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RESEARCH ARTICLE

Comparative metabolite profiling of citrus species affected by greening disease

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Abstract

Citrus greening disease (CGD), devastating phloem residing proteobacteria, *Candidatus* Liberibacter asiaticus, has significantly impacted various citrus species in Tamil Nadu, India. A roving survey of citrus species revealed that the infected *Citrus aurantifolia* from Palamedu, Madurai district, exhibited the highest susceptibility, with a disease incidence (DI) of 67.89 % and disease severity (DS) of 65.89 %. In contrast, *Poncirus trifoliata* from Thadiyankudisai, Dindigul district, demonstrated the highest tolerance with a DI of 10.46 % and DS of 12.56 %. Molecular assessments using PCR and LAMP techniques confirmed the infection of CGD in symptomatic samples of *Citrus aurantifolia, Citrus reticulata* and *Citrus* × *limonia*, while asymptomatic samples tested negative. Biochemical analyses revealed substantial changes in metabolite compositions due to infection. Healthy acid lime contained 79 metabolites, with organic acids (27.84 %) and sugars (20.25 %) being the most dominant groups, whereas infected samples showed reduced diversity with only 60 metabolites. Similarly, healthy mandarin orange contained 93 metabolites, while infected samples exhibited 68 metabolites, significantly reducing sugars and organic acids. *Poncirus* and Rangpur lime also demonstrated metabolic shifts under infection, reducing the number and proportion of various metabolites. Comparative metabolic analysis highlighted the changes in key metabolites, with significant upregulation of mannose, hexadecenoic acid and maltose in healthy acid lime and downregulation of talose and shikimic acid in infected samples. Metabolomics revealed that compounds like tetramethoxyflavone, quinic acid and linoleic acid were consistently more abundant in healthy citrus samples. These findings provide insights into the biochemical responses of different citrus species to CGD, emphasizing the potential of tolerant varieties such as *Poncirus trifoliata* and guiding future disease management strategies through targeted metabolomic interventions.

Keywords: *Citrus* spp; GC-MS analysis, greening disease; metabolite profiling

Introduction

Huanglongbing (HLB), also called citrus greening disease (CGD), is one of the most destructive diseases of citrus, caused by the phloem-limited bacteria *Candidatus* Liberibacter asiaticus (CLas), which is transmitted to the host by Asian citrus psyllids (*Diaphorina citri*) (1, 2). HLB was first discovered in China and spread to Brazil in 2004 and the United States in 2005 (3-5). Symptoms of the disease include leaf vein yellowing, foliar blotchy mottle and asymmetrical chlorosis, followed by tree decline, leaf loss and premature fruit drop. HLB reduces fruit quality and yield, ultimately destroying millions of citrus trees. Due to ineffective control methods, this has significantly impacted global citrus production (6, 7). Despite extensive research, HLB management faces significant challenges, including the lack of a cure and the difficulty of early detection. A primary challenge is that there is no effective cure for HLB. Although chemical application and management strategies have been implemented, they have proven ineffective in controlling the disease. In addition, CLas has not been successfully isolated and cultured *in vitro*, making it more challenging to characterize the disease and fully understand pathogenic mechanisms, hindering efforts to describe the disease, particularly in the early stage of host-microbe interaction (8-10).

Metabolomic studies have emerged as a powerful tool to elucidate the biochemical mechanisms underlying plant responses to HLB infection, offering insights into host-pathogen interactions and potential resistance pathways. The disease disrupts normal physiological and metabolic processes in citrus plants. A comparative analysis of metabolite profiles in healthy and infected citrus species, such as Mandarin orange (*Citrus reticulata*), Poncirus (*Poncirus trifoliata*), Acid Lime (*Citrus aurantiifolia*) and Rangpur Lime (*Citrus* × *limonia*), reveals critical differences that contribute to disease tolerance or susceptibility. HLB infection profoundly alters carbohydrate metabolism in citrus plants, disrupting sugar transport and leading to excessive starch accumulation in leaves. This metabolic imbalance impairs photosynthetic efficiency and

results in nutrient starvation in sink tissues. Elevated levels of soluble sugars such as sucrose and glucose and increased invertase activity exacerbate the diseases' impact by breaking down sucrose into glucose and fructose, ultimately affecting plant growth and defence responses (11).

HLB infection significantly alters amino acid profiles, increasing stress-related amino acids such as glutamine, asparagine and proline. These amino acids are crucial in nitrogen remobilization and osmotic adjustment (12). However, essential amino acids such as alanine and tryptophan showed a marked decline, reflecting protein biosynthesis and secondary metabolite production disturbances.

Secondary metabolites, including flavonoids, alkaloids and terpenoids, play a vital role in the citrus plants' defence against HLB by exhibiting antioxidant, anti-microbial and signalling properties. Similarly, terpenoids such as limonene and β-caryophyllene exhibit anti-microbial properties and attract plant growth-promoting rhizobacteria that support plant health (13). Comparative metabolomic analysis of resistant and susceptible citrus species has provided valuable insights into natural resistance mechanisms, including enhanced secondary metabolite production and stress response pathways. *Poncirus trifoliata*, known for its HLB tolerance, exhibits higher levels of defence-related metabolites. In contrast, susceptible species like Mandarin orange and Acid Lime display more significant metabolic disruptions, including imbalances in carbohydrate metabolism and oxidative stress accumulation (14).

Understanding these metabolic responses is crucial for developing disease-resistant citrus varieties and improving disease management strategies. Metabolite based biomarkers can serve as early diagnostic tools for HLB detection, facilitating timely intervention. Metabolomic research provides a foundation for breeding HLB-resistant citrus cultivars. Additionally, these findings support implementing sustainable agricultural practices to combat this destructive disease effectively. Utilizing advances in metabolomics, researchers and citrus growers can gain deeper insights into plant-pathogen interactions. This knowledge can be used to develop targeted strategies that enhance citrus resilience against HLB, ensuring citrus orchards' long-term sustainability and productivity.

Materials and methods

Survey and sampling

A comprehensive survey was conducted across prominent citrus cultivating districts *viz.,* Madurai, Dindigul, Tenkasi, Tirunelveli, Perambalur, Trichy, Tuticourin, Virudhunagar in Tamil Nadu to document the incidence and severity of Citrus Greening Disease (CGD) in different species. Symptomatic and asymptomatic leaf samples were collected @ three samples each from four different citrus species: *Citrus aurantifolia* (Acid Lime), *Citrus reticulate* (Mandarin Orange), *Citrus limonia* (Rangpur Lime) and *Poncirus trifoliate* (Poncirus). The collected samples were stored at 4 °C for subsequent analyses. From each species, three symptomatic and three asymptomatic leaves were selected randomly in the chosen tree and each leaf was cut into two halves. The first half was designated for molecular detection, utilizing techniques such as conventional PCR and LAMP to identify the presence of pathogens associated with CGD. The second half was processed for metabolomic analysis using Gas Chromatography-Mass Spectrometry (GC-MS) to profile metabolic changes related to the disease.

Molecular confirmation of *Candidatus* Liberibacter asiaticus (*C*Las)

Total DNA was extracted from 100 mg of midribs of the upper half from symptomatic and asymptomatic leaf samples using the CTAB method. The 50 S ribosomal protein L10 (*rplJ*) gene was amplified using primers CQULA03F (5'-3': CAAGGAAAGAGCGTAGAA) and CQULA03R (5'-3': CCTCAAGATCGGGTAAAG), producing a 382 bp product (15). A selected portion of DNA from the three samples mentioned was further analyzed using the colourimetric Loop-Mediated Isothermal Amplification (LAMP) methodology (16). A 25 µL reaction mixture was prepared containing 1.6 µM of inner primers (FIPH1 and BIPH1), 0.4 µM of outer primers (F3H1 and B3H1) and 0.2 µM of loop primers (FLH1 and BLH1). The mixture also included 8 U of Bst DNA polymerase, 5 mM MgSO4, 1.4 mM dNTP mix, 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM (NH4)2SO4, 0.1 % Triton X-100 and 0.8 M betaine, along with 2 µL of template DNA (100 ng). The reaction was incubated at 65 °C for 60 min and then terminated by heating to 80 °C for 10 min in a thermocycler. Follow-ing this, 2 µL of SYBR Green I (100X, Takara) was added to the LAMP product and the resulting colour change from orange to green was observed under UV light and with the naked eye. The amplified products were subsequently separated on a 1.5 % agarose gel and visualized under UV light.

GC-MS analysis

Sample extraction

Leaf samples were frozen and ground using liquid nitrogen. A 0.3 g portion of the ground material was transferred to an Eppendorf tube, adding 1.4 mL of 100 % methanol and vortexing. Then, 50 µL of ribitol (0.2 mg/mL in water) was added and the mixture was incubated at 70 °C with continuous shaking for 15 mins. The sample was centrifuged at 12000 rpm for 20 mins at 4 °C. The supernatant was filtered through a 0.2 µm filter. The supernatant was transferred to a fresh Eppendorf tube. Then, 1.4 mL of distilled water and 750 µl of chloroform were added to facilitate phase separation. After thorough mixing, the sample was centrifuged at 12000 rpm for 10 mins.

Fractionation

One mL of supernatant was transferred to a new tube, while the remaining solution was transferred to other tubes for further analysis. The supernatant was concentrated using a vacuum concentrator at 45 °C for 3 hrs.

Derivitization

After concentration, 50 µL of methoxamine hydrochloride (20 mg /mL of pyridine) was added and the tube was incubated for 3 hrs with continuous shaking for 2 hrs. Then, 80 µL of MSTFA was added to the sample, followed by incubation at 37 °C for 30 min. The derivatized sample was centrifuged again at 10000 rpm for 3 min. The super-natant was then transferred to GC-MS vials for analysis using a GC-MS/MS system (Shimadzu Nexis GC -2030 MS - TQ8040 NX) at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Statistical analysis

A Venn diagram was used to interpret metabolite differences between symptomatic and asymptomatic CGD samples, generated using the tool available at http://www.interactivenn.net/. Heat maps and pathway enrichment analysis were generated from MetaboAnalyst 6.0 software. Marker metabolites were selected based on their mean peak area values, with those >1 being high and those <1 being low.

Results

Assessment of HLB incidence in *Citrus* spp.

Acid lime (*Citrus aurantifolia* (local variety) from Palamedu, Madurai district, showed the highest susceptibility, with a disease incidence (DI) of 67.89 % and dis-ease severity (DS) of 65.89 %. In contrast, *Citrus reticulata* (Kodai orange) from Kaanalkadu, Dindigul district, exhi-bited moderate susceptibility with a DI of 49.67 % and a DS of 48.43 %. *Poncirus trifoliata* from Thadiyankudisai exhibited the highest tolerance, with a DI of 10.46 % and a DS of 12.56 %, highlighting its potential as a resistant rootstock. *Citrus* × *limonia* (Rangpur lime) also demon-strated moderate resistance with lower DI (15.38 %) and DS (17.43 %). Older trees like *Citrus reticulata* exhibited more severe disease due to prolonged exposure, while younger trees, such as *Citrus* × *limonia,* showed lower disease levels (Table 1; Fig. 1).

Molecular assessment of *citrus* greening disease sensitivity in *Citrus* spp.

Polymerase Chain Reaction (PCR) and Loop-Mediated Isothermal Amplification (LAMP) assay revealed that symptomatic samples from Acid Lime, Mandarin orange and Rangpur Lime consistently showed positive reactions for both tests, confirming their sensitivity to CGD. In contrast, asymptomatic samples from these species exhibited negative results, indicating no infection. Notably, the symptomatic sample of *Poncirus trifoliata* showed a negative result in PCR while testing positive in the LAMP assay. Asymptomatic leaves from *P. trifoliata* also showed negative results in both tests (Table 2; Fig. 2-3).

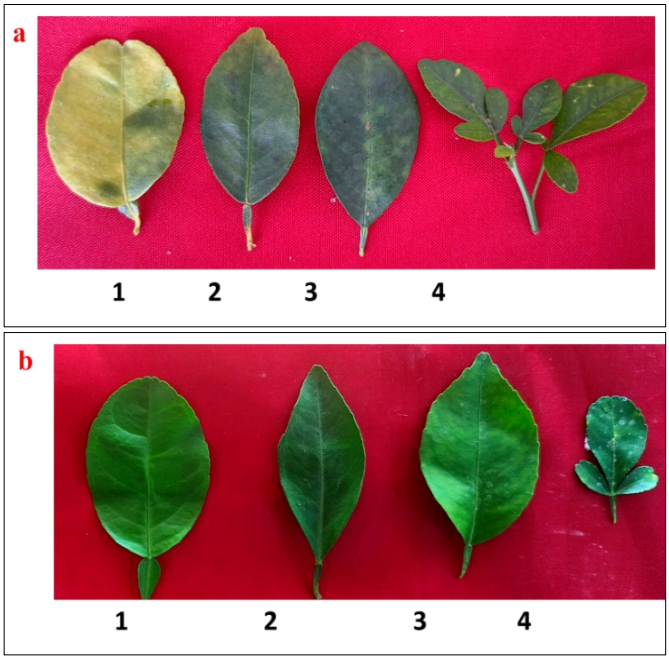

Fig . 1. Collection of leaf samples from different *Citrus* species. **a**) CGD infected samples appeared asymmetrical blotch mottling on **1** acid lime, **2**- mandarin orange, **3**- Rangpur lime, **4**- Poncirus; **b**) healthy leaf samples without any symptoms on **1**-acid lime, **2**-mandarin orange, **3**-Rangpur lime, **4**- Poncirus.

Biochemical changes in citrus species due to infection with citrus greening disease

Acid lime

In healthy acid lime, 79 identifiable metabolites were detected. Organic acids were the most prominent group, comprising 22 compounds (27.84 % of the total compo-sition). Sugars and their derivatives followed closely, contributing 16 compounds (20.25 %), while amino acids and their derivatives accounted for 9 (11.39 %). Alcohols and polyols were moderately represented, with 7 compounds (8.86 %). Steroids and lipids, phenols and flavonoids, aromatic and heterocyclic compounds and miscellaneous compounds each contributed 6.32 %. Terpenes and aromatics comprised a smaller proportion, with 3 compounds (3.79 %), while cyclic compounds and fatty acids, esters and derivatives were the least represented, with only 1 compound each (1.26 %). This composition reflected a healthy acid limes' diverse and complex chemical makeup.

Table 1. Greening disease incidence (DI) and disease severity (DS) of *Citrus* species in the sampled locations of Tamil Nadu, India

Districts	Village name	Latitude	Longitude	Scientific name	Variety	Age of the tree	DI (%)	DS (%)
Madurai	Palamedu	10.066	78.0952	*Citrus aurantifolia* (Acid lime)	Balaji	10	67.89	65.89
Dindigul	Kanalkadu	10.3022	77.6911	*Citrus reticulata* (Mandarin orange)	Kodai orange	12	49.67	48.43
	Thadiyankudisai	10.296	77.7107	*Poncirus trifoliata* (Poncirus)	Poncirus	7	10.46	12.56
	Thadiyankudisai	10.296	77.7107	*Citrus* × *limonia*	Rangpur lime	5	15.38	17.43

Table 2. CGD sensitivity analysis of Citrus cultivars

Citrus spp.	Cultivars	Sample code	PCR	LAMP	Status
Citrus aurantifolia	Acid lime	S/AL	+ve	+ve	Symptomatic
		A/AL	-ve	-ve	Asymptomatic
Citrus reticulata	Mandarin orange	S/MN	+ve	+ve	Symptomatic
		A/MN	-ve	-ve	Asymptomatic
Citrus limonia	Rangpur lime	S/RL	+ve	+ve	Symptomatic
		A/RL	-ve	-ve	Asymptomatic
Poncirus trifoliata	Poncirus	S/PT	-ve	+ve	Symptomatic
		A/PT	-ve	-ve	Asymptomatic

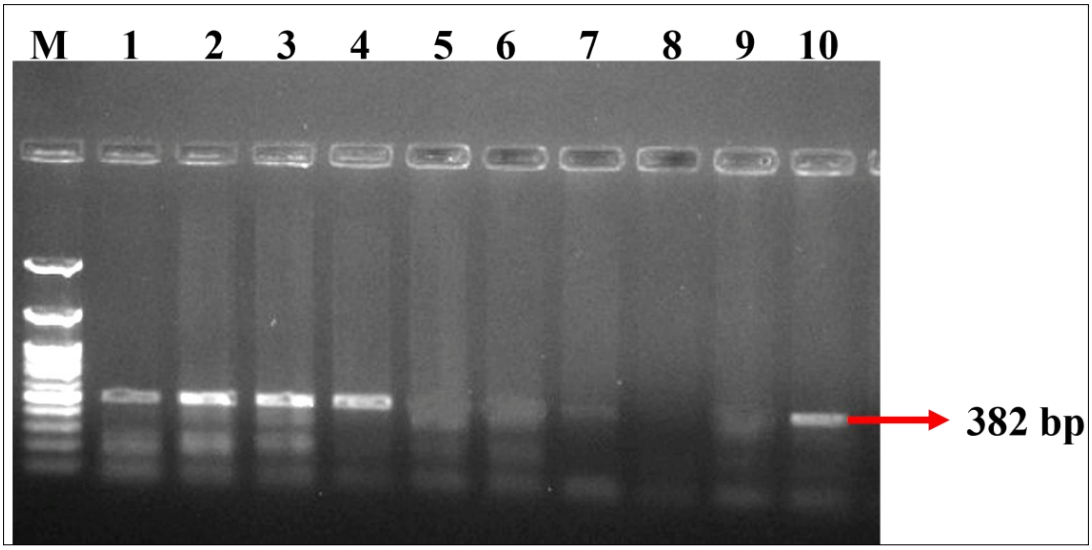

Fig. 2. Amplification of rplJ-50S ribosomal protein L10 gene of *C*Las. **M**- 100 bp ladder, (**1-4** CLas infected samples of different Citrus var.) **1**- acid lime, **2**- mandarin orange, **3**- Rangpur lime, **4**- Poncirus; (**5-8** healthy samples from different Citrus var.) **5**- acid lime, **6**- mandarin orange, **7**- Rangpur lime, **8**- Poncirus. 9- Negative control, 10- Positive control.

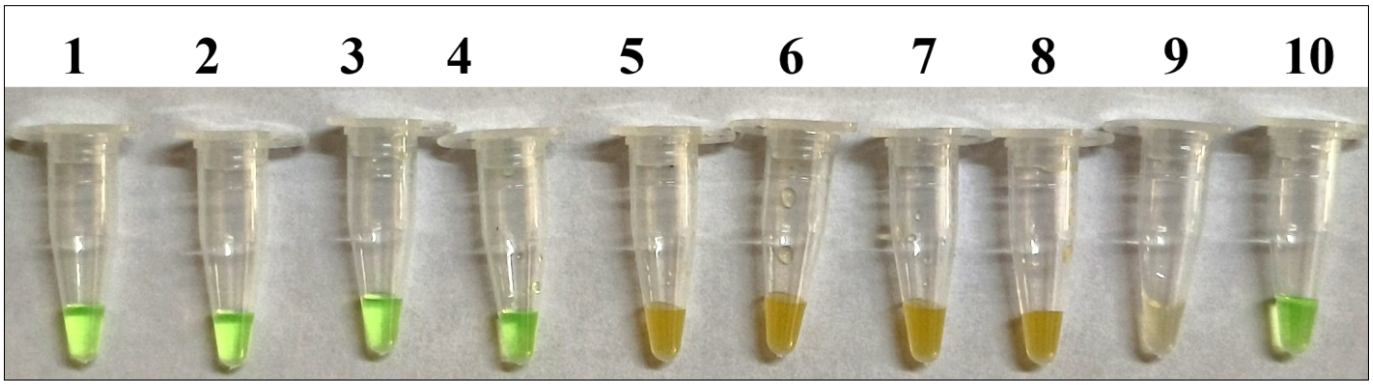

Fig. 3. LAMP assay for confirmation of *C*Las infection. (**1-4** *C*Las infected samples from different Citrus var.) **1**- acid lime, **2**- mandarin orange, **3**- Rangpur lime, **4**-Poncirus; (**5-8** healthy samples from different Citrus var.) **5**- acid lime, **6**- mandarin orange, **7**- Rangpur lime, **8**- Poncirus. **9**- Negative control, **10**- Positive control.

The composition of an infected acid lime consisted of 60 identifiable compounds, with organic acids being the most dominant group, comprising 16 compounds or 26.66 % of the total. Sugars and their derivatives were the second most abundant group, with 10 compounds (16.66 %), followed by terpenes and sterols, which accounted for 8 compounds (13.33 %). Other unclassified compounds comprised 7 (11.66 %), representing metabolites not grouped into specific categories. Fatty acids and lipids comprised 6 compounds (10 %), indicating moderate presence. Smaller contributions were observed from phenols and flavonoids, as well as pyridine and pyrimidine compounds, each accounting for 4 compounds (6 %). Alcohols and polyols were less abundant, contributing 3 compounds (5 %) (Supplementary Table. S1-S2; Fig. 4).

Mandarin orange

The metabolite profile of a healthy mandarin orange comprised 93 identifiable compounds. Sugars and their derivatives were the most abundant category, comprising 21 compounds (22.58 %). Organic acids were the second most prevalent group, consisting of 12 compounds (12.9 %), while the unclassified metabolites included 11 compounds (11.82 %). Alcohols and polyols, as well as steroids and lipids, each accounted for 9 compounds (9.67 %). Other groups, such as terpenes, aromatics, fatty acids, esters and aromatic and heterocyclic compounds, account-ted for 6 compounds (6.45 %). Additionally, phenols, flavonoids, amino acids and their derivatives contributed 5 compounds each (5.37 %). Cyclic com-pounds, with only 3 compounds (3.22 %), represented the smallest category.

The composition of an infected mandarin orange consisted of 68 identifiable compounds. Organic acids were the most abundant group, comprising 16 compounds and accounting for 23.52 % of the total. Sugars, sugar derivatives, terpenes and sterols contributed 9 compounds (13.23 %), indicating their substantial presence in the metabolomic profile. Fatty acids and lipids followed with 7 compounds (10.29 %), while phenols and flavonoids accounted for 6 compounds (8.82 %), indicating moderate abundance. Aromatics and miscellaneous compounds comprised 5 compounds (7.35 %), representing diverse metabolite classes. Smaller proportions were noted for amino acids, alcohols and polyols and cyclic and poly-cyclic compounds, each comprising 3 (4.41 %). Pyrimidines and related compounds were the least represented, with 2 (2.94 %). These metabolomic alterations highlight the biochemical changes induced by infection in mandarin orange (Supplementary Table. S3-S4 & Fig. 4)

Poncirus

The composition of healthy Poncirus comprised 96 identifiable compounds, with sugars and sugar derivatives being the most abundant at 25 %. Organic and fatty acids, esters and derivatives were present; each functional group contributed 10.41 %, while miscellaneous compounds accounted for 11.45 %. Alcohols and

polyols represented 9.37 %, followed by aromatic and heterocyclic compounds at 8.33 %. Moderate contributions came from steroids and lipids (7.29 %) and amino acids and derivatives (6.25 %). Smaller proportions included terpenes, aromatics, phenols and flavonoids, each at 4.16 %, with cyclic compounds being the least represented at 3.12 %.

The metabolite composition of infected Poncirus consisted of 77 identifiable compounds. Sugars and sugar derivatives were the most abundant category, accounting for 21 compounds (27.27 %) of the total composition. Organic acids followed, with 16 compounds contributing 20.77 % of the total. Steroids and lipids comprised 8 compounds, representing 10.38 % of the composition. Alcohols, polyols, phenols and flavonoids accounted for 6 compounds, contributing 7.79 % each. Aromatic and heterocyclic compounds and amino acids were present in smaller quantities, with 4 compounds contributing 5.19 % of the total. Cyclic compounds comprised 3 compounds, representing 3.89 %. Fatty acids, esters, derivatives and lipids were the least represented categories, each contributing just 1 compound (1.29 % of the total composition) (Supplementary Table. S5-S6 & Fig. 4).

Rangpur lime

Total of 116 identifiable compounds were detected in healthy Rangpur lime. Sugars and sugar derivatives were the most abundant category, comprising 19 compounds and accounting for 16.37 % of the total composition. Organic acids, steroids and lipids each contributed 15 compounds, making up 12.93 %. Fatty acids, esters and derivatives were also significant, with 14 compounds representing 12.06 %. Alcohols, polyols and miscellaneous compounds were present in moderate amounts, contributing 10 (8.62 %) and 11 (9.48 %) compounds, respectively. Smaller categories included phenols and flavonoids, cyclic compounds and aromatic and hetero-cyclic compounds, with their contributions ranging between 2.58 % and 6.89 % of the total composition.

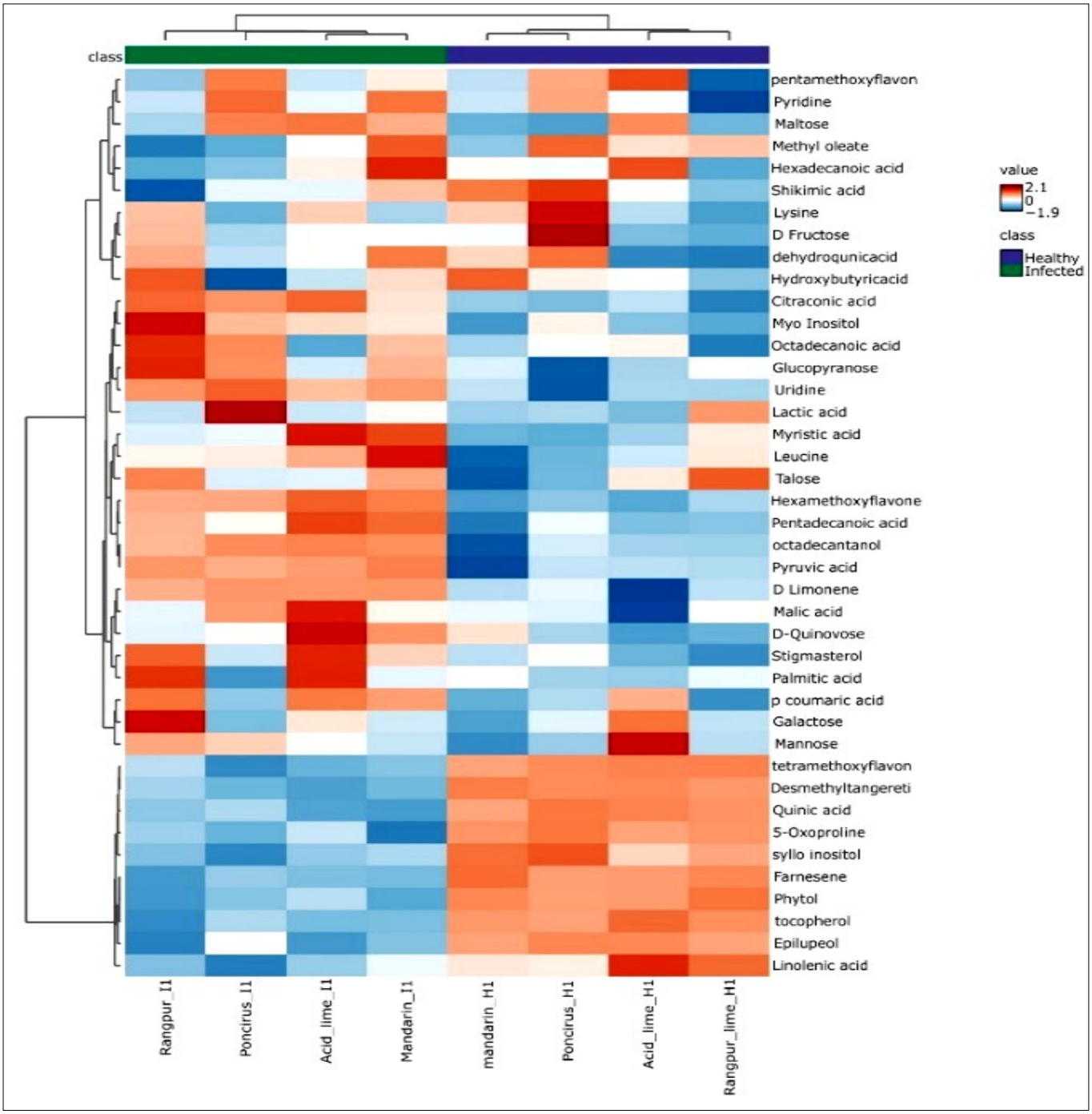

Fig. 4. Comparison of metabolites in healthy and CGD infected samples of *Citrus* spp.

The metabolite composition of infected Rangpur lime was altered, with various chemical groups represented and organic acids being the most dominant, comprising 66 compounds (19.69 %). Sugars, sugar derivatives, fatty acids and lipids accounted for 8 compounds (12.12 %), reflecting their significant presence. Terpenes, sterols, phenols and flavonoids contributed 7 compounds each (10.6 %), highlighting their roles in the fruits' altered chemical profile. Smaller proportions were noted for cyclic, polycyclic and miscellaneous compounds, each representing 5 compounds (7.57 %). Amino acids, alcohols, polyols and aromatics each contributed 4 compounds (6.06 %), indicating moderate representation. Pyrimidines and related compounds were the least abundant, with only 1 compound (1.51 %), indicating the altered metabolic profile due to infection (Supplementary Table. S7-S8 & Fig. 4).

Metabolic shifts in different citrus spp. under cgd infection: a comparative analysis

Healthy leaf samples of four various *Citrus* species contained significant amounts of tetramethoxyflavone, desmethyltangeton, quinic acid, 5-oxyproline, scylloinositol, farnesene, phytol, tocopherol, epilupeol and linoleic acid. However, these 10 metabolites were present in only trace amounts or neatly absent in infected samples, suggesting their potential as health biomarkers in *Citrus* spp. Interestingly, acid lime exhibited a significantly higher linolenic acid concentration among the four healthy Citrus samples than other species. A unique observation was the markedly high presence of mannose in healthy acid lime leaves. At the same time, it was either absent or found in trace amounts in the nutritious leaves of the other three *Citrus* species and all four infected samples. Additionally, healthy *Poncirus* tissues, known for their tolerance to CGD, contained significantly higher levels of D-fructose and lysine. These compounds were either completely absent or present in trace amounts in the other three healthy tissues and all infected samples across the four species.

Discussions

The extraction of biologically active compounds from plant sources has gained attention as an eco-friendly approach for obtaining valuable compounds with therapeutic potential. This study documented quinic acid (QA) as a potential bioactive compound derived from phenyl propanoid biosynthesis. It was found to be increased in healthy conditions but decreased in infected conditions. Several findings supported the therapeutic properties of QA as an antimicrobial, antifungal, cytotoxic, antidiabetic, insecticidal, anticancer, antioxidant and analgesic agent (17-21). However, limited studies have explored the antibacterial potential of QA derivatives or their synergistic interaction with standard antibiotics, antibiofilm activity, toxicity analysis and drug-likeness properties (22, 23). Under *in vitro* conditions, it was tested against several bacterial pathogens and exhibited potent antibacterial activity (24).

In this study, healthy leaves of four Citrus species contained high levels of 10 key metabolites, which were significantly reduced or nearly absent in infected samples, suggesting their role as health indicators. Acid lime showed notably higher levels of linolenic acid and uniquely high mannose levels than other species. CGD-tolerant Poncirus tissues had significantly elevated D-fructose and lysine, absent or found in trace amounts in different species and infected samples. It also has maltose and uridine in higher abundance, indicating robust carbohydrate and nucleotide metabolism. These metabolites are critical for energy storage, structural integrity and RNA synthesis, which support normal growth and maintenance. D-limonene, malic acid and stigmasterol were relatively lower, reflecting baseline metabolic activity without needing heightened defence responses or stress adaptation.

Metabolite profiling of infected Poncirus revealed that upregulated metabolites include D-limonene, malic acid and stigmasterol. The increase in D-limonene, a secondary metabolite, suggests that infection triggers chemical defence mechanisms, likely as part of a protective strategy against the pathogen (12). Malic acid levels increased, indicating enhanced TCA cycle activity to meet the higher energy demands induced by infection stress (11). Stigmasterol levels increased, which may contribute to membrane reinforcement or signalling processes essential for defence (13). Downregulated meta-bolites include maltose and uridine, suggesting infection diverts resources from carbohydrate storage and nucleotide synthesis to support defence and stress-response pathways (14). The depletion of these meta-bolites could limit normal growth and cellular repair mechanisms during infection. In healthy Mandarin Orange, metabolic pathways related to energy storage, nucleotide synthesis and signalling remain stable, with higher levels of myo-inositol, maltose and uridine (12).

Myo-inositol plays a crucial role in maintaining cellular integrity and signalling pathways. Maltose indicates robust carbohydrate storage and energy reserves, while uridine supports nucleotide synthesis for growth and repair (12). In contrast, during infection, the metabolic profile shifts significantly. The upregulation of p-coumaric acid, malic acid and pyruvic acid reflects an enhanced defensive response and increased energy metabolism to counteract infection (11). P-coumaric acid, a precursor for lignin synthesis, helps strengthen the cell wall to prevent pathogen spread (13).

Meanwhile, malic acid and pyruvic acid suggest heightened activity in the TCA cycle to meet the increased energy demands during stress (14). Concurrently, key metabolites like Myo-inositol, maltose and uridine are downregulated, indicating a shift away from growth and maintenance processes. This suppression reflects a resource reallocation strategy that prioritizes energy production and defence mechanism over cellular maintenance, enhancing the plants' ability to combat infection (12). In addition to the observed metabolic shifts during HLB infection, several other factors contribute to the complex response of citrus cultivars to the disease. Metabolic reprogramming during HLB infection is driven by defence mechanisms and the plants' need to adapt to pathogen-induced stress. For instance, studies have reported alterations in the lipid profile, with significant changes in the fatty acid composition of infected citrus leaves. These changes may help enhance the integrity of the cell membrane and mediate signalling pathways necessary for stress response (25).

Furthermore, reactive oxygen species (ROS) accumulate in response to *C*Las infection, triggering oxidative stress that can damage cellular structures. The plants' antioxidant defence

system, including compounds like ascorbate and glutathione, is crucial in mitigating this damage and maintaining cellular homeostasis (26). In addition, the alteration of secondary metabolites like flavonoids, which are upregulated during infection, suggests a role in antimicrobial defence and the regulation of signalling pathways that control stress responses (27). These findings support the idea that the citrus plants' response to HLB is multifaceted, involving metabolic changes, enhanced antioxidant activity and secondary metabolite production to combat oxidative stress and pathogen attack.

Conclusion

This study identifies substantial metabolic shifts in citrus species affected by CGD, demonstrating distinct metabo-lite profiles under healthy and infected conditions. Key metabolites, such as quinic acid and epilupeol, were common across all the healthy citrus species. Metabolomic analyses revealed potential resistance mechanisms in species like *Poncirus*, characterized by elevated levels of defence-related metabolites. Specific metabolites in healthy Citrus species suggest their potential as bio-markers for *Citrus* health. The notably higher levels of linolenic acid and mannose in acid lime and elevated D-fructose and lysine in CGD-tolerant Poncirus highlight species-specific metabolic traits that may contribute to disease resistance. These findings could aid in developing diagnostic tools and improving disease management strategies for *Citrus* crops through targeted metabolic profiling and biomarker identification.

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Authors' contributions

RR contributed to conceptualising the study and revising the draft, MK contributed to writing the original draft, Project administration and Conceptualisation of the work. SR and AM edited the draft and incorporated tables and figures, KK and TM engaged in the formal analysis and KP and AR contributed to the data curation. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Bové JM. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. J Plant Pathol. 2006;88(1):7–37.
2. Halbert SE, Manjunath KL, Ramadugu C, Brodie MW, Webb SE, Lee RF. Trailers transporting oranges to processing plants move Asian citrus psyllids. Fla Entomol. 2010;93:33–38. https://doi.org/10.1653/024.093.0104
3. López-Arroyo JI, Peña MA, Rocha-Peña MA, Loera J. Ocurrenciaen México del psílidoasiático *Diaphorina citri* (Homoptera: Psyllidae). Memorias del VII Congreso Internacional de Fitopatología, Chihuahua; 2005. p. 68
4. Halbert SE. The discovery of Huanglongbing in Florida. In: Proceedings of the 2nd International Citrus Canker and Huanglongbing Research Workshop, Orlando, FL; 2005. p. 50.
5. Bové JM, Rogers ME. Huanglongbing-control. Acta Horti. 2015;1065:869–89. https://doi.org/10.17660/ActaHortic.2015.1065.109
6. Inoue H, Yamashita-Muraki S, Fujiwara K, Honda K, Ono H, Nonaka T, et al. Fe^{2-} ions alleviate the symptom of citrus greening disease. Int J Mol Sci. 2020;21(11):4033. https://doi.org/10.3390/ijms21114033
7. McCollum G, Baldwin E. Huanglongbing: devastating disease of citrus. In: Janick J, editor. Horticultural Reviews. 2016;43:315–61 https://doi.org/10.1002/9781119281269.ch7
8. Davis MJ, Mondal SN, Chen H, Rogers ME, Brlansky RH. Co-cultivation of *'Candidatus Liberibacter asiaticus'* with Actinobacteria from citrus with Huanglongbing. Plant Dis. 2008;92:1547–50. https://doi.org/10.1094/PDIS-92-11-1547
9. Parker JK, Wisotsky SR, Johnson EG, Hijaz FM, Killiny N, Hilf ME, De La Fuente L. Viability of *'Candidatus Liberibacter asiaticus*' prolonged by addition of citrus juice to culture medium. Phytopathol. 2014;104:15–26. https://doi.org/10.1094/PHYTO-05-13-0119-R
10. Sechler A, Schuenzel EL, Cooke P, Donnua S, Thaveechai N, Postnikova E, et al. Cultivation of *'Candidatus Liberibacter* asiaticus', 'Ca. *L. africanus'* and 'Ca. *L. americanus'* associated with Huanglongbing. Phytopathol. 2009;99:480–86. https://doi.org/10.1094/PHYTO-99-5-0480
11. Etxeberria E, Gonzalez P, Achor D, Albrigo G. Anatomical distribution of abnormally high levels of starch in HLB-affected Valencia orange trees. Physiol Mol Plant Patho. 2009;74:76–83. https://doi.org/10.1016/j.pmpp.2009.09.004
12. Zhang LH, Ren SL, Su ZQ, Xu PP, Ou D, Wang LJ, et al. Impact of Huanglongbing pathogen infection on the amino acid composition in both citrus plants and the Asian citruspsyllid. FrontPhysiol. 2021;12:777908. https://doi.org/10.3389/fphys.2021.777908
13. Kumar R, Sariga R, Manonmani K, Ayyandurai M, Senthilraja C, Akila R, et al. Assessment of Huanglongbing - induced changes in primary and secondary metabolites of acid lime. Physiol Mol Plant Patho. 2025;136:102547. https://doi.org/10.1016/j.pmpp.2024.102547
14. Naser TZ, Mohammad B. Physiological and morphological response to drought stress in seedlings of ten citrus. Trees. 2016;30:985–93. https://doi.org/10.1007/s00468-016-1372-y
15. Wang Z, Yin Y, Hu H, Yuan Q, Peng G, Xia Y. Development and application of molecular-based diagnosis for *'Candidatus Liberibacter asiaticus'*, the causal pathogen of citrus huanglongbing. Plant Patho. 2006;55(5):630–38. https://doi.org/10.1111/j.1365-3059.2006.01438.x
16. Ghosh DK, Bhose S, Warghane A, Motghare M, Sharma AK, Dhar AK, Gowda S. Loop-mediated isothermal amplification (LAMP) based method for rapid and sensitive detection of *'Candidatus Liberibacter asiaticus*' in citrus and the psyllid vector, *Diaphorina*

citri Kuwayama. J Plant Biochem. 2016;25:219–23. https://doi.org/10.1007/s13562-015-0332-8

17. Karaman M, Tesanovic K, Gorjanovic S, Pastor FT, Simonovic M, Glumac M, et al. Polarography as a technique of choice for the evaluation of total antioxidant activity: the case study of selected *Coprinus comatus* extracts and quinic acid, their antidiabetic ingredient. Nat Prod Res. 2021;35(10):1711–16. https://doi.org/10.1080/14786419.2019.1628753
18. Pero RW, Lund H. *In vivo* treatment of humans with quinic acid enhances DNA repair and reduces the influence of lifestyle factors on risk to disease. Int J Biotechnol Biochem. 2009;5(3):293–305.
19. Bai J, Wu Y, Wang X, Liu X, Zhong K, Huang Y, et al. *In vitro* and *in vivo* characterization of the antibacterial activity and membrane damage mechanism of quinic acid against *Staphylococcus aureus*. J Food Saf. 2018;38(1):e12416. https://doi.org/10.1111/jfs.12416.
20. Samimi S, Ardestani MS, Dorkoosh FA. Preparation of carbon quantum dots-quinic acid for drug delivery of gemcitabine to breast cancer cells. J Drug Deliv Sci Technol. 2021;61:102287. https://doi.org/10.1016/j.jddst.2020.102287
21. Liu L, Liu Y, Zhao J, Xing X, Zhang C, Meng H. Neuroprotective effects of D-(-)-quinic acid on aluminum chloride-induced dementia in rats. Evid Based Complement Altern Med. 2020:1–10. https://doi.org/10.1155/2020/5602597
22. Gohari AR, Saeidnia S, Mollazadeh K, Yassa N, Malmir M, Shahverdi AR. Isolation of a new quinic acid derivative and its antibacterial modulating activity. Daru. 2010;18(1):69–73.
23. Nirmal NP, Khanashyam AC, Mundanat AS, Shah K, Babu KS, Thorakkattu P, et al. Valorization of fruit waste for bioactive compounds and their applications in the food industry. Foods. 2023;12:556. https://doi.org/10.3390/foods12030556
24. Heena B, Kaushal S, Kalia A, Kaur V. Proximate, mineral, chemical composition, antioxidant and antimicrobial potential of dropped fruits of *Citrus reticulata* Blanco. J Food Meas Charact. 2022;16:4303–17. https://doi.org/10.1007/s11694-022-01532-w
25. Meng LL, Liu RC, Yang L, Zou YN, Srivastava AK,et al. The change in fatty acids and sugars reveals the association between trifoliate orange and endophytic fungi. J Fungi. 2021;7(9):716. https://doi.org/10.3390/jof7090716
26. Hasanuzzaman M, Bhuyan MB, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, et al. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. Antioxidants. 2020;9(8):681. https://doi.org/10.3390/antiox9080681
27. Hijaz F, Al-Rimawi F, Manthey JA, Killiny N. Phenolics, flavonoids and antioxidant capacities in citrus species with different degree of tolerance to Huanglongbing. Plant Signal. Behav. 2020;15 (5):1752447. https://doi.org/10.1080/15592324.2020.1752447

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