



Time-resolved network metabolomics reveals metabolic reprogramming and network stability in cowpea leaves during virus infection

Raissa S. C. Bret · Silvio A. Cândido-Sobrinho · Samuel G. Abreu · Nicole P. Porto · David B. Medeiros · Leonardo Perez Souza · Murilo S. Alves · Alisdair R. Fernie · Danilo M. Daloso 

Received: 20 January 2025 / Accepted: 7 April 2026
© The Author(s) 2026

Abstract Cowpea severe mosaic virus (CPSMV) represents a major constraint for cowpea (*Vigna unguiculata* (L.) Walp.) cultivation. However, the metabolic basis underlying CPSMV resistance remains poorly understood. Given the importance of metabolism for plant defence against pathogens, we hypothesized that cowpea resistance to CPSMV is associated to the activation of defence-related pathways from both primary and secondary metabolisms. We investigated the metabolic dynamics associated with CPSMV infection in the resistant cowpea genotype Macaibo using a time-resolved, network-based

metabolomics approach. Conventional RT-PCR suggests that the intensity of the CPSMV coat protein band increased in the first eight Hours After CPSMV Inoculation (HAI) but appeared reduced in the following 72 HAI. Metabolomic analyses revealed that CPSMV infection had little impact on secondary metabolism, whereas several primary metabolites were significantly altered over time. Transient increases in fumarate, pyruvate, and several amino acids, including alanine, asparagine, glutamine, glutamate, isoleucine, leucine, proline, serine, threonine, and valine, were observed at 48 HAI compared with mock-treated leaves. Network analysis indicated that overall metabolic network density and heterogeneity remained relatively stable during infection. However, a highly connected metabolic module composed of the branched-chain amino acids (BCAAs) isoleucine, leucine, and valine emerged in infected leaves. Our results indicate that CPSMV infection reshapes primary metabolic dynamics without extensive alterations in secondary metabolism during early infection in cowpea leaves. Despite these metabolic adjustments, the overall metabolic network structure remained stable, suggesting that a robust metabolic reprogramming, including the activation of BCAA-associated pathways, may help buffer viral perturbation in cowpea leaves.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s40626-026-00401-x>.

R. S. C. Bret · S. A. Cândido-Sobrinho · S. G. Abreu · N. P. Porto · M. S. Alves · D. M. Daloso (✉)
Departamento de Bioquímica E Biologia Molecular,
Universidade Federal do Ceará, 60451-970 Fortaleza, CE,
Brazil
e-mail: daloso@ufc.br

D. B. Medeiros · L. P. Souza · A. R. Fernie
Max-Planck-Institute of Molecular Plant Physiology,
14476 Potsdam-Golm, Germany

D. M. Daloso
Instituto Nacional de Ciência e Tecnologia em Fisiologia
de Plantas em Condições de Estresse, 36570-900 Viçosa,
MG, Brazil

D. M. Daloso
ForCE Metabolomics, Fortaleza, CE 60451-970, Brazil

Keywords Metabolic fingerprinting · Metabolic network · Metabolite profiling · Plant-virus interaction · Systems biology

1 Introduction

The current climate change scenario predicts that periods of drought and high temperature, among other (a)biotic unfavourable conditions, will be more frequent and intense for crop cultivation (Zandalinas et al. 2021). It is thus crucial to find strategies to improve yield and/or resilience to abnormal conditions in crops (Evans and Lawson 2020). However, there is a clear yield-stress tolerance trade-off in plants, in which the tolerance to unfavourable conditions is generally achieved at expenses of productivity loss, and *vice-versa* (Fernandez et al. 2021; Cândido-Sobrinho et al. 2022). For instance, the model of our study—cowpea (*Vigna unguiculata* (L.) Walp.) Macaibo genotype—is resistant to most cowpea severe mosaic virus (CPSMV) isolates described until now (Lima et al. 2012; Teixeira et al. 2021), but has much lower yield than other sensitive and high productive cowpea genotypes (Vale and Lima 1995; Lima et al. 2011; Paiva et al. 2014). Cowpea is a leguminous crop with great importance for regions with dry soil, thanks to its low-cost production, high nutritional value, and high tolerance to extreme environments (Kareem and Taiwo 2007; Singh et al. 2011). However, infection by CPSMV, a Comovirus (*Secoviridae*) that infects *Fabaceae* plants, can reduce yield by up to 85%, representing a major bottleneck for cowpea cultivation (Booker et al. 2005; Kareem and Taiwo 2007). Understanding the mechanisms by which cowpea plants respond to CPSMV is thus important to find strategies to maintain the cultivation and productivity of this important staple food (Jha et al. 2023).

The severe mosaic disease caused by CPSMV is characterised by yellowish, mosaic-like spots on the leaves. In more advanced stages of infection, CPSMV may lead to plant dwarfism and cell death spots on leaves (Rys et al. 2014; Laliberté and Zheng 2014). CPSMV is an obligatorily intracellular biotrophic pathogen, meaning that it cannot be controlled by prophylactic or pesticide treatments, which makes its control difficult in large scale cultivations (Hull 2009; Nicaise 2014). Furthermore, the resistance to CPSMV is polygenic, which hampers the development of resistant cultivars (Young 2000). In incompatible interactions, no severe symptoms appear after virus infection (Lima et al. 2011; Magalhães 2011). Macaibo, therefore, represents an important genetic

resource to understand the mechanisms of plant-virus interaction, which is crucial to obtain not only virus-resistant cowpea but also other virus-resistant *Fabaceae* genotypes.

It has been shown that the CPSMV-resistant BRS-Marataoã cowpea genotype displays no morphological symptoms 6 days after CPSMV infection. However, significant alterations in the content of compounds and activity of enzymes related to the redox metabolism were observed (Varela et al. 2017). Furthermore, proteomics analysis of this incompatible interaction unveiled that proteins related to both energy and carbohydrate metabolisms were among the main functional categories altered by CPSMV infection (Varela et al. 2017). These results suggest that the cowpea tolerance to CPSMV involves changes in primary metabolism. However, no study has yet used any metabolomics approach to study the cowpea-CPSMV pathosystem. Furthermore, although several secondary metabolites such as those pertaining to the classes of alkaloids, terpenes, and phenols have been identified as plant chemical defenses against pathogens, little is known concerning their role in the battle against viruses (Islam et al. 2019), especially for the cowpea-CPSMV pathosystem. It is clear therefore that several pieces of the cowpea-CPSMV puzzle are yet to be discovered.

Here, we hypothesized that the resistance of *V. unguiculata* to CPSMV is also associated to the activation of defence-related pathways of both primary and secondary metabolisms. To test this hypothesis, we carried out an extensive leaf metabolic characterization to unravel the metabolic responses of the virus-resistant cowpea Macaibo genotype during the progression of CPSMV infection. Contrary to the hypothesized, the resistance to CPSMV doesn't seem to be related to the activation of secondary metabolism, at least in the time period analysed here. Notably, however, CPSMV infection altered the dynamic of accumulation of primary metabolites and the topology of the primary metabolic network, with the appearance of a subnetwork containing the amino acids leucine, isoleucine, and valine. These amino acids were upregulated by CPSMV infection and have been previously associated to resistance to other pathogens. The results are discussed in the context of the physiological relevance of the metabolic changes triggered by CPSMV infection and the possible implications for plant virus resistance.

2 Material and methods

2.1 Plant material and growth conditions

We used the cowpea (*Vigna unguiculata* (L.) Walp.) Macaibo genotype, which is resistant to most CPSMV isolates described until now. Notably, 11 CPSMV isolates were described and present sequences deposited in GenBank (Teixeira et al. 2021), in which only one of them has been reported to be capable to infecting the Macaibo genotype (Lima et al. 2012). Macaibo seeds were disinfected by adding sodium hypochlorite (Sigma-Aldrich®) and germinated in filter paper rolls (28×38 cm; Germitest) kept in a growth chamber for 7 days with a 12 h photoperiod, 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD). The seedlings were transferred to 6 L plastic pots (6 seedlings per pot) and maintained under hydroponic system using Hoagland and Arnon's nutrient solution with modifications (Hoagland and Arnon 1950; Silveira et al. 2001). Plants were kept under greenhouse conditions with natural sunlight (300–650 $\mu\text{mol PPFD m}^{-2} \text{s}^{-1}$), with daily temperature ranging from 27 to 31 °C and $79.8 \pm 10.9\%$ of average relative humidity.

2.2 Isolation of CPSMV

The inoculum of cowpea severe mosaic virus (CPSMV), strain CE, was isolated from previously infected leaves of cowpea, genotype CE-31, as described previously (Paz et al. 1999). Virus-infected leaves of cowpea CE-31 were macerated in 10 mM potassium phosphate buffer (Sigma-Aldrich®), pH 7.0, containing sodium sulfite (1:10, m/v) for 10 min. Carborudum (600 mesh) was added to the inoculum (1:10, m/v) to facilitate infection in the leaf tissues. The CPSMV inoculum used in this study corresponds to an isolate previously characterized in the literature (Paiva et al. 2016; Silva et al. 2016).

2.3 Inoculation of CPSMV into cowpea leaves

Cowpea leaves are disposed as trifoliate, composed by a central leaf surrounded by two lateral leaves (Fig. 1A). The inoculation of Mock and CPSMV were performed on the central leaf of the first fully expanded trifoliate by friction with thumb and index fingers. Control plants were Mock inoculated with same solution, but without CPSMV (Fig. 1A). Central

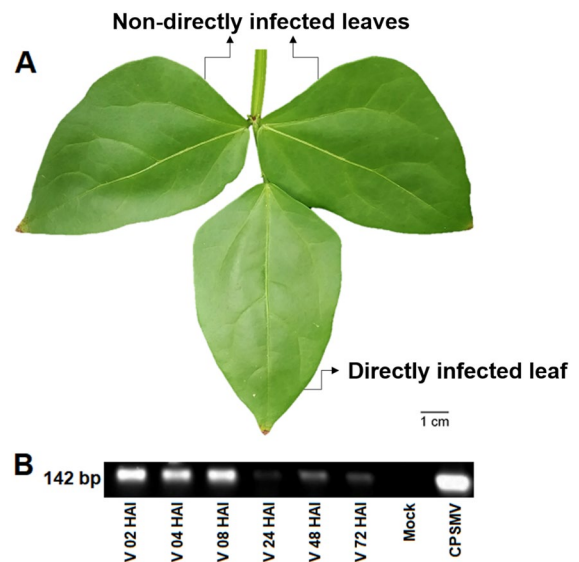


Fig. 1 Representative leaf infected with cowpea severe mosaic virus (CPSMV) and the detection of the CPSMV in leaf tissues of the Macaibo genotype. **A** Representative image of a cowpea first true leaf 15 days after CPSMV inoculation, highlighting the leaves that have been directly (DIL) and non-directly (NDIL) infected with CPSMV. **B** Detection of the coat protein of CPSMV by PCR analysis using CPSMV inoculated leaves after 2, 4, 8, 24, 48 and 72 h after inoculation (HAI). Mock and CPSMV lanes represent negative and positive controls. The band found in the CPSMV spot corresponds to the expression of the CPSMV coat protein extracted from a leaf material of a CPSMV-susceptible genotype

(called herein as directly infected leaf—DIL) and lateral (called herein as non-directly infected leaves—NDIL) of cowpea Macaibo were harvested after 02, 04, 08, 24, 48 and 72 h after virus or mock inoculation (HAI) and frozen in liquid nitrogen for metabolomics analysis.

2.4 Virus detection in cowpea leaves

Frozen cowpea leaves were macerated with liquid nitrogen until a fine powder. Total RNA was extracted using SV Total RNA Isolation System kit (Promega®) according to the manufacturer's instructions. To confirm the absence and/or the presence of CPSMV in the uninfected and virus infecting cowpea plants, virus detection by RT-PCR was performed as described previously (Paiva et al. 2016). Briefly, the first-strand cDNA product that corresponds to a fragment of the viral coat protein gene obtained was amplified by RT-PCR using two degenerated

universal oligonucleotide primers for the Comovirus genus: 25 5'-GCATGGTCCACWCAGGT-3'(forward) and 5'-YTCRAAWCCVYTRTTKGGMCCACA-3'(reverse). The RT-PCR reaction thermal cycler programmed was an initial denaturation step at 95 °C for 5 min, 40 cycles consisting of denaturation at 94 °C for 20 s, annealing at 41 °C for 20 s, and extension at 72 °C for 45 s, followed by an extension step at 72 °C for 10 min. Upon completion of the reaction, the products were visualized (gel-red staining) after a 1% (m/v) agarose gel electrophoresis run carried out on a Pharmacia Biotec electrophoresis unit for 40 min, at 25 °C, 100 V, 50 mA. Virus detection was performed using CPSMV-specific primers previously validated (Paiva et al. 2016; Silva et al. 2016). The viral identity was then inferred based on this previously characterized isolate and the PCR detection, without additional DNA sequencing.

2.5 GC-TOF/MS-based metabolite profiling analysis

The extraction of polar metabolites and the gas chromatography time of flight mass spectrometry (GC-TOF/MS) analysis were carried out exactly as described previously (Lisec et al. 2006). Leaves were macerated with liquid nitrogen until a fine powder. 50 mg was transferred to a microtube (2.0 mL), where 1.4 mL of methanol (LC-MS grade, Sigma-Aldrich®) and 60 µL of ribitol (0.2 mg/mL in deionized water) (Sigma-Aldrich®) were added. Ribitol is used as an internal quantitative standard, which corrects for any experimental error during sample preparation as well as for variations in the sensitivity of the GC-MS (Lisec et al. 2006). Microtubes were shaken (950 r.p.m.) for 15 min at 37 °C, followed by centrifugation (11,000 g) for 10 min. The supernatant was transferred to a new microtube (2.0 mL), where 375 µL of cold chloroform (LC-MS grade, Sigma-Aldrich®) and 700 µL of deionized water were added. After mixing by vortex and centrifugation (10,000 g, 15 min), aliquots of 150 µL from upper (polar) phase were taken and dried using speed vac. Subsequently, samples were derivatized by adding 40 µL of methoxyamine hydrochloride (Sigma-Aldrich®), previously dissolved in 20 mg/mL in pure pyridine (Sigma-Aldrich®) and shaken at 37 °C for 2 h. We next added 70 µL of *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) (Sigma-Aldrich®) and tubes were shaken again at 37 °C for further 30 min. After that,

a centrifugation was performed, and the supernatant was transferred to vials and injected in a GC-TOF/MS (Agilent 6890N, LECO Pegasus III TOF-MS). Analysis of the chromatograms was carried out using the Xcalibur 2.1 software (Thermo Fisher Scientific, Waltham, MA, USA). Metabolites were identified using the Golm Metabolome Database (<http://gmd.mpimp-golm.mpg.de/>) (Kopka et al. 2005).

2.6 LC/MS analysis

Ultra-high-performance liquid chromatography high-resolution mass spectrometry (LC/MS) analysis was carried exactly out as described earlier (Tohge and Fernie 2010). This analysis was carried out using the same leaf material of the GC-TOF/MS analysis, but the sample preparation was made following the protocol for LC-MS. The LC/MS platform used mostly detect medium polarity secondary metabolites (Perez de Souza et al. 2021). We first used a *metabolic fingerprinting* approach, which provides a global overview of the features detected by LC/MS without the need for metabolite identification (Perez de Souza et al. 2019). This analysis was carried out by using 13,586 features (peaks found in the chromatograms). After that, the metabolites were annotated by using MZmine (Pluskal et al. 2010), which resulted in 98 metabolites identified.

2.7 Metabolic network analysis

Debiased sparse partial correlation (DSPC) were calculated among the metabolites of the metabolic networks using Lasso Penalty Method and the CorrelationCalculator software. Correlation-based metabolic networks were built using MetScape on Cytoscape software considering $-0.5 > r > 0.5$. The parameters density and heterogeneity of the metabolic networks were obtained as described earlier (Assenov et al. 2008). Both network density and heterogeneity were calculated from the aggregate metabolic network constructed for each condition using the correlation matrix of all metabolites. As these metrics describe global topological properties of the reconstructed network rather than replicate-level measurements, they produce single values and do not include measures of variability such as standard deviation. We further calculated and identified the number of hubs of the network as described earlier (Freire et al. 2021). Briefly,

a node was considered a hub when its degree of connection (number of links) was higher than the average of the degree of connection of the network in a given mock-treated leaf and time point. For example, if the mean degree of connection of the mock-treated local leaf at 02 HAI is 3, all nodes with degree of connection higher than 3 at 02 HAI are considered hubs.

2.8 Experimental design and data analysis

Experiments were completely randomized in a factorial arrangement consisted of two treatments (mock and virus inoculation), two organs (DIL and NDIL), six time points (02, 04, 08, 24, 48 and 72 h after infection (HAI)) and five biological replicates ($n=5$). The metabolomics data was evaluated using analysis of variance (ANOVA) followed by the Tukey's test ($P<0.05$) and partial least squares-discriminant analysis (PLS-DA) using the MetaboAnalyst platform (Chong et al. 2018). This data was also analysed using a time series approach named clustering dynamic time warping (dtw) through the TSclust on R software (Montero and Vilar 2015). Heat maps were build using the MetaboAnalyst platform or the MultiExperiment Viewer 4.9.0 software and manually integrated into a metabolic pathway.

3 Results

3.1 CPSMV infects cowpea Macaibo leaves, but no visual symptom was observed

No visual symptoms appeared after 15 days of CPSMV inoculation either in DIL or in NDIL (Fig. 1A). Conventional RT-PCR analysis of the CPSMV coat protein suggests an increase in the infection in the first day of infection (from 2 to 8 Hours After CPSMV Inoculation (HAI)), as compared to the negative control (Mock). Interestingly, the intensity of the CPSMV coat protein band measured by RT-PCR decreased from 8 to 24, 48 and 72 HAI, resembling more the negative than the positive CPSMV control (Fig. 1B). This result suggests that CPSMV can infect Macaibo leaves, but the intensity of the CPSMV coat protein band reduces throughout time. It is important to highlight, however, that the RT-PCR approach used here provides only qualitative or semi-quantitative information based on band

intensity. Thus, although this result coupled to the lack of symptoms after 15 days of CPSMV infection suggest that Macaibo struggle CPSMV infection, further quantitative RT-PCR analysis are required to confirm this hypothesis.

3.2 Leaf primary metabolism is altered in CPSMV-infected leaves

We carried out an extensive metabolic characterization of leaves inoculated with mock or CPSMV for 02, 04, 08, 24, 48 and 72 HAI. GC-TOF/MS analysis highlights that the primary metabolism was substantially modified upon CPSMV infection (Fig. 2). 22 and 17 metabolites were differentially accumulated over time in DIL and NDIL, respectively (Table 1). The content of 21 and 14 metabolites was significantly ($P<0.05$) altered upon CPSMV infection in DIL and NDIL in at least one time point, when compared to their respective mock control, respectively (Table 1; Fig. 2). Partial least squares-discriminant analysis (PLS-DA) clearly separated Mock from CPSMV-infected leaves, with a great extent to the DIL, in which a clearer separation by the first component was observed (Fig. 3). PLS-DA using the data of DIL and NDIL normalized according to their respective mock control further distinguished the metabolic responses of DIL and NDIL to CPSMV, as evidenced by the separation by the first components (Fig. 4). This analysis provides a variable importance in projection (VIP) score for each parameter used in the PLS model. Metabolites with VIP score higher than 1 are those that most contributed to the separation found in the PLS-DA graph (Xia and Wishart 2011). Citrate, nicotinic acid, Val, Leu and Ile showed VIP score higher than 1 in both DIL and NDIL in at least one time point (Table S1). The contents of citrate decreased while fumarate, pyruvate, and the amino acids Ala, Asn, Gln, Glu, homoserine, Ile, Leu, Pro, Ser, Thr, and Val increased after CPSMV infection in DIL at 48 HAI (Fig. 5). Nicotinic acid, Val, Leu and Ile are also included in the VIP score list of the PLS model that discriminated DIL from NDIL (Table S1). This analysis indicates that the primary metabolism was transiently modified upon CPSMV infection, given that the differences between mock and CPSMV treated leaves observed at 48 HAI disappear at 72 HAI.

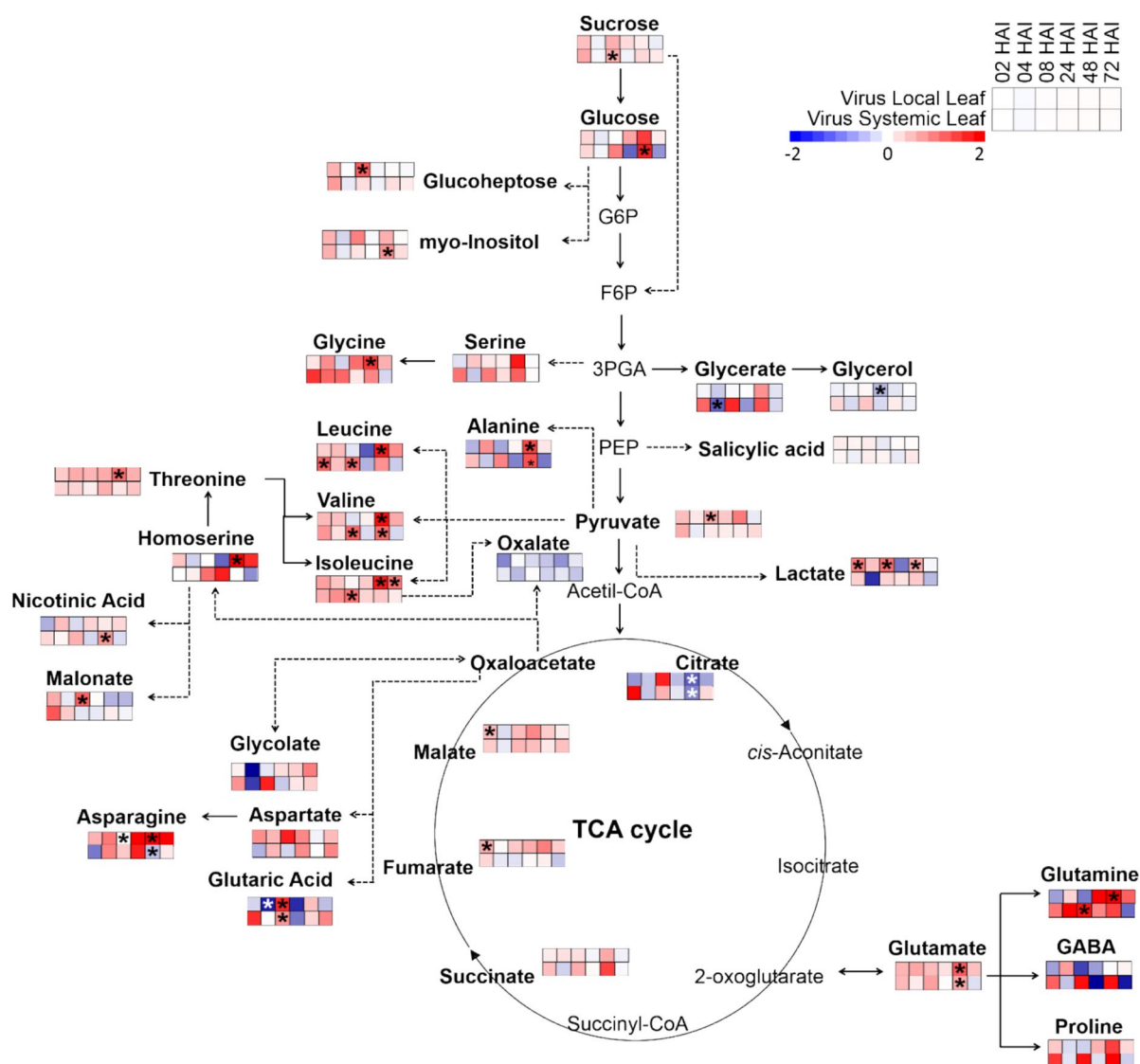


Fig. 2 Changes in the content of primary metabolites in leaves that have been directly (DIL) or non-directly (NDIL) inoculated with Mock (control group) or CPSMV (cowpea severe mosaic virus) for 02, 04, 08, 24, 48 and 72 h. The content of each metabolite is represented by the colour scale of the leg-

end. Red and blue squares represent metabolites with increased and decreased content in DIL (upper squares) and NDIL (lower squares) leaves, as compared to their mock control, respectively. Dashed lines indicate non-sequential metabolic reactions. Abbreviation: HAI, hours after inoculation

3.3 Metabolic fingerprinting analysis highlights that leaf secondary metabolism was slightly affected by CPSMV inoculation

We next carried out a LC-MS-based metabolic fingerprinting analysis (Figures S1A-B), which is useful to discriminate samples based in the intensity of the features (peaks) identified in the chromatograms

(Kosmides et al. 2013; Cândido-Sobrinho et al. 2022). Most of the features detected belongs to pathways of the secondary metabolism (Figure S2). None of them were statistically different over time and between Mock and CPSMV (Table S2A). When the data of DIL and NDIL were normalized according to the values found in their respective mock control, 34 and 35 features were found to display differential

Table 1 List of metabolites identified by gas chromatography mass spectrometry (GC/MS) analysis in leaves that have been directly (DIL) or non-directly (NDIL) inoculated with Mock (control group) or CPSMV (cowpea severe mosaic virus)

Metabolites	Time		Mock vs CPSMV	
	DIL	NDIL	DIL	NDIL
Alanine	$P < 0.05$	ns	$P < 0.05$	ns
Asparagine	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Aspartate	Ns	ns	$P < 0.05$	ns
Butyrate	ns	$P < 0.05$	ns	ns
Citrate	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Fumarate	$P < 0.05$	$P < 0.05$	$P < 0.05$	ns
Glucoheptose	ns	ns	$P < 0.05$	ns
Glucose	$P < 0.05$	$P < 0.05$	ns	$P < 0.05$
Glutamate	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Glutamine	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Glutarate	$P < 0.05$	ns	$P < 0.05$	$P < 0.05$
Glycerate	ns	ns	ns	$P < 0.05$
Glycerol	$P < 0.05$	ns	$P < 0.05$	ns
Glycine	$P < 0.05$	$P < 0.05$	$P < 0.05$	ns
Glycolate	ns	ns	ns	ns
Homoserine	$P < 0.05$	$P < 0.05$	$P < 0.05$	ns
<i>myo</i> -inositol	ns	ns	ns	$P < 0.05$
Isoleucine	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Lactate	$P < 0.05$	ns	$P < 0.05$	ns
Leucine	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Malate	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Malonate	ns	ns	$P < 0.05$	
Nicotinic_acid	ns	$P < 0.05$	ns	$P < 0.05$
Oxalate	$P < 0.05$	$P < 0.05$	ns	ns
Proline	$P < 0.05$	$P < 0.05$	ns	ns
Pyruvate	$P < 0.05$	ns	$P < 0.05$	ns
Salicylic_acid	ns	ns	ns	ns
Serine	$P < 0.05$	$P < 0.05$	ns	ns
Succinate	ns	ns	ns	ns
Sucrose	$P < 0.05$	ns	ns	$P < 0.05$
Threonine	$P < 0.05$	$P < 0.05$	$P < 0.05$	ns
Valine	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

The pattern of accumulation of the metabolites over time was analysed by analysis of variance (ANOVA) ($P < 0.05$) using the entire data set (all time points) of DIL and NDIL leaves infected with Mock or CPSMV. Metabolites with no changes over time are identified with ns (non-significant). These results are displayed in the Time columns. Metabolites with differential accumulation between Mock and CPSMV-infected leaves were compared by Student's *t* test ($P < 0.05$) in each time point (02, 04, 08, 24, 48 and 72 h after infection). The columns Mock vs CPSMV display metabolites in which the level was significantly ($P < 0.05$) or non-significantly (ns) altered in CPSMV compared to Mock in at least one time point

accumulation over time in DIL and NDIL, respectively (Table S2B). However, only one feature was significantly different between mock and CPSMV treatments (Table S2C), indicating that the secondary metabolism was not affected by CPSMV inoculation in the period analysed here. We next identified 97 metabolites from the LC/MS data (Figures S3A–B). None of these 97 metabolites were significantly altered over time and between the treatments (Table S3). When the data from CPSMV-infected leaves were normalized according to the values found in mock-treated leaves, only one and two metabolites changed over time in DIL and NDIL, respectively (Table S3). Our metabolomics analyses collectively suggest that the major metabolic changes induced by CPSMV infection are observed in the primary rather than the secondary metabolism. We then next deeply explored the GC-TOF/MS data using other systemic analyses.

3.4 The dynamic of accumulation/degradation of primary metabolites was altered in CPSMV-infected leaves

We next adopted a time-series approach based on dynamic time warping (DTW) and hierarchical clustering to better investigate the changes in primary metabolites in DIL and NDIL over time of CPSMV infection. DTW analysis cluster metabolites with similar dynamic over time using a maximum-minimum transformation, i.e. the content of the metabolites is transformed to a 0 (lowest average value) to 1 (highest average value) scale (Deans et al. 2019; Cândido-Sobrinho et al. 2022). Twenty clusters were generated including data from DIL and NDIL (Figure S4). The dynamic of accumulation/degradation of 22 and 14 metabolites was altered in DIL and NDIL over time, respectively (Table S4). For example, the dynamic of Leu changed from the cluster 5 to 3 after CPSMV infection (Figures S5A, C). By contrast, Ala changed from the cluster 7 to the clusters 13 and 3 in DIL and NDIL after CPSMV infection (Figures S5B, D). Similar differences were also observed in certain sugars (glucose, sucrose), organic acids (malate and succinate), amino acids (Pro, Ser, and homoserine), GABA, glycerate and glycolate, especially in DIL (Figures S5A–D). Nicotinic acid and salicylic acid were grouped in different clusters in DIL, whilst only salicylic acid was grouped in a different cluster

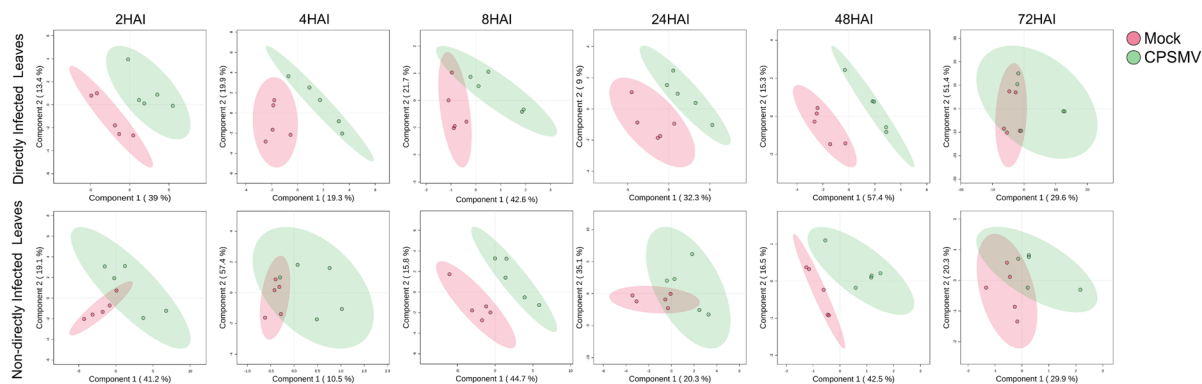


Fig. 3 Partial least-squares discriminant analysis (PLS-DA) using the gas chromatography mass spectrometry (GC/MS)-based metabolite profiling data from leaves that have been directly (DIL) or non-directly (NDIL) inoculated with Mock (control group) or CPSMV (cowpea severe mosaic virus). Raw data refer to the content of the metabolites normalized by the ribitol and the fresh mass used in the extraction. Upper

and lower panels refer to leaves subjected to mock (in red) or CPSMV (in green) treatments and harvest after 02, 04, 08, 24, 48 and 72 h after infection (HAI). The percentage variation explained by the components 1 and 2 are represented in each axis. PLS-DA was carried out using the Metaboanalyst platform (n=5)

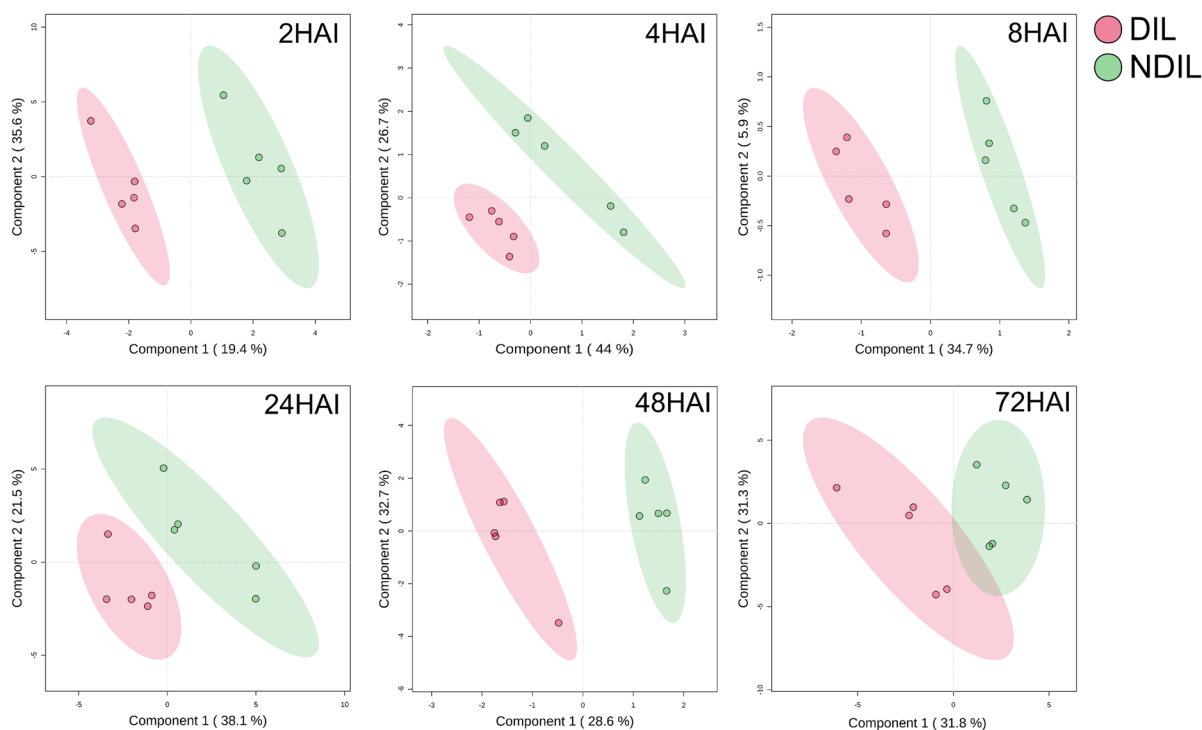


Fig. 4 Partial least-squares discriminant analysis (PLS-DA) using the gas chromatography mass spectrometry (GC/MS)-based metabolite profiling data from leaves that have been directly (DIL) or non-directly (NDIL) inoculated with Mock (control group) or CPSMV (cowpea severe mosaic virus). DIL (in red) and NDIL (in green) leaves were subjected to mock or CPSMV treatments and harvest after 02, 04, 08, 24, 48 and

72 h of infection (HAI). This PLS-DA was carried out using data from DIL and NDIL infected with CPSMV normalized by their respective mock control. The percentage variation explained by the components 1 and 2 are represented in each axis. PLS-DA was carried out using the Metaboanalyst platform (n=5)

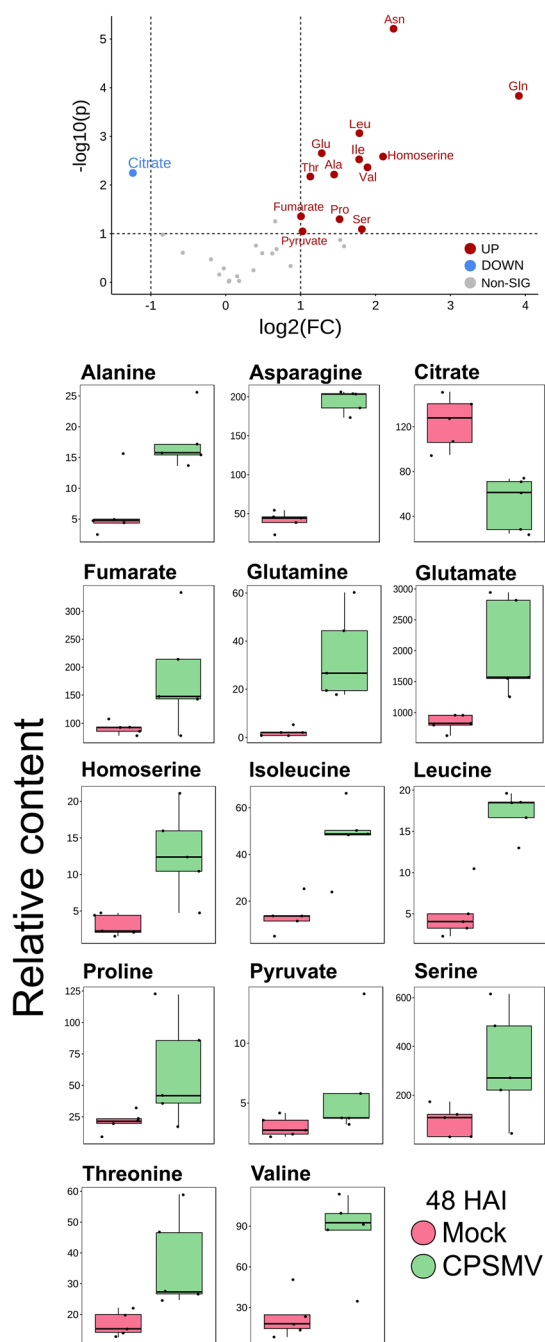


Fig. 5 Volcano and box plots of selected metabolites identified by gas chromatography mass spectrometry (GC/MS) analysis in leaves that have been directly (DIL) or non-directly (NDIL) inoculated with Mock (control group) or CPSMV (cowpea severe mosaic virus) for 48 h. These metabolites showed a differential accumulation between mock and CPSMV treatments by Student's *t* test ($P < 0.05$). This analysis was carried out using the Metaboanalyst platform

after virus infection in NDIL (Table S4). Beyond the changes in the content of metabolites, CPSMV infection substantially altered the dynamic of accumulation/degradation of primary metabolites over time.

3.5 The density and the topology of the leaf primary metabolic network was relatively unaffected by CPSMV infection

We next applied a correlation-based network approach to investigate the changes in both density and topology of leaf metabolic networks induced by CPSMV infection. Nodes and links correspond to the metabolites identified by GC-TOF/MS and the debiased sparse partial correlation (DSPC) among them, respectively (Freire et al. 2021). Given that network density and heterogeneity are descriptive properties of the reconstructed metabolic network, these parameters are represented by single values and do not allow statistical inference between treatments and time points. CPSMV infection increased and decreased the density and the heterogeneity of the networks, respectively, in DIL and NDIL (Figures S6A–B). Interestingly, Ile, Leu and Val appear as a module highly connected to each other in DIL (Figure S6A). This is even more evident when the links of the networks are restricted to the significant correlations ($P < 0.05$), in which an isolated, highly connected module (subnetwork) composed by Ile, Leu, Val and homoserine is formed (Figs. 6A–B).

These network analyses suggest that CPSMV infection leads to metabolic networks more connected (higher density) and with the presence of more hubs (lower heterogeneity). However, when the networks were analysed in each time point (Figures S7–S8), no clear pattern of changes in either density or heterogeneity of the networks were observed in CPSMV-infected leaves (Figures S9A–D). This result highlights that, although the primary metabolism was altered by CPSMV, the topology of the metabolic network is relatively unaffected.

3.6 Branched-chain amino acids were the major hubs of CPSMV-infected leaf metabolic networks

We next identified the hubs of these networks and investigated their behaviour throughout the time of CPSMV infection. Interestingly, the number of hubs and the number of exclusive hubs was substantially

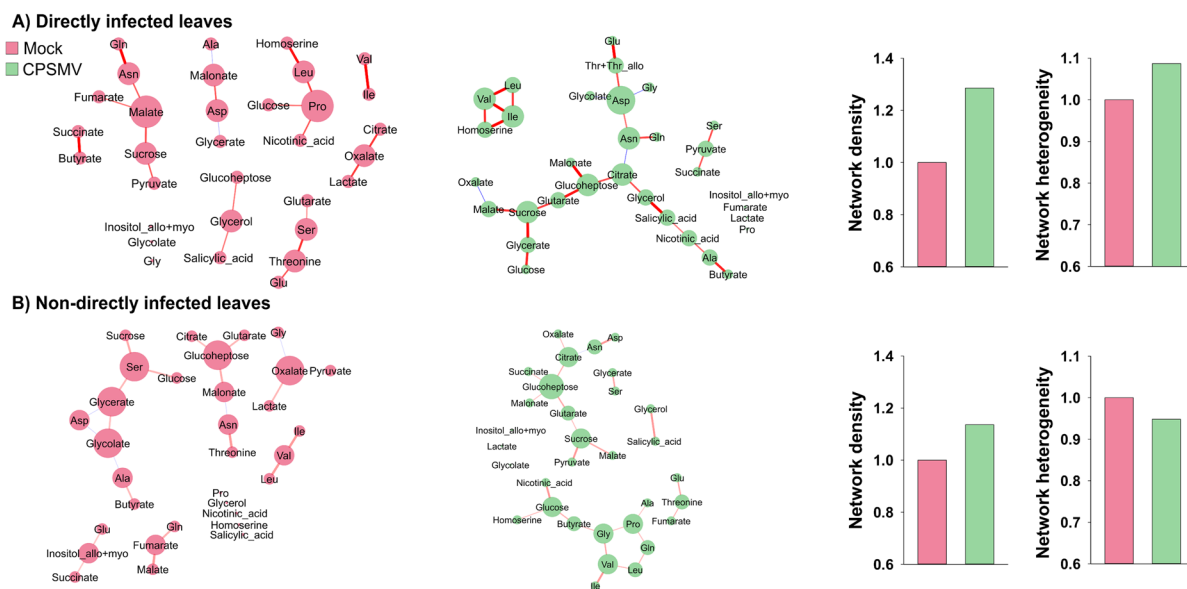


Fig. 6 Correlation-based metabolic networks of leaves that have been directly (DIL) or non-directly (NDIL) inoculated with Mock (control group) or CPSMV (cowpea severe mosaic virus). The metabolic networks were created combining gas chromatography mass spectrometry (GC/MS)-based metabolite profiling data from all time points (from 02 to 72 h after inoculation). Nodes and links represent metabolites and the correlation among them, respectively. Bigger nodes and thicker

lines imply more connections and stronger debiased sparse partial correlation (DSPC) coefficient (r), respectively. Red and blue lines represent positive and negative r . The networks were build using MetScape on Cytoscape software considering only the significant correlations ($P < 0.05$). Both density and heterogeneity of the networks were obtained by using NetworkAnalyzer on Cytoscape

higher in mock-treated leaves, when compared to DIL (Figures S9E, G). In NDIL, these parameters oscillated over time in both mock and CPSMV-treated leaves, with exception of the number of hubs in mock-treated leaves that remained relatively stable over time (Figures S9F, H). Among the hubs identified in the different time points, nicotinic acid is the one that most appeared in the different time points in DIL, followed by glucoheptose and Gln (Figure S10). Glucose, sucrose, fructose, and several metabolites related to the TCA cycle such as fumarate, pyruvate, oxalate, malate, malonate, succinate, and citrate were found as hubs in DIL. The branched-chain amino acids (BCAA) Ile, Leu, and Val were identified as hubs after 72 HAI in DIL (Figure S10). Similarly, Ile and nicotinic acid at 48 HAI and Val at 72 HAI were also found as hubs in NDIL (Figure S11).

CPSMV infection modified the metabolic network of cowpea differently in DIL and NDIL (Figure S12). Even though a few important hubs (e.g. glucose at 4 HAI, lactate at 24 HAI, and nicotinic acid at 48 HAI) were found as hubs in both DIL and NDIL, the number of hubs range substantially between them over time (Figures S9E-F). Ile appeared as hub in all time points in DIL, whilst Val was identified as a hub at 2, 4, 24, 48 and 72 HAI and Leu appears at 4, 8 and 48 HAI. Homoserine, Thr, butyrate, and nicotinic acid appeared as hubs only in DIL. Ile, glycolate, Gly, Val and *myo*-inositol appeared as hubs in NDIL in different time points (Figure S13). These results suggest that nicotinic acid and BCAAs are prominent hubs in cowpea leaves under CPSMV infection, although they do not necessarily indicate a causal relationship with viral resistance.

4 Discussion

4.1 CPSMV infection alters especially the primary rather than the secondary metabolism

Plant metabolism is separated in primary and secondary metabolisms. Whilst the first is related to essential metabolic pathways such as those associated with carbon and nitrogen metabolisms, the former is especially activated under unfavourable conditions (Saito et al. 2013). However, a growing body of evidence highlights that the primary metabolism is of paramount importance for plant acclimation to drought, salinity, flooding and other abnormal abiotic conditions (Pires et al. 2016; Barros et al. 2017; Fàbregas et al. 2018; Fàbregas and Fernie 2019; Balfagón et al. 2022; Zandalinas et al. 2022; Cardoso et al. 2023). Our results highlight that the major changes induced by CPSMV infection was observed at the primary rather than the secondary metabolism. This is evidenced by the low number of features and metabolites derived from the LC/MS platform that have been significantly altered in response to CPSMV (Tables S2–S3), in contrast to the more drastic changes observed in primary metabolites identified by GC/MS (Table 1).

The higher stability of the secondary metabolism could be related to the incompatible nature of the CPSMV-Macaibo pathosystem. This interaction is characterized by a rapid elimination of the virus, as evidenced by the lack of CPSMV coat protein expression in leaves of a resistant cowpea genotype six days after CPSMV infection (Varela et al. 2019). In this context, our RT-PCR result showed that the signal intensity of the CPSMV coat protein band reduced after 24, 48 and 72 HAI (Fig. 1B). Together with the lack of symptoms 15 days after virus inoculation (Fig. 1A), this reinforces the findings from previous works highlighting the incompatibility of the CPSMV-Macaibo pathosystem (Vale and Lima 1995; Lima et al. 2012; Paiva et al. 2016). However, it is important to highlight that the conventional RT-PCR used here to detect viral presence provides only qualitative or semi-quantitative information based on band intensity. Therefore, RT-qPCR analyses would be required to accurately quantify viral load dynamics and confirm potential reductions in virus accumulation. It seems like that changes in the expression of genes and proteins (Varela et al. 2017; Jha et al. 2023)

and in the content of primary metabolites are sufficient to struggle CPSMV infection, safeguarding the secondary metabolism.

It is noteworthy that the modifications in the content of primary metabolites triggered by unfavourable conditions are faster than that observed in secondary metabolites (Angelovici and Kliebenstein 2022; Brand and Tissier 2022), whose biosynthesis and accumulation is mostly transcriptionally regulated (Yang et al. 2012). Thus, we cannot exclude the possibility that the concentration of secondary metabolites is constitutively high in Macaibo leaves, which could contribute to CPSMV resistance without the need to further increase the concentration of these compounds after CPSMV infection. Additionally, it is known that the phytohormones ethylene, salicylic acid, and jasmonic acid are important against viruses (Collum and Culver 2016; Pan et al. 2021). However, how these and other phytohormones contribute to CPSMV resistance remains to be investigated. Further LC/MS analysis comparing susceptible and resistant cowpea genotypes to CPSMV may unveil which metabolism and phytohormone-mediated mechanisms contribute to cowpea resistance to CPSMV infection.

4.2 On the stability of the primary metabolic network under CPSMV infection

Plant metabolism operates as a highly integrated network (Sweetlove and Fernie 2005). It is thus important to analyse the metabolic changes at network level. Here, the values of both density and heterogeneity of the DIL metabolic network oscillated throughout the time of CPSMV infection (Figure S9). Although DIL leaves had lower density and higher heterogeneity than mock-treated leaves in the first day of CPSMV infection (i.e. 2, 4 and 8 HAI) (Figures S9A, C), which was associated to a clear reduction in the number of hubs (Figure S9E), the values of density and heterogeneity were similar between mock and CPSMV DIL at 24 and 72 HAI (Figures S9A, C). These results indicate that the dynamic of the topology and the density of the metabolic networks were not substantially altered by CPSMV infection. Similarly, a recent multi-species/stress condition meta-analysis indicate that the changes in topology and density of metabolic networks are species and stress intensity specific (Cardoso et al. 2023).

Thus, although the changes in the content of primary metabolites seems to be a common response to (a) biotic unfavourable conditions (Obata and Fernie 2012; Pires et al. 2016; Fàbregas and Fernie 2019; Fonseca-Pereira et al. 2019; Zandalinas et al. 2022), as observed here, the metabolic network of cowpea leaves directly infected with CPSMV remained relatively stable throughout the time of CPSMV infection. These results suggest that CPSMV infection triggers adjustments in metabolite abundance without substantially disrupting the topology of the cowpea metabolic network. This suggests that Macaibo primary metabolism remains structurally robust during the early stages of CPSMV infection, potentially contributing to its ability to buffer viral perturbations during this period.

4.3 Looking to the subnetworks: metabolic pathways contributing to CPSMV resistance

Scale-free networks, such as plant metabolic networks, are characterized by the presence of few nodes with high number of connections (links), called hubs (Barabási 2009). In plants, amino acids associated to acclimation to unfavourable conditions such as Asn, Val, Leu, Ile, Pro, Trp, and Gly have been identified as hubs in metabolic networks from numerous species under different abnormal conditions (Cardoso et al. 2023). Here, when the metabolomics data was analysed combining all time points (from 2 to 72 HAI), both DIL and NDIL networks showed higher density and lower heterogeneity, when compared to mock-treated leaves (Figure S6). Interestingly, Val and Ile were significantly and positively correlated in mock-treated leaves (Fig. 6A). This strong interaction was maintained after CPSMV infection with addition of Leu and homoserine, creating a highly interconnected subnetwork (Fig. 6A). These amino acids were present in several VIP score lists (Table S1) and had higher content in DIL in different time points, especially at 48 HAI (Fig. 5), when compared to mock-treated leaves. Homoserine is synthesized from Asp and is a metabolic intermediate of Thr biosynthesis (Aubert et al. 1998). Thr can be also used to the synthesis of Val, which justify the high correlation among them. Leu, Ile, and Val can be synthesized from pyruvate and from protein degradation (Araújo et al. 2011).

BCAAs play an important role defending plants against abiotic unfavourable conditions (Hildebrandt et al. 2015; Batista-Silva et al. 2019), specially by feeding alternative pathways of respiration (Araújo et al. 2010; Pires et al. 2016). Increases in Leu, Ile, and Val were also associated to the tolerance of rice and wheat against the fungus *Ustilagi-noidea virens* and *Puccinia striiformis*, respectively (Corredor-moreno et al. 2021; Liu et al. 2023). The mechanism by which these BCAAs modulated plant resistance to these pathogens involved the activation of salicylic acid-mediated defence responses in wheat (Corredor-moreno et al. 2021) and by stimulating cell death in the fungi, especially when Leu was exogenously applied (Liu et al. 2023). Therefore, although the accumulation of BCAAs has been commonly observed in plants under unfavourable growth conditions, they are highly associated to plant pathogen resistance in different species. The increase in the content of BCAAs observed here could either be related to a transient response to the stress caused by CPSMV or is part of the mechanism that confers the resistance of Macaibo against CPSMV. It is important to note, however, that the increases in BCAAs observed during CPSMV infection does not necessarily indicate a causal relationship with viral resistance. Future studies will be required to determine whether BCAA metabolism contributes directly to CPSMV resistance, for instance through exogenous BCAA application or by manipulating the metabolic flux through this pathway using pharmacological or genetic approaches. Thus, whether BCAAs can be used as markers for CPSMV resistance in breeding programs or metabolic engineer strategies remains to be determined in future studies.

5 Conclusion

This study demonstrates that CPSMV infection primarily affects the dynamics of primary metabolism, while secondary metabolism remains largely unchanged during the early stages of infection. Although transient metabolic changes were observed, the overall topology of the primary metabolic network remained relatively stable. Notably, network analyses revealed the emergence of a highly connected module composed of the BCAAs isoleucine, leucine, and valine. This suggests that BCAA-related

metabolic adjustments may be part of the metabolic reprogramming associated with CPSMV resistance. Future studies investigating the functional role of these pathways may help clarify whether BCAA metabolism contributes directly to antiviral defence mechanisms and whether these metabolic traits could be explored in metabolic engineer strategies to obtain virus-resistant cowpea genotypes.

Acknowledgements This work was supported by the National Institute of Science and Technology in Plant Physiology under Stress Conditions (INCT Plant Stress Physiology–Grant: 406455/2022–8). We also thank the research fellowship granted by CNPq to DMD and the scholarships granted by Federal Agency for Support and Evaluation of Graduate Education (CAPES–Brazil) to RCSB and SACS.

Author contributions RCSB, MSA and DMD designed the research and experiments. RCSB, SACS, SGA, NPP, DBM and LPS performed the experiments and all analyses with the supervision of DMD, MSA and ARF. All authors contributed to analysing the data, establishing the figures, and writing the manuscript. DMD obtained funding and is responsible for this article.

Funding The Article Processing Charge (APC) for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614). The funding was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico, 406455/2022–8, Danilo M. Daloso.

Data availability All relevant data can be found within the manuscript and its supporting materials. The data from GC–MS and LC–MS analyses can be obtained by request.

Declarations

Conflict of interest The authors declare no potential conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Angelovici R, Kliebenstein D (2022) A plant balancing act: meshing new and existing metabolic pathways towards an optimized system. *Curr Opin Plant Biol* 66:102173. <https://doi.org/10.1016/j.pbi.2022.102173>
- Araújo WL, Ishizaki K, Nunes-Nesi A et al (2010) Identification of the 2-hydroxyglutarate and isovaleryl-CoA dehydrogenases as alternative electron donors linking lysine catabolism to the electron transport chain of *Arabidopsis* mitochondria. *Plant Cell Online* 22:1549–1563. <https://doi.org/10.1105/tpc.110.075630>
- Araújo WL, Tohge T, Ishizaki K et al (2011) Protein degradation – an alternative respiratory substrate for stressed plants. *Trends Plant Sci* 16:489–498. <https://doi.org/10.1016/j.tplants.2011.05.008>
- Assenov Y, Ramírez F, Schelhorn S-E et al (2008) Computing topological parameters of biological networks. *Bioinformatics* 24:282–284. <https://doi.org/10.1093/bioinformatics/btm554>
- Aubert S, Curien G, Bligny R et al (1998) Transport, compartmentation, and metabolism of homoserine in higher plant cells: carbon-13- and phosphorus-31-nuclear magnetic resonance studies. *Plant Physiol* 116:547–557. <https://doi.org/10.1104/pp.116.2.547>
- Balfagón D, Gómez-Cadenas A, Rambla JL et al (2022) γ -Aminobutyric acid plays a key role in plant acclimation to a combination of high light and heat stress. *Plant Physiol*. <https://doi.org/10.1093/plphys/kiac010>
- Barabási AL (2009) Scale-free networks: a decade and beyond. *Science* 325:412–413. <https://doi.org/10.1126/science.1173299>
- Barros JAS, Cavalcanti JHF, Medeiros DB et al (2017) Autophagy deficiency compromises alternative pathways of respiration following energy deprivation in *Arabidopsis thaliana*. *Plant Physiol* 175:62–76. <https://doi.org/10.1104/pp.16.01576>
- Batista-Silva W, Heinemann B, Rugen N et al (2019) The role of amino acid metabolism during abiotic stress release. *Plant Cell Environ* 42:1630–1644. <https://doi.org/10.1111/pce.13518>
- Booker HM, Umaharan P, McDavid CR (2005) Effect of Cowpea severe mosaic virus on crop growth characteristics and yield of cowpea. *Plant Dis* 89:515–520. <https://doi.org/10.1094/PD-89-0515>
- Brand A, Tissier A (2022) Control of resource allocation between primary and specialized metabolism in glandular trichomes. *Curr Opin Plant Biol* 66:102172. <https://doi.org/10.1016/j.pbi.2022.102172>
- Cândido-Sobrinho SA, Lima VF, Freire FBS et al (2022) Metabolism-mediated mechanisms underpin the differential stomatal speediness regulation among ferns and angiosperms. *Plant Cell Environ* 45:296–311. <https://doi.org/10.1111/pce.14232>
- Cardoso LL, Freire FBS, Daloso DM (2023) Plant metabolic networks under stress: a multi-species/stress condition meta-analysis. *J Soil Sci Plant Nutr* 23:4–21. <https://doi.org/10.1007/s42729-022-01032-2>
- Chong J, Soufan O, Li C et al (2018) MetaboAnalyst 4.0: towards more transparent and integrative metabolomics

- analysis. *Nucleic Acids Res* 46:W486–W494. <https://doi.org/10.1093/nar/gky310>
- Collum TD (2016) Culver JN The impact of phytohormones on virus infection and disease. *Curr Opin Virol* 17:25–31. <https://doi.org/10.1016/j.coviro.2015.11.003>
- Corredor-moreno P, Minter F, Davey PE et al (2021) The branched-chain amino acid aminotransferase TaBCAT1 modulates amino acid metabolism and positively regulates wheat rust susceptibility. *Plant Cell*. <https://doi.org/10.1093/plcell/koab049>
- Deans RM, Brodribb TJ, Busch FA, Farquhar GD (2019) Plant water-use strategy mediates stomatal effects on the light induction of photosynthesis. *New Phytol* 222:382–395. <https://doi.org/10.1111/nph.15572>
- Evans JR, Lawson T (2020) From green to gold: agricultural revolution for food security. *J Exp Bot* 71:2211–2215. <https://doi.org/10.1093/jxb/eraa110>
- Fàbregas N, Fernie AR (2019) The metabolic response to drought. *J Exp Bot* 70:1077–1085. <https://doi.org/10.1093/jxb/ery437>
- Fàbregas N, Lozano-Elena F, Blasco-Escámez D et al (2018) Overexpression of the vascular brassinosteroid receptor BRL3 confers drought resistance without penalizing plant growth. *Nat Commun* 9:1–13. <https://doi.org/10.1038/s41467-018-06861-3>
- Fernandez AR, Sáez A, Quintero C et al (2021) Intentional and unintentional selection during plant domestication: herbivore damage, plant defensive traits and nutritional quality of fruit and seed crops. *New Phytol* 231(4):1586–1598
- Fonseca-Pereira P, Daloso DM, Gago J et al (2019) The mitochondrial thioredoxin system contributes to the metabolic responses under drought episodes in *Arabidopsis*. *Plant Cell Physiol* 60:213–229. <https://doi.org/10.1093/pcp/pcy194>
- Freire FBS, Bastos RL, Bret RSC et al (2021) Mild reductions in guard cell sucrose synthase 2 expression leads to slower stomatal opening and decreased whole plant transpiration in *Nicotiana tabacum* L. *Environ Exp Bot* 184:104370. <https://doi.org/10.1016/j.envexpbot.2020.104370>
- Hildebrandt TM, Nunes Nesi A, Araújo WL, Braun HP (2015) Amino acid catabolism in plants. *Mol Plant* 8:1563–1579. <https://doi.org/10.1016/j.molp.2015.09.005>
- Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. *Calif Agric Exp Stn Circ*. 347:1–32
- Hull R (2009) Comparative plant virology, 2nd edn. Elsevier, Norwich
- Islam W, Naveed H, Zaynab M et al (2019) Plant defense against virus diseases; growth hormones in highlights. *Plant Signal Behav* 14:1596719. <https://doi.org/10.1080/15592324.2019.1596719>
- Jha UC, Nayyar H, Chattopadhyay A et al (2023) Major viral diseases in grain legumes : designing disease resistant legumes from plant breeding and OMICS integration. *Front Plant Sci*. <https://doi.org/10.3389/fpls.2023.1183505>
- Kareem KT, Taiwo MA (2007) Interactions of viruses in cowpea: effects on growth and yield parameters. *Virol J* 4:1–7. <https://doi.org/10.1186/1743-422X-4-15>
- Kopka J, Schauer N, Krueger S et al (2005) GMD@CSB.DB: the golm metabolome database. *Bioinformatics* 21:1635–1638. <https://doi.org/10.1093/bioinformatics/bti236>
- Kosmides AK, Kamisoglu K, Calvano SE et al (2013) Metabolomic fingerprinting: challenges and opportunities. *Crit Rev Biomed Eng* 41:205–221. <https://doi.org/10.1615/CritRevBiomedEng.2013007736>
- Laliberté JF, Zheng H (2014) Viral manipulation of plant host membranes. *Annu Rev Virol* 1:237–259. <https://doi.org/10.1146/ANNUREV-VIROLOGY-031413-085532>
- Lima JAA, da Silva AKF, Aragão MdoL et al (2011) Simple and multiple resistances to viruses in cowpea genotypes. *Pesqui Agropecu Bras* 46:1432–1438. <https://doi.org/10.1590/S0100-204X2011001100003>
- Lima JAA, Nascimento AKQdo, Da Silva AKF, Aragão MdoL (2012) Biological stability of a strain of Cowpea severe mosaic virus over 20 years. *Rev Cienc Agron* 43:105–111. <https://doi.org/10.1590/S1806-66902012000100013>
- Lisec J, Schauer N, Kopka J et al (2006) Gas chromatography mass spectrometry-based metabolite profiling in plants. *Nat Protoc* 1:387–396. <https://doi.org/10.1038/nprot.2006.59>
- Liu X, Matsumoto H, Lv T et al (2023) Phyllosphere microbiome induces host metabolic defence against rice false-smut disease. *Nat Microbiol*. <https://doi.org/10.1038/s41564-023-01379-x>
- Magalhães VG (2011) prospecção bioquímica e molecular de fatores possivelmente envolvidos na defesa de feijão-de-corda [*Vigna unguiculata* (L.) walp] ao vírus do mosaico severo do caupi (CPSMV). Federal University of Ceará
- Montero P, Vilar JA (2015) TSclust: an R package for time series clustering. *J Stat Softw* 62:1–43. <https://doi.org/10.18637/JSS.V062.I01>
- Nicaise V (2014) Crop immunity against viruses: outcomes and future challenges. *Front Plant Sci* 5:1–18. <https://doi.org/10.3389/fpls.2014.00660>
- Obata T, Fernie AR (2012) The use of metabolomics to dissect plant responses to abiotic stresses. *Cell Mol Life Sci* 69:3225–3243. <https://doi.org/10.1007/s00018-012-1091-5>
- Paiva JB, Freire Filho FR, Teófilo EM, Ribeiro VQ (2014) Feijão-caupi: melhoramento genético no Centro de Ciências Agrárias. Edições UFC, Fortaleza
- Paiva ALS, Oliveira JTA, De Souza GA, Vasconcelos IM (2016) Label-free proteomic reveals that Cowpea Severe Mosaic Virus transiently suppresses the host leaf protein accumulation during the compatible interaction with cowpea (*Vigna unguiculata* [L.] Walp.). *J Proteome Res*. <https://doi.org/10.1021/acs.jproteome.6b00211>
- Pan LL, Miao H, Wang Q et al (2021) Virus-induced phytohormone dynamics and their effects on plant–insect interactions. *New Phytol* 230:1305–1320. <https://doi.org/10.1111/nph.17261>
- Paz CD, Lima JAA, Pio-Ribeiro G et al (1999) Purificação de um isolado do vírus do mosaico severo do caupi, obtido em perna-buco, produção de antissoros e determinação de fontes de resistência em caupi. *Summa Phytopathologica* 25(4):285–288
- Perez de Souza L, Alseikh S, Naake T, Fernie A (2019) Mass spectrometry-based untargeted plant metabolomics. *Curr*

- Protoc Plant Biol 4:e20100. <https://doi.org/10.1002/cppb.20100>
- Perez de Souza L, Alseekh S, Scossa F, Fernie AR (2021) Ultra-high-performance liquid chromatography high-resolution mass spectrometry variants for metabolomics research. *Nat Methods*. <https://doi.org/10.1038/s41592-021-01116-4>
- Pires MV, Pereira AA Jr, Medeiros DB et al (2016) The influence of alternative pathways of respiration that utilize branched-chain amino acids following water shortage in *Arabidopsis*. *Plant Cell Environ* 39:1304–1319. <https://doi.org/10.1111/pce.12682>
- Pluskal T, Castillo S, Villar-Briones A, Orešič M (2010) MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinformatics* 11:1–11. <https://doi.org/10.1186/1471-2105-11-395/TABLES/3>
- Rys M, Juhász C, Surówka E et al (2014) Comparison of a compatible and an incompatible pepper-tobamovirus interaction by biochemical and non-invasive techniques: chlorophyll a fluorescence, isothermal calorimetry and FT-Raman spectroscopy. *Plant Physiol Biochem* 83:267–278. <https://doi.org/10.1016/j.plaphy.2014.08.013>
- Saito K, Yonekura-Sakakibara K, Nakabayashi R et al (2013) The flavonoid biosynthetic pathway in *Arabidopsis*: structural and genetic diversity. *Plant Physiol Biochem* 72:21–34. <https://doi.org/10.1016/j.plaphy.2013.02.001>
- Silva RGG, Vasconcelos IM, Martins TF et al (2016) Drought increases cowpea (*Vigna unguiculata* [L.] Walp.) susceptibility to cowpea severe mosaic virus (CPSMV) at early stage of infection. *Plant Physiol Biochem* 109:91–102. <https://doi.org/10.1016/j.plaphy.2016.09.010>
- Silveira JAGD, Costa RCD, Oliveira JTA (2001) Drought-induced effects and recovery of nitrate assimilation and nodule activity in cowpea plants inoculated with *Bradyrhizobium* spp. under moderate nitrate level. *Braz J Microbiol* 32:187–194
- Singh A, Baoule AL, Ahmed HG et al (2011) Influence of phosphorus on the performance of cowpea (*Vigna unguiculata* (L) Walp.) varieties in the Sudan savanna of Nigeria. *Agric Sci* 02:313–317. <https://doi.org/10.4236/as.2011.23042>
- Sweetlove LJ, Fernie AR (2005) Regulation of metabolic networks: understanding metabolic complexity in the systems biology era. *New Phytol* 168:9–24. <https://doi.org/10.1111/j.1469-8137.2005.01513.x>
- Teixeira KJML, Cascardo RDS, Leal LL et al (2021) First complete genome sequence of an isolate of cowpea severe mosaic virus from South America. *Virus Genes* 57:238–241. <https://doi.org/10.1007/s11262-021-01831-2>
- Vale CC, Lima JAA (1995) Herança da imunidade da cultivar Macaibo de *Vigna unguiculata* ao vírus do mosaico severo do caupi. *Fitopatol Bras* 20:30–32
- Varela ALN, Komatsu S, Wang X et al (2017) Gel-free/label-free proteomic, photosynthetic, and biochemical analysis of cowpea (*Vigna unguiculata* [L.] Walp.) resistance against Cowpea severe mosaic virus (CPSMV). *J Proteomics* 163:76–91. <https://doi.org/10.1016/j.jprot.2017.05.003>
- Varela ALN, Oliveira JTA, Komatsu S et al (2019) A resistant cowpea (*Vigna unguiculata* [L.] Walp.) genotype became susceptible to Cowpea severe mosaic virus (CPSMV) after exposure to salt stress. *J Proteomics* 194:200–217. <https://doi.org/10.1016/j.jprot.2018.11.015>
- Xia J, Wishart DS (2011) Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst. *Nat Protoc* 6:743–760. <https://doi.org/10.1038/nprot.2011.319>
- Yang C-Q, Fang X, Wu X-M et al (2012) Transcriptional regulation of plant secondary metabolism. *J Integr Plant Biol* 54:703–712. <https://doi.org/10.1111/j.1744-7909.2012.01161.x>
- Young ND (2000) The genetic architecture of resistance. *Curr Opin Plant Biol* 3:285–290
- Zandalinas SI, Fritschi FB, Mittler R (2021) Global warming, climate change, and environmental pollution: recipe for a multifactorial stress combination disaster. *Trends Plant Sci* 26:588–599. <https://doi.org/10.1016/j.tplants.2021.02.011>
- Zandalinas SI, Balfagón D, Gómez-Cadenas A, Mittler R (2022) Responses of plants to climate change: metabolic changes during abiotic stress combination in plants. *J Exp Bot*. <https://doi.org/10.1093/jxb/erac073>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.