Sample Preparation

All plant samples were lyophilized for 72 hours and then homogenized using an OMNI Bead Ruptor Elite bead mill homogenizer (6 m/s, 3 cycles of 10 seconds run, 10 seconds dwell time). Approx 10±0.5 mg of the plant material was weighed for each analysis.

a. Untargeted Lipidomics and polar metabolites assay

Lipids and polar metabolites were extracted from samples using a biphasic approach[1]. All the samples were freeze-dried and extracted with 100 µL/mg of ice-cold 3:2 methyl tert-butyl ether: 80% methanol in a glass vial. Each sample was sonicated in a cold-water bath for 20 min followed by a 90 min vortex at 4 $^\circ$C. The 200 µL/mg of LC-MS grade water was added to the extracting vial to induce the phase separation, followed by a 5 min vortex at 4 $^\circ$C. After centrifugation for 15 min at 4 $^\circ$C at 4000 x g, 200 µL of the top, the nonpolar layer was transferred to a 2 mL glass vial and 400 µL of the bottom polar layer was transferred to a new 2 mL glass vial. Both fractions were dried under a gentle stream of nitrogen at room temperature. The nonpolar fraction was resuspended in 200 µL of 9:1 methanol: toluene for LC-MS-based lipidomics analysis. The polar fraction was resuspended in 80 µL of 80% methanol, then analyzed by GC-MS and LC-MS for polar metabolites. A 20 µl from each sample was pooled to prepare a quality control sample for lipidomics and polar metabolites assay.

b. Phytohormones assay

100 µL/mg of 6:3:1 methyl tert-butyl ether (MTBE): methanol: water with deuterated internal standards extraction solvent was added. Samples were vortexed for 90 minutes at 4 $^\circ$C then centrifuged 15 minutes at 3500 x g and 4 $^\circ$C, and 400 µL supernatant was transferred to a new 2 mL autosampler vial. Extracts were then dried under nitrogen gas, resuspended in 200 µL methanol, vortexed for 5 minutes to mix, and transferred to an glass insert for LC-MS analysis. A 20 µl from each sample was pooled to prepare a quality control sample.

Standard Curve – Phytohormones

A standard curve was prepared by first adding 40 µL of phytohormones standards mix at 10 µg/mL to 760 µL 6:3:1 MTBE:methanol:water with deuterated internal standards, then performing 2-fold serial dilutions. 400 µL aliquots were dried under nitrogen gas, resuspended in 200 uL methanol, vortexed for 5 minutes to mix, and transferred to inserts for analysis.

c. Untargeted Secondary metabolites assay

100 µL/mg extraction solvent (30:30:20:20; ACN: MeOH: H2O: IPA) was added to each sample in an eppendorf tube. All samples were vortexed for 90 minutes at 4 $^\circ$C and then

centrifuged for 15 minutes at 16,000 x g at 4 °C. The 200 µL supernatant was then transferred to a 2 mL autosampler vial and a pooled quality control sample was prepared.

Data Acquisition

a. Untargeted Lipidomics

LC-MS lipidomics analysis was performed at the UC Riverside Metabolomics Core Facility as described previously[1]. Briefly, analysis was performed on a G2-XS quadrupole time-of-flight mass spectrometer (Waters) coupled to an I-class UPLC system (Waters). Separations were carried out on a CSH C18 column (2.1 x 100 mm, 1.7 µM) (Waters). The mobile phases were (A) 60:40 acetonitrile:water with 10 mM ammonium formate and 0.1% formic acid and (B) 90:10 isopropanol:acetonitrile with 10 mM ammonium formate and 0.1% formic acid. The flow rate was 400 µL/min and the column was held at 65° C. The injection volume was 1 µL. The gradient was as follows: 0 min, 10% B; 1 min, 10% B; 3 min, 20% B; 5 min, 40% B; 16 min, 80% B; 18 min, 99% B; 20 min 99% B; 20.5 min, 10% B. The MS was scan range was (50 to 1600 m/z) with a 100 ms scan time. MS/MS was acquired in data-dependent fashion. Source and desolvation temperatures were 150° C and 600° C, respectively. Desolvation gas was set to 1100 L/hr and cone gas to 150 L/hr. All gases were nitrogen except the collision gas, which was argon. The capillary voltage was 1 kV in positive ion mode and 2 kV in negative ion mode. A quality control sample, generated by pooling equal aliquots of each sample, was analyzed periodically to monitor system stability and performance. Samples were analyzed in random order. Leucine enkephalin was infused continuously and used for mass correction.

b. Targeted polar metabolites

Targeted metabolomics of polar, primary metabolites was performed on a TQ-XS triple quadrupole mass spectrometer (Waters) coupled to an I-class UPLC system (Waters)[1]. Separations were carried out on a ZIC-pHILIC column (2.1 x 150 mm, 5 µM) (SIgma Millipore). The mobile phases were (A) water with 15 mM ammonium bicarbonate adjusted to pH 9.6 with ammonium hydroxide and (B) acetonitrile. The flow rate was 200 µL/min and the column was held at 50 °C. The injection volume was 2 µL. The gradient was as follows: 0 min, 90% B; 1.5 min, 90% B; 16 min, 20% B; 18 min, 1% B; 22 min, 1% B; 23 min, 90% B; 33 min, 90% B. Source and desolvation temperatures were 150 °C and 500 °C, respectively. Desolvation gas was set to 1000 L/hr and cone gas to 150 L/hr. The collision gas was set to 0.15 mL/min. All gases were nitrogen except the collision gas, which was argon. The Capillary voltage was 1 kV in positive ion mode and 2 kV in negative ion mode. System stability was monitored by analyzing a quality control sample in between the sample injections. Samples were analyzed in random order.

c. **Phytohormones assay**

Phytohormone quantitation was performed at the UC Riverside Metabolomics Core Facility as described previously with minor modifications[2]. Briefly, analysis was performed on a TQ-XS triple quadrupole mass spectrometer (Waters) coupled to an I-class UPLC system (Waters). Separations were carried out on a T3 C18 column (2.1 x 100 mm, 1.8 µM) (Waters). The mobile phases were (A) water and (B) acetonitrile, both with 0.1% formic acid. The flow rate was 300 µL/min and the column was held at 45 $^{\circ}$C. The injection volume was 2 µL. The gradient was as follows: 0 min, 0.1% B; 1 min, 0.1% B; 6 min, 55% B; 7 min, 100% B; 8 min, 100% B; 8.5 min, 0.1% B; 13 min, 0.1% B. The MS was operated in a selected reaction monitoring mode. Source and desolvation temperatures were 150 $^{\circ}$C and 600 $^{\circ}$C, respectively. The desolvation gas was set to 1100 L/hr and cone gas to 150 L/hr. The collision gas was set to 0.15 mL/min. All gases were nitrogen except the collision gas, which was argon. The capillary voltage was 1 kV in positive ion mode and 2 kV in negative ion mode. A quality control sample, generated by pooling equal aliquots of each sample, was analyzed periodically to monitor system stability and performance. Samples were analyzed in random order.

d. **Untargeted Secondary metabolites assay**

LC-MS metabolomics analysis was performed at the UC Riverside Metabolomics Core Facility as described previously[3]. Briefly, analysis was performed on a Synapt G2-Si quadrupole time-of-flight mass spectrometer (Waters) coupled to an I-class UPLC system (Waters). Separations were carried out on a CSH phenyl-hexyl column (2.1 x 100 mm, 1.7 µM) (Waters). The mobile phases were (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. The flow rate was 250 µL/min and the column was held at 40 $^{\circ}$C. The injection volume was 2 µL. The gradient was as follows: 0 min, 1% B; 1 min, 1% B; 8 min, 40% B; 24 min, 100% B; 26.5 min, 100% B; 27 min, 1% B. The MS was scan range was (50 to 1600 m/z) with a 100 ms scan time. MS/MS was acquired in a data-dependent fashion. Source and desolvation temperatures were 150 $^{\circ}$C and 600 $^{\circ}$C, respectively. The desolvation gas was set to 1100 L/hr and cone gas to 150 L/hr. All gases were nitrogen except the collision gas, which was argon. The capillary voltage was 1 kV in positive ion mode and 2 kV in negative ion mode. A quality control sample, generated by pooling equal aliquots of each sample, was analyzed periodically to monitor system stability and performance. Samples were analyzed in random order. Leucine enkephalin was infused continuously and used for mass correction.

e. **Untargeted Polar analysis by GC-MS**

The dried-down polar fraction was resuspended in 50 µl of methoxyamine hydrochloride solution in GC grade pyridine (20 mg ml^{-1}) and vortexed for 2 h at 4 °C. Shaking continued for another 30 min after adding 50 ul of MSTFA +1% TMCS solution (ThermoTS48915)[4]. Samples were injected (2 ul) directly into the GC-MS. The analysis of small metabolites by GC–MS was performed using Thermo 1300 coupled with Thermo Fisher ISQ7000 mass spectrometers. Chromatographic separations of metabolites were carried out on a 30m×0.25mmx0.25 µm Thermo TG5SilMS column. Chromeleon software was used to process the chromatographic and mass spectrometric data. The GC oven temperature was maintained at 50 °C for 1 min, then gradually raised at the rate of 7 °C min−1 to 300 °C and maintained for 5 min. The sample was injected in the split mode at a splitting ratio of 1:20. Helium was used as a carrier gas and set at a constant flow rate of 1 mL min^{-1}. The mass selective detector was run in the electron impact (EI) mode, with an electron energy of 70 eV. The temperature of the ion source, the transmission line and the inlet temperature was set to 300 °C, 280 °C, and 290 °C, respectively. Full scan mode (SCAN) was employed and the mass scan range was 50~700 m/z. All samples were analyzed in random order. The QC sample was prepared by mixing aliquots of the samples to be a pooled sample. To monitor the GC-MS system and data acquisition stability, an injection of QC sample was tested every 10 injections of the random biological samples.

Data processing and analysis

Targeted polar metabolites and phytohormones assay data processing was performed with the open-source Skyline software[5]. The untargeted lipidomics and secondary metabolites data processing (peak picking, alignment, deconvolution, integration, and spectral matching) was performed in Progenesis Qi software (Nonlinear Dynamics). Ions included for deconvolution were [M+H], [M+NH$_4$], [M+Na] and [M+H-H$_2$O] in positive ion mode, and [M-H], [M-H-H2O] and [M-H+HCOOH]. The untargeted data was normalized to total ion count. Features with a CV greater than 30% across QC injections and average abundance of less than 500 in quality control injections were removed[6,7]. To group features belonging to the same metabolite together, features were assigned a cluster-ID using RAMClust[8]. An extension of the metabolomics standard initiative guidelines was used to assign annotation level confidence[9,10]. Annotation level 1a indicates an MS, MS/MS, and retention time match to an in-house database. Level 2a indicates an MS and MS/MS match to an external database. Level 2b indicates an MS and MS/MS match to the Lipidblast in-silico database[11]. Level 3 indicates an MS match potentially with a partial MS/MS match. Several mass spectral metabolite databases were searched against including Metlin[12], Mass Bank of North America[13], and an in-house database.

The GC-MS raw spectral data files were converted to analysis base file (Abf) format by ABF Converter and then were imported into MS-DIAL[14]. The peak picking, peak identification, alignment, deconvolution, integration, TIC normalization, and spectral

matching were performed in MS-DIAL. The feature values with a CV greater than 30% across pooled QC injections were removed[6,7]. The mass spectral library GC-MS DB-Public-KovatsRI-VS3 available from the MS-Dial repository was used for the data annotation.

References

1. Hollin, T. *et al.* Functional genomics of RAP proteins and their role in mitoribosome regulation in Plasmodium falciparum. *Nat. Commun.* **13**, 1275–1275 (2022).

2. Sheflin, A. M. *et al.* High-throughput quantitative analysis of phytohormones in sorghum leaf and root tissue by ultra-performance liquid chromatography-mass spectrometry. *Anal. Bioanal. Chem.* **411**, 4839–4848 (2019).

3. A., R. J., Laura, L., S., K. J. & S., M. Q. Cadmium and selenate exposure affects the honey bee microbiome and metabolome, and bee-associated bacteria show potential for bioaccumulation. *Appl. Environ. Microbiol.* **85**, e01411-19 (2019).

4. Bhatia, A. *et al.* Metabolic profiling of *Commiphora wightii* (guggul) reveals a potential source for pharmaceuticals and nutraceuticals. *Phytochemistry* **110**, 29–36 (2015).

5. MacLean, B. *et al.* Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics* **26**, 966–968 (2010).

6. Barupal, D. K. *et al.* Generation and quality control of lipidomics data for the alzheimer's disease neuroimaging initiative cohort. *Sci. Data* **5**, 180263–180263 (2018).

7. Dunn, W. B. *et al.* Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. *Nat. Protoc.* **6**, 1060–1083 (2011).

8. Broeckling, C. D., Afsar, F. A., Neumann, S., Ben-Hur, A. & Prenni, J. E. RAMClust: A novel feature clustering method enables spectral-matching-based annotation for metabolomics data. *Anal. Chem.* **86**, 6812–6817 (2014).

9. Sumner, L. W. *et al.* Proposed minimum reporting standards for chemical analysis. *Metabolomics* **3**, 211–221 (2007).

10. Schymanski, E. L. *et al.* Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environ. Sci. Technol.* **48**, 2097–2098 (2014).

11. Kind, T. *et al.* LipidBlast in silico tandem mass spectrometry database for lipid identification. *Nat. Methods* **10**, 755–758 (2013).

12. Smith, C. A. *et al.* METLIN: A metabolite mass spectral database. *Ther. Drug Monit.* **27**, (2005).

13. Horai, H. *et al.* MassBank: a public repository for sharing mass spectral data for life sciences. *J. Mass Spectrom.* **45**, 703–714 (2010).

14. Tsugawa, H. *et al.* A lipidome atlas in MS-DIAL 4. *Nat. Biotechnol.* **38**, 1159–1163 (2020).