold at which CLas-induced metabolic perturbation exceeds the plant's capacity for metabolic accommodation.

Sugar analysis revealed critical transport and storage carbohydrates whose metabolism is severely impacted by phloem-limited diseases like HLB (Suh, Guha, et al., 2021). In HLB-symptomatic leaves, sucrose exhibits abnormally high accumulation due to phloem blockage caused by callose deposition, preventing normal transport to sink organs. This hyperaccumulation is a major pathological indicator of phloem dysfunction (Freitas et al., 2015). The CLas bacterium utilises specific carbohydrates, causing sugar imbalance in infected tissues. HLB-tolerant cultivars typically exhibit reduced glucose availability, potentially limiting pathogen nutrition. In pathogen-infected plants, glucose concentration can be rapidly reduced as it is consumed to form defence compounds.

The depletion of α -linolenic acid (9,12,15-octadecatrienoic acid) is biologically significant because it serves as the primary substrate for the lipoxygenase (LOX) pathway, leading to the biosynthesis of Green Leaf Volatiles (GLVs) and jasmonic acid (JA), as documented by Asai et al. (2016) and Wen et al. (2024). The reduction in these precursors in

FMS suggests they are being rapidly consumed to fuel downstream defence cascades (Pérez-Hedo et al., 2024). Unsaturated fatty acids are precursors for oxylipins and jasmonic acid (JA), critical signalling molecules for plant defence against herbivores and pathogens (Pérez-Hedo et al., 2024).

The specific volatile markers emerging in symptomatic tissues reflect genotype-distinct mechanisms of stress perception and response. Phthalide derivatives, such as 3a-methyl-cis-hexahydrophthalide found exclusively in symptomatic FM samples, exhibit antifungal activity by inducing ROS overproduction and mitochondrial dysfunction in pathogens, supporting their emergence in infected plant or microbial samples as defence compounds. Conjugated octadecadienoic acids, such as (9E,11E)-octadecadienoic acid identified in CTS, associate with bacterial infections and oxidative stress, potentially resulting from lipid peroxidation during pathogen invasion. Indole-related structures, such as 1H-Indene, octahydro-, cis- found in SPS, prime plant defences via H₂O₂ bursts and antioxidant responses against necrotrophs (Jack et al., 1994). This shared activation at a common retention time across all three genotypes implies activation of a common pathway, such as lipoxygenase-mediated fatty acid oxidation or terpenoid degradation under infection stress, yielding structurally related volatiles. In plant metabolomics, such peaks in pathogen-infected tissues indicate defence reprogramming. This metabolite could serve as a diagnostic marker, but quantification across replicates and pathogen controls is needed to confirm specificity (Li et al., 2025).

Compounds like β -ocimene and β -caryophyllene have been identified as broad-spectrum stress responders, induced by diverse stressors including HLB, canker, and mites, as documented by Wen et al. (2024). In tolerant varieties like *Poncirus trifoliata* (a parent of Carrizo), constitutive levels of these sesquiterpenes are naturally higher, providing a "primed" defence state (Hijaz et al., 2016). The tolerant rootstocks like Carrizo can regulate callose deposition through the upregulation of β -1,3-glucanase, thereby preventing excessive phloem blockage (Pérez-Hedo et al., 2024).

The depletion of sucrose in the stress samples of FM and SP may result from such phloem blockage, preventing sugar transport from source leaves to sinks, a typical symptom of susceptible varieties. Furthermore, specific volatiles like α -farnesene serve as markers for mechanical wounding and JA signalling, distinguishing abiotic stress from biotic infection (Asai et al., 2016). The loss of methylated compounds such as (Z)-5-methoxy-3,5-dimethyl-2-hexenyltrimethylsilane, unique to asymptomatic control samples of CT and FM, may reflect a redirection of metabolic resources away from non-essential volatile production toward dedicated defence biosynthesis.

The comprehensive metabolite profile provides insights into the primary metabolism of citrus leaves, encompassing pathways related to amino acid biosynthesis for defence precursors, energy metabolism through TCA cycle intermediates, and carbohydrate metabolism disruption indicative of phloem dysfunction. These metabolites serve as potential biomarkers for physiological status and reflect the metabolic reprogramming that occurs in symptomatic versus asymptomatic plant tissues in response to biotic stress conditions. The presence of osmoprotectants, stress-signalling compounds, and defence precursors suggests an active metabolic response to pathogen challenge or environmental stress.

The dichotomy observed in the NMR data, wherein primary metabolite profiles segregate by genotype rather than symptom status, contrasts with the LC-MS secondary metabolite data and GC-MS volatile data, both of which clearly distinguish symptomatic from asymptomatic tissues. This three-tiered pattern suggests a fundamental principle: genotype determines the basal capacity for stress-responsive metabolism (primary metabolite pools of amino acids, organic acids, and transport sugars), while symptom expression results from the deployment of these resources toward secondary metabolite and volatile biosynthesis coupled with physical barrier formation (callose deposition).

Citron's elevated proline betaine, distinct primary metabolomic

signature, and metabolic stability (minimal volatile disruption) may indicate a genotype-encoded tolerance mechanism that permits active maintenance of osmotic balance, membrane integrity, and baseline metabolic function despite CLas colonisation. By contrast, Fremont (despite displaying volatile stability) and susceptible Sai-Nam-Phueng may rapidly exhaust metabolic reserves in the synthesis of defence compounds, volatile signals, and structural barriers, triggering the wholesale secondary metabolite reprogramming detected in the LC-MS analysis, volatile stress-response signatures, and observable macroscopic symptoms of HLB disease. The convergence of three genotypes onto a common volatile marker at ~56.39 min suggests that despite genotype-specific differences in metabolic capacity and symptom severity, all infected tissues activate a shared stress-sensing and signalling mechanism, one that bridges the primary-to-secondary metabolic transition and marks the commitment to visible symptom development.

5. Conclusion

This multi-system metabolomic analysis reveals that HLB tolerance is not determined by a single metabolic trait but by genotype-encoded capacity for coordinated resource allocation across four integrated systems: primary metabolism (amino acids, organic acids), antioxidant defence (phenolics, flavonoids), polysaccharide remodelling (callose vs. starch strategies), and secondary metabolite deployment. Tolerant and susceptible citrus varieties possess fundamentally different primary metabolite pools that pre-exist infection and determine their ability to sustain phenolic defence, antioxidant capacity, and cellular homeostasis under CLas pressure. Susceptible varieties rapidly exhaust metabolic reserves in mounting secondary metabolite responses, leading to metabolic collapse and visible symptoms. Tolerant varieties maintain metabolic flexibility and reserve capacity, enabling sustained defence even under severe phloem obstruction.

Future work should pursue: (1) identification of genetic basis through GWAS/QTL mapping of tolerance-associated genes (amino acid biosynthesis, flavonoid pathways) enabling marker-assisted selection; (2) field validation of the convergent volatile marker (~56.39 min) as a pre-symptom CLas diagnostic; (3) development of primary metabolite profiling as a rapid, non-invasive biomarker for tolerance capacity in breeding programs; (4) testing agronomic interventions (rootstocks, defence primers, exogenous metabolites) to enhance metabolic reserves in susceptible commercial varieties; (5) temporal spatial metabolomics of infected phloem tissue to map defence compound localisation and quantify CLas-induced pathway suppression; (6) functional validation through CRISPR knockdowns and metabolite supplementation to establish causal relationships; (7) multi-environment field trials integrating metabolomic data with epidemiological models to predict tolerance stability across diverse stress combinations and CLas populations.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the authors used Claude AI (2026, Anthropic PBC) to refine language mechanics, enhance structural flow, and cross-check for typographical errors. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article

CRediT authorship contribution statement

Sarana Rose Sommano: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Pirawan Chaiwan:** Investigation. **Jiraporn Sangta:** Writing – review & editing, Investigation, Formal analysis. **Tibet Tangpao:** Writing – review & editing, Validation. **Malaiporn Wongkaew:** Methodology, Investigation. **Tuan Nguyen:** Methodology. **Mark S. Turner:** Writing – review & editing. **Marlize Z.**

Bekker: Writing – review & editing. **Ieva Lelenaite:** Writing – review & editing, Methodology, Investigation. **William Willats:** Methodology.

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Declaration of Competing 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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2026.106458.

Data availability

Data supporting the findings are available upon reasonable request from the corresponding author.

References

- Achor, D., Welker, S., Ben-Mahmoud, S., Wang, C., Folimonova, S.Y., Dutt, M., Gowda, S., Levy, A., 2020. Dynamics of *Candidatus Liberibacter asiaticus* movement and sieve-pore plugging in citrus sink cells. *Plant. Physiol.* 182 (2), 882–891. <https://doi.org/10.1104/pp.19.01391>.
- Aksenov, A.A., Blacutt, A., Ginnan, N., Rolshausen, P.E., Melnik, A.V., Ali, L., Gentry, E. C., Ramasamy, M., Zuniga, C., Zengler, K., Mandadi, K.K., McCollum, G., Dorrestein, P.C., Roper, M.C., 2024. Spatial chemistry of citrus reveals molecules bactericidal to *Candidatus Liberibacter asiaticus*. *Sci. Rep.* 14, 20306. <https://doi.org/10.1038/s41598-024-70499-z>.
- Al Kadhi, O., Melchini, A., Mithen, R., Saha, S., 2017. Development of a LC-MS/MS method for the simultaneous detection of tricarboxylic acid cycle intermediates in a range of biological matrices. *J. Anal. Methods Chem.* 2017, 5391832. <https://doi.org/10.1155/2017/5391832>.
- Albrecht, C., Hicks, A., Hornbaker, V., Khalid, S., Kumagai, L., Morgan, D., Okasaki, K., 2020. Action plan for Asian Citrus Psyllid and Huanglongbing (citrus greening) in California. *J. Citrus Pathol.* 7 (1).
- Albrecht, U., Bowman, K.D., 2008. Gene expression in Citrus sinensis fruit tissues harvested from huanglongbing-infected trees: Comparison with girdled fruit. *J. Exp. Bot.* 59 (13), 3531–3544.
- Amsbury, S., Marcus, S., Yeshvekar, R., Barber, J., German, L., Ross, J., Lelenaite, I., Moraes, T., Wickramanayake, J., Klebanovych, A., Czymmek, K., Burch-Smith, T., Bayer, E., Willats, W., Manfield, I., Knox, P., Benitez-Alfonso, Y., 2024. Struct. Divers. calloses/ β -13-glucans *Plant. Cell. wall Micro.* <https://doi.org/10.1101/2024.07.04.602027>.
- Asai, T., Matsukawa, T., Kajiyama, S. i, 2016. Metabolic changes in Citrus leaf volatiles in response to environmental stress. *J. Biosci. Bioeng.* 121 (2), 235–241. <https://doi.org/10.1016/j.jbiosc.2015.06.004>.
- Bakshani, C.R., Sangta, J., Sommano, S., Willats, W.G.T., 2023. Microarray Polymer Profiling (MAPP) for High-Throughput Glycan Analysis. *JoVE* (199), e65443. <https://doi.org/10.3791/65443>.
- Bernardini, C., Franco, A., Russo, R., Livingston, T., Gmitter, F.G., Levy, A., Vashisth, T., 2024. An efficient method for the extraction and the quantitative determination of callose from HLB-affected and healthy citrus [Methods]. *Front. Agron.* 6, 2024. <https://doi.org/10.3389/fagro.2024.1307566>.
- Blake, A.W., Marcus, S.E., Copeland, J.E., Blackburn, R.S., Knox, J.P., 2008. situ analysis of cell wall polymers associated with phloem fibre cells in stems of hemp, Cannabis sativa L. *Planta* 228 (1), 1–13. <https://doi.org/10.1007/s00425-008-0713-5>.
- Boraston, A.B., Revett, T.J., Boraston, C.M., Nurizzo, D., Davies, G.J., 2003. Structural and Thermodynamic Dissection of Specific Mannan Recognition by a Carbohydrate Binding Module. *Structure* 11 (6), 665–675. [https://doi.org/10.1016/S0969-2126\(03\)00100-X](https://doi.org/10.1016/S0969-2126(03)00100-X).
- Bunyarit, T., Huanhong, K., Tangpao, T., Sunanta, P., Sangta, J., Muangsanguan, A., Ruksiriwanich, W., Prasad, S.K., Sommano, S.R., Wongkaew, M., 2025. Exploring value-adding components from different varieties of arabica coffee pulps for

- sustainable resource utilisation. *Circ. Agric. Syst.* 5 (1). <https://doi.org/10.48130/cas-0025-0009>.
- Chai, N., Xu, J., Zuo, R., Sun, Z., Cheng, Y., Sui, S., Li, M., Liu, D., 2021. Metabolic and Transcriptomic Profiling of Liliun Leaves Infected With Botrytis elliptica Reveals Different Stages of Plant Defence Mechanisms [Original Research]. *Front. Plant. Sci.* 12, 2021. <https://doi.org/10.3389/fpls.2021.730620>.
- Chaiwan, P., Rachtanapun, P., Phimolsiripol, Y., Ruksirivanich, W., Li, C., Luo, L., Shen, D., Chung, H.-H., George, D., Tangpao, T., Sommano, S.R., Sunanta, P., 2024. Physicochemical Marker for Determination of Value-Adding Component in Over-Ripe Thai Mango Peels. *Horticulturae* 10 (10).
- Chen, X.-Y., Kim, J.-Y., 2009. Callose synthesis in higher plants. *Plant. Signal. & Behav.* 4 (6), 489–492. <https://doi.org/10.4161/psb.4.6.8359>.
- Clark, K., Franco, J.Y., Schwizer, S., Pang, Z., Hawara, E., Liebrand, T.W.H., Pagliaccia, D., Zeng, L., Douglas, C.J., Wang, N., 2018. An effector from the Huanglongbing-associated pathogen targets citrus proteases. *Nat. Commun.* 9, 1718. <https://doi.org/10.1038/s41467-018-04140-9>.
- Da Graça, J., Korsten, L., 2004. Citrus huanglongbing: Review, present status and future strategies. *Dis. Fruits Veg. I*, 229–245 (Diagnosis and Management).
- Darolt, J.D., Raiol-Junior, L.L., Carvalho, E.V., Alves, M.N., Wulff, N.A., Bagéa, O., Gros, B., Hufnagel, R.M., Morillon, R., Peña, L., Girardi, E.A., 2026. Can an HLB-resistant interstock block the long-distance movement of 'Candidatus Liberibacter asiaticus' within citrus trees? *Front. Plant. Sci.* 17, 1733981. <https://doi.org/10.3389/fpls.2026.1733981>.
- do Prado Aparecido, R., Carlos, E.F., Lião, L.M., Vieira, L.G.E., Alcantara, G.B., 2017. NMR-based metabolomics of transgenic and non-transgenic sweet orange reveals different responses in primary metabolism during citrus canker development. *Metabolomics* 13 (2), 20.
- Fiori, J., Amadesi, E., Fanelli, F., Tropeano, C.V., Rugolo, M., Gotti, R., 2018. Cellular and mitochondrial determination of low molecular mass organic acids by LC-MS/MS. *J. Pharm. Biomed. Anal.* 150, 33–38. <https://doi.org/10.1016/j.jpba.2017.11.071>.
- Freitas, D. d S., Carlos, E.F., Gil, M. r C.S. d S., Vieira, L.G.E., Alcantara, G.B., 2015. NMR-based metabolomic analysis of Huanglongbing-asymptomatic and-symptomatic citrus trees. *J. Agric. Food Chem.* 63 (34), 7582–7588.
- Gamir, J., Pastor, V., Sánchez-Bel, P., Agut, B., Mateu, D., García-Andrade, J., Flors, V., 2018. Starch degradation, abscisic acid and vesicular trafficking are important elements in callose priming by indole-3-carboxylic acid in response to *Plectosphaerella cucumerina* infection. *Plant. J.* 96 (3), 518–531.
- German, L., Yeshvekar, R., Benitez-Alfonso, Y., 2023. Callose metabolism and the regulation of cell walls and plasmodesmata during plant mutualistic and pathogenic interactions. *Plant. Cell. Environ.* 46 (2), 391–404. <https://doi.org/10.1111/pce.14510>.
- Gonzalez, P., Reyes-De-Corcuera, J., Etxeberria, E., 2011. Starch analysis of HLB-affected and control healthy citrus leaves reveal variations in the amylose/amylopectin ratio. *Proc. Fla. State Hort. Soc.*
- González-Mas, M.C., Rambla, J.L., Alamar, M.C., Gutiérrez, A., Granell, A., 2011. Comparative Analysis of the Volatile Fraction of Fruit Juice from Different Citrus Species. *PLoS. One* 6 (7), e22016. <https://doi.org/10.1371/journal.pone.0022016>.
- Grafton-Cardwell, E.E., Stelinski, L.L., Stansly, P.A., 2013. Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens. *Annu. Rev. Entomol.* 58 (1), 413–432.
- Granato, L.M., D'Alessandre, N.R.D., Boscariol-Camargo, R.L., Machado, M.A., 2026. RNAi-Mediated Attenuation of CsCalS7-Related Transcripts Mitigates Callose Accumulation and Huanglongbing Symptoms. *Mol. Plant. Pathol.* 27 (6), e70253. <https://doi.org/10.1111/mpp.70253>.
- Granato, L.M., Galdeano, D.M., D'Alessandre, N.R.D., Breton, M.C., Machado, M.A., 2019. Callose synthase family genes plays an important role in the Citrus defense response to *Candidatus Liberibacter asiaticus*. *Eur. J. Plant. Pathol.* 155 (1), 25–38. <https://doi.org/10.1007/s10658-019-01747-6>.
- Hamade, K., Fliniaux, O., Fontaine, J.-X., Molinié, R., Petit, L., Mathiron, D., Sarazin, V., Mesnard, F., 2024. NMR and LC-MS-based metabolomics to investigate the efficacy of a commercial bio stimulant for the treatment of wheat (*Triticum aestivum*). *Metabolomics* 20 (3), 58. <https://doi.org/10.1007/s11306-024-02131-0>.
- He, T., Chen, L., Wu, Y., Wang, J., Wu, Q., Sun, J., Ding, C., Zhou, T., Chen, L., Jin, A., 2024. Combined Metabolome and Transcriptome Analyses of Maize Leaves Reveal Global Effect of Biochar on Mechanisms Involved in Anti-Herbivory to *Spodoptera frugiperda*. *Metabolites* 14 (9), 498.
- He, Y., Li, Z., Tan, F., Liu, H., Zhu, M., Yang, H., Bi, G., Wan, H., Wang, J., Xu, R., Wen, W., Zeng, Y., Xu, J., Guo, W., Xue, S., Cheng, Y., Deng, X., 2019. Fatty acid metabolic flux and lipid peroxidation homeostasis maintain the biomembrane stability to improve citrus fruit storage performance. *Food Chem.* 292, 314–324. <https://doi.org/10.1016/j.foodchem.2019.04.009>.
- Hijaz, F., Nehela, Y., Killiny, N., 2016. Possible role of plant volatiles in tolerance against huanglongbing in citrus. *Plant. Signal. Behav.* 11 (3), e1138193. <https://doi.org/10.1080/15592324.2016.1138193>.
- Hong, T.Y., Meng, M., 2003. Biochemical characterization and antifungal activity of an endo-1,3-beta-glucanase of *Paenibacillus* sp. isolated from garden soil. *Appl. Microbiol. Biotechnol.* 61 (5-6), 472–478. <https://doi.org/10.1007/s00253-003-1249-z>.
- Huang, G., et al., 2024. SDE19, a Sec-dependent effector from *Candidatus Liberibacter asiaticus* suppresses plant immunity and targets citrus Sec12 to interfere with vesicle trafficking. *PLOS Pathog.* 20, e1012542.
- Jack, C.I., Ridgway, E., Jackson, M.J., Hind, C.R., 1994. Serum octadeca-9,11 dienoic acid—an assay of free radical activity or a result of bacterial production? *Clin. Chim. Acta* 224 (2), 139–146. [https://doi.org/10.1016/0009-8981\(94\)90180-5](https://doi.org/10.1016/0009-8981(94)90180-5).
- Kim, J.-S., Sagaram, U.S., Burns, J.K., Li, J.-L., Wang, N., 2009. Response of sweet orange (*Citrus sinensis*) to *Candidatus Liberibacter asiaticus* infection: Microscopy and microarray analyses. *Phytopathology* 99 (1), 50–57.
- Koh, E.-J., Zhou, L., Williams, D.S., Park, J., Ding, N., Duan, Y.-P., Kang, B.-H., 2012. Callose deposition in the phloem plasmodesmata and inhibition of phloem transport in citrus leaves infected with 'Candidatus Liberibacter asiaticus'. *Protoplasma* 249 (3), 687–697. <https://doi.org/10.1007/s00709-011-0312-3>.
- Li, W.-Y., Gu, H.-B., Yang, R.-H., Xu, A.-G., Zhang, J.-C., Yu, Z.-Q., He, W.-J., Yang, X.-D., 2024. UPLC-ESI-MS/MS-based widely targeted metabolomics reveals differences in metabolite composition among four *Ganoderma* species. *Front. Nutr.* 11, 1335538. <https://doi.org/10.3389/fnut.2024.1335538>.
- Li, X.M., Wang, H.L., Wei, L.Y., Li, G., Qu, Y., Liu, D.Q., 2025. Integrated Analysis of GC-MS-Based Metabolomics and Proteomics Reveals the Importance of Volatile Metabolite Stigmaterol in the Defence Response of *Panax notoginseng* Against Root Rot. *Mol. Plant. Pathol.* 26 (9), e70144. <https://doi.org/10.1111/mpp.70144>.
- Liu, C., Cheng, Y., Zhang, H., Deng, X., Chen, F., Xu, J., 2012. Volatile Constituents of Wild Citrus *Mangshanyegan* (*Citrus nobilis* Lauriro) Peel Oil. *J. Agric. Food Chem.* 60 (10), 2617–2628. <https://doi.org/10.1021/jf2039197>.
- Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25 (4), 402–408.
- López-Gresa, M.P., Maltese, F., Bellés, J.M., Conejero, V., Kim, H.K., Choi, Y.H., Verpoorte, R., 2010. Metabolic response of tomato leaves upon different plant-pathogen interactions. *Phytochem. Anal. Int. J. Plant. Chem. Biochem. Tech.* 21 (1), 89–94.
- Ma, W., Pang, Z., Huang, X., Xu, J., Pandey, S.S., Li, J., Achor, D.S., Vasconcelos, F.N.C., Hendrich, C., Huang, T., Wang, N., Lee, R.F., Stanton, D., Wang, N., 2022. Citrus Huanglongbing is a pathogen-triggered immune disease that can be mitigated with antioxidants and gibberellin. *Nat. Commun.* 13, 529. <https://doi.org/10.1038/s41467-022-28189-9>.
- Martinelli, F., Uratsu, S.L., Albrecht, U., Reagan, R.L., Phu, M.L., Britton, M., Buffalo, V., Dandekar, A.M., 2013. Gene regulatory networks elucidating huanglongbing disease mechanisms. *PLoS. ONE* 8 (9), e74256.
- Meikle, P.J., Bonig, I., Hoogenraad, N.J., Clarke, A.E., Stone, B.A., 1991. The location of (1→3)-β-glucans in the walls of pollen tubes of *Nicotiana glauca* using a (1→3)-β-glucan-specific monoclonal antibody. *Planta* 185 (1), 1–8. <https://doi.org/10.1007/BF00194507>.
- Merfà, M.V., De La Fuente, L., 2020. Why we should care about culturing the Huanglongbing associated bacterium 'Candidatus'. *J. Citrus Pathol.* 7 (1).
- Munir, S., Li, Y., He, P., He, P., Ahmed, A., Wu, Y., He, Y., 2020. Unraveling the metabolite signature of citrus showing defence response towards *Candidatus Liberibacter asiaticus* after application of endophyte *Bacillus subtilis* L1-21. *Microbiol. Res.* 234, 126425.
- Najmudin, S., Guerreiro, C.I.P.D., Carvalho, A.L., Prates, J.A.M., Correia, M.A.S., Alves, V.D., Ferreira, L.M.A., Romão, M.J., Gilbert, H.J., Bolam, D.N., Fontes, C.M.G. A., 2006. Xyloglucan Is Recognized by Carbohydrate-binding Modules That Interact with β-Glucan Chains. *J. Biol. Chem.* 281 (13), 8815–8828. <https://doi.org/10.1074/jbc.M510559200>.
- Pagliaccia, D., Shi, J., Pang, Z., Hawara, E., Clark, K., Thapa, S.P., De Francesco, A., Liu, J.X., Wang, N., 2017. A pathogen secreted protein as a detection marker for citrus Huanglongbing. *Front. Microbiol.* 8, 2041.
- Pandey, S.S., Nogueira da Costa Vasconcelos, F., Wang, N., 2021. Spatiotemporal Dynamics of 'Candidatus Liberibacter asiaticus' Colonization Inside Citrus Plant and Huanglongbing Disease Development. *Phytopathology* 111 (6), 921–928. <https://doi.org/10.1094/PHYTO-09-20-0407-R>.
- Pereira-Caro, G., Borges, G., Ky, I., Ribas, A., Calani, L., Del Rio, D., Clifford, M.N., Roberts, S.A., Crozier, A., 2015. vitro colonic catabolism of orange juice (poly) phenols. *Mol. Nutr. & Food Res.* 59 (3), 465–475. <https://doi.org/10.1002/mnfr.201400779>.
- Pérez-Hedo, M., Gallego-Giraldo, C., Forner-Giner, M.Á., Ortells-Fabra, R., Urbaneja, A., 2024. Plant volatile-triggered defence in citrus against biotic stressors [Original Research]. *Front. Plant. Sci.* 15, 2024. <https://doi.org/10.3389/fpls.2024.1425364>.
- Pirselová, B., Matusíková, I., 2013. Callose: The plant cell wall polysaccharide with multiple biological functions. *Acta Physiol. Plant.* 35, 635–644.
- Rao, M.J., Ding, F., Wang, N., Deng, X., Xu, Q., 2018. Metabolic mechanisms of host species against citrus Huanglongbing (Greening Disease). *Crit. Rev. Plant. Sci.* 37 (6), 496–511.
- Rao, M.J., Feng, B., Ahmad, M.H., Tahir ul Qamar, M., Aslam, M.Z., Khalid, M.F., Hussain, S., Zhong, R., Ali, Q., Xu, Q., Ma, C., Wang, L., 2023. LC-MS/MS-based metabolomics approach identified novel antioxidant flavonoids associated with drought tolerance in citrus species [Original Research]. *Front. Plant. Sci.* 14, 2023. <https://doi.org/10.3389/fpls.2023.1150854>.
- Rao, M.J., Wu, S., Duan, M., Wang, L., 2021. Antioxidant Metabolites in Primitive, Wild, and Cultivated Citrus and Their Role in Stress Tolerance. *Molecules* 26 (19), 5801.
- Sangta, J., Tangpao, T., Piraonapicha, K., Chinachanta, K., Chainanti, C., Nguyen, T., Sommano, S.R., 2026. Field Epidemiology of Huanglongbing: High Psyllid Density, Disease Severity, and an Alternative Host in Northern Thailand. *Crops* 6 (3), 58. <https://doi.org/10.3390/crops6030058>.
- Sivager, G., Calvez, L., Bruyere, S., Boissenois, R., Brat, P., Gros, O., Ollitrault, P., Morillon, R., 2021. Specific physiological and anatomical traits associated with polyploidy and better detoxification processes contribute to improved Huanglongbing tolerance of the Persian lime compared with the Mexican lime. *Front. Plant. Sci.* 12, 685679. <https://doi.org/10.3389/fpls.2021.685679>.
- Stover, E., McCollum, G., 2011. Incidence and Severity of Huanglongbing and *Candidatus Liberibacter asiaticus* Titer among Field-Infected Citrus Cultivars. *HortScience* 46 (10), 1344–1348.

- Suh, J.H., Guha, A., Wang, Z., Li, S.Y., Killiny, N., Vincent, C., Wang, Y., 2021. Metabolomic analysis elucidates how shade conditions ameliorate the deleterious effects of greening (Huanglongbing) disease in citrus. *Plant. J.* 108 (6), 1798–1814.
- Suh, J.H., Tang, X., Zhang, Y., Gmitter Jr, F.G., Wang, Y., 2021. Metabolomic analysis provides new insight into tolerance of Huanglongbing in citrus. *Front. Plant. Sci.* 12, 710598.
- Teutschbein, J., Gross, W., Nimtz, M., Milkowski, C., Hause, B., Strack, D., 2010. Identification and localization of a lipase-like acyltransferase in phenylpropanoid metabolism of tomato (*Solanum lycopersicum*). *J. Biol. Chem.* 285 (49), 38374–38381.
- Torode, T.A., O'Neill, R., Marcus, S.E., Cornuault, V., Pose, S., Lauder, R.P., Kračun, S.K., Rydahl, M.G., Andersen, M.C.F., Willats, W.G.T., Braybrook, S.A., Townsend, B.J., Clausen, M.H., Knox, J.P., 2018. Branched Pectic Galactan in Phloem-Sieve-Element Cell Walls: Implications for Cell Mechanics. *Plant. Physiol.* 176 (2), 1547–1558. <https://doi.org/10.1104/pp.17.01568>.
- Van Der Maarel, M.J., Van der Veen, B., Uitdehaag, J.C., Leemhuis, H., Dijkhuizen, L., 2002. Properties and applications of starch-converting enzymes of the α -amylase family. *J. Biotechnol.* 94 (2), 137–155.
- Venditto, I., Luis, A.S., Rydahl, M., Schückel, J., Fernandes, V.O., Vidal-Melgosa, S., Bule, P., Goyal, A., Pires, V.M.R., Dourado, C.G., Ferreira, L.M.A., Coutinho, P.M., Henrissat, B., Knox, J.P., Baslé, A., Najmudin, S., Gilbert, H.J., Willats, W.G.T., Fontes, C.M.G.A., 2016. Complexity of the *Ruminococcus flavefaciens* cellulosome reflects an expansion in glycan recognition. *Proc. Natl. Acad. Sci.* 113 (26), 7136–7141. <https://doi.org/10.1073/pnas.1601558113>.
- Wang, Y., Li, X., Fan, B., Zhu, C., Chen, Z., 2021. Regulation and Function of Defence-Related Callose Deposition in Plants. *Int. J. Mol. Sci.* 22 (5), 2393. <https://www.mdpi.com/1422-0067/22/5/2393>.
- Wang, Z., Yin, Y., Hu, H., Yuan, Q., Peng, G., Xia, Y., 2006. Development and application of molecular-based diagnosis for 'Candidatus Liberibacter asiaticus', the causal pathogen of citrus huanglongbing. *Plant. Pathol.* 55, 630–638.
- Wen, H., Zhang, S., Liu, Y., Hu, Z., Zhu, C., Zeng, J., Song, Z., Chen, J., Xu, J., 2024. Screening universal stress-response terpenoids and their biosynthetic genes via volatile and transcriptomic profiling in citrus. *J. Agric. Food Chem.* 72 (1), 351–362. <https://doi.org/10.1021/acs.jafc.3c06109>.
- Yang, C., Zhong, Y., Powell, C.A., Doud, M.S., Duan, Y., Huang, Y., Zhang, M., 2018. Antimicrobial Compounds Effective against Candidatus Liberibacter asiaticus Discovered via Graft-based Assay in Citrus. *Sci. Rep.* 8, 17288. <https://doi.org/10.1038/s41598-018-35461-w>.
- Yin, L., Fu, S., Wu, R., Wei, S., Yi, J., Zhang, L.-M., Yang, L., 2020. A neutral polysaccharide from green tea: Structure, effect on α -amylase activity and hydrolysis property. *Arch. Biochem. Biophys.* 687, 108369. <https://doi.org/10.1016/j.abb.2020.108369>.
- Zavaliev, R., Ueki, S., Epel, B.L., Citovsky, V., 2011. Biology of callose (β -1,3-glucan) turnover at plasmodesmata. *Protoplasma* 248 (1), 117–130. <https://doi.org/10.1007/s00709-010-0247-0>.
- Zhang, H., Xie, Y., Liu, C., Chen, S., Hu, S., Xie, Z., Deng, X., Xu, J., 2017. Comprehensive comparative analysis of volatile compounds in citrus fruits of different species. *Food Chem.* 230, 316–326. <https://doi.org/10.1016/j.foodchem.2017.03.040>.
- Zhang, T., Klair, A., Tang, Z., Tripka, A., Luo, S., Reyes, A.V., Lee, J., Gundran, K., Noll, S. E., Wang, X., Zare, R.N., Xu, S.-L., Garay-Arroyo, A., Wang, C., Dickinson, A.J., 2025. The metabolite itaconate is a transcriptional and posttranslational modulator of plant metabolism, development, and stress response. *Sci. Adv.* 11 (23), eadt7463. <https://doi.org/10.1126/sciadv.adt7463>.
- Zhou, Y., Xu, J., Xie, J., Yao, S., Deng, L., Zeng, K., 2017. Involvement of membrane degradation in response to oleocellosis induced by exogenous orange oil in citrus fruit. *Acta Physiol. Plant.* 39 (8), 163. <https://doi.org/10.1007/s11738-017-2464-z>.