




Cite this: DOI: 10.1039/d6lc00201c

Capillary microsampling enables on-site collection and storage of plant sap

 Ellinor Hedberg, ^a Jaime Sebastián-Azcona,^b Federico Ribet, ^a
 Virginia Hernandez-Santana,^b Göran Stemme, ^a
 Antonio Diaz Espejo^b and Niclas Roxhed^{*ac}

Plant sap analysis typically relies on destructive sampling and immediate freezing, limiting field deployment and longitudinal studies. We introduce a minimally invasive microfluidic device that extracts sap from the stem of *Solanum lycopersicum* and dries it *in situ*, enabling storage analogous to dried blood spots in humans. Using both artificial phytohormone mixtures and tomato sap, we assessed the stability of dried samples stored at room temperature for up to seven days and observed no substantial degradation of key phytohormones. Device performance was further validated in a paired sampling experiment, showing strong agreement with a conventional stem severing method for tZR and ABA quantification. These findings demonstrate that dried sap sampling *via* a microfluidic device provides a practical, field ready alternative to destructive methods, supporting repeated sampling from the same plant and enabling longitudinal metabolic monitoring.

 Received 2nd March 2026,
Accepted 21st April 2026

DOI: 10.1039/d6lc00201c

rsc.li/loc

Introduction

Collecting plant samples in the field is a widely used approach to evaluate both crop nutritional status and gain insight into the physiology of plants. Metabolic profiling, including the assessment of responses to biotic and abiotic stressors, can be achieved by analysing plant phytohormonal concentrations. Even at very low concentrations, these signalling molecules exert a significant influence on the plant physiological state.^{1–4}

When collecting samples for phytohormonal or metabolomic analysis, the sampling strategy and subsequent handling are critical determinants of the quality of the obtained results.⁵ Several conventional approaches are used to extract plant sap, with the choice depending both on the plant species and the sap type of interest. Examples include pressing/crushing tissue,⁶ making incisions in self-bleeding plants, applying EDTA treatments to prevent sieve element blockage by P-proteins,³ aphid stylectomy to extract pure phloem sap,⁷ centrifugation⁸ or application of external pressure.^{9,10}

In collected samples, enzymes can cause degradation of metabolites or other analytes of interest. To prevent this, the standard approach is to freeze the sample, thereby halting the enzymatic activity. Freezing can be achieved through lyophilization (freeze-drying) or by using liquid nitrogen (fresh-frozen).^{11–14} An alternative approach has been suggested, which involves drying samples at room temperature. For example, cornflower petals subjected to this method exhibited low water activity, a parameter closely related to enzymatic activity, compared to hot-air convective drying and lyophilization.^{15–17} Equally important as sample collection are the subsequent storage and transport conditions, which must be carefully controlled to ensure sample stability.^{5,12,14} Successful metabolomic analysis requires an optimized protocol for sample collection, a reproducible sampling procedure, and robust transport and storage conditions.

Inspired by human sampling, dried blood spot (DBS) microsampling has become a common procedure for easy blood sample collection and long-term storage. Due to the low enzymatic activity in dried samples, proteins can remain stable for up to 30 years with minimal destruction of the analyte and only minor changes in detection levels.^{18–20} Hormones²¹ and metabolites²² have also been successfully analysed in DBS samples, with hormones shown to remain stable for four weeks at room temperature and up to a year in cold storage.²¹

Thus, DBS enables easy sampling procedures without greatly compromising the analyte integrity. Given that plant

^a Department of Micro and Nanosystems, KTH Royal Institute of Technology, Stockholm, Sweden. E-mail: roxhed@kth.se

^b Plant Ecophysiology and Irrigation Group, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC), Sevilla, Spain

^c MedTechLabs, Karolinska University Hospital, Solna, Sweden



sap functions as the transport medium for phytohormones and metabolites in a manner that could be seen as somewhat analogous to the human blood stream, we hypothesize that a paper-based drying approach could be applied to plant sap for the analysis of phytohormones.

Here we show a microsampling device that combines the approach of drying plant sap samples in room temperature, similar to the approach of DBS, using a sampling procedure that allows for sample collection in a more low-invasive manner compared to conventional approaches. The microsampling device consists of a microfluidic channel connected to a paper matrix enclosed in a plastic case. This

configuration allows for quick and easy sap sampling, with minimal manual intervention, and with integrated sample drying inside the plastic casing through a vent.

Experimental

The microfluidic sampling device

The microfluidic sampling device is manufactured from two hydrophilic (DI water contact angle $<12^\circ$), sheets of plastic (Xerox type C laser printing transparency, Xerox) that are bonded together with a double-sided adhesive (8132LE, 3M, Digi-key) with thickness 50 μm . The hydrophilic plastic has

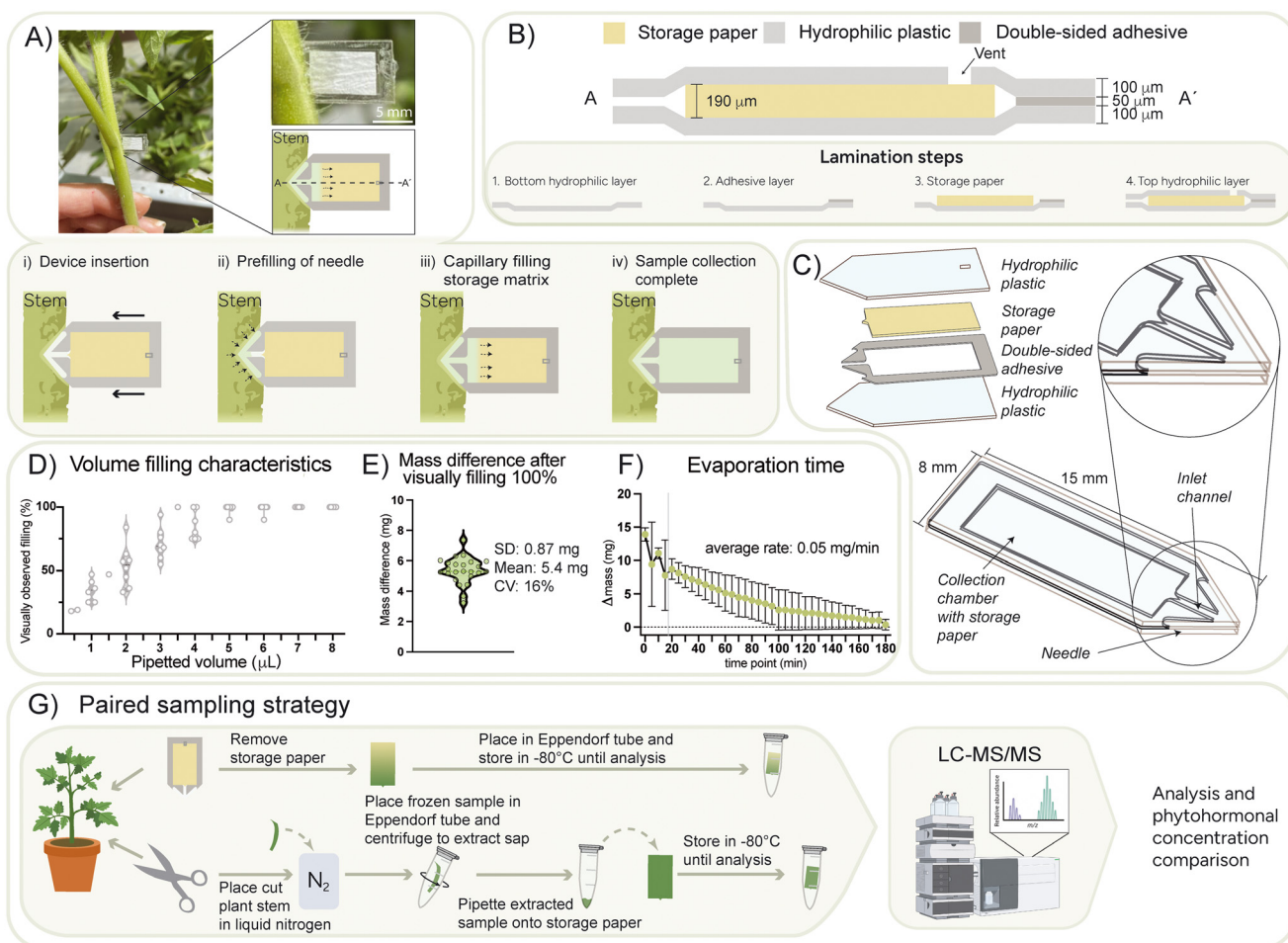


Fig. 1 A) Microfluidic sampling device inserted into tomato stem. Below is a schematic of the extraction procedure of sap being collected in the device. i) Device insertion into stem. ii) Capillary pre-filling of needle, the large surface area of the needle in contact with the plant enables greater sap extraction. iii) Capillary filling of the storage matrix. iv) Sample collection complete. B) Schematic overview of the device layers and lamination steps of the fabrication. C) 3D and exploded view of the sampling device. D) Volume collection distribution used to determine the extracted sap volume based on visually observing the filling characteristics. E) Gravimetrically measured mass difference upon collection of plant sap into fully filled microsampling devices. F) Evaporation curve obtained from microfluidic devices evaluated through gravimetric measurements of mass loss over time. The evaporated mass of water is plotted against time for $n = 4$ devices. The average evaporation time is 0.05 $\text{mg } \mu\text{L}^{-1}$, which for water is approximately 0.05 $\mu\text{L min}^{-1}$. The non-linear and linear range are separated with a grey line. This indicates the evaporation from water that has adhered to the device when filling it up (non-linear range) and evaporation from inside the device (linear range). Values presented as average \pm SD. G) The paired sampling strategy and analysis steps. The sampling is conducted by first sampling with the microsampler followed by destructive sampling through cutting using a scalpel. The microfluidic sample is directly removed from the plastic casing and placed in -80°C until further analysis. The destructive sample is placed in liquid nitrogen followed by centrifugation and pipetting of the extracted sap onto storage paper. The sample is then stored in -80°C until further analysis. UHPLC-QQQ-MS/MS analysis is conducted on the samples to enable phytohormonal concentration comparisons. The LC-MS/MS image in Fig. 1G was created using <https://www.Biorender.com>.



been previously evaluated and implemented in a variety of capillary microfluidic devices.^{23–27} The thickness of the adhesive layer determines the geometries and the height of the capillary inlet channel, needle and collection chamber. The adhesive keeps the two hydrophilic plastic layers sandwiched around the storage paper and provides structural integrity to the device, enabling puncturing of the plant stem. In the collection chamber, there is a storage paper (601-N, Sartorius, VWR) with dimensions (5.5 mm, 8.5 mm, 0.19 mm) used to collect and store the sample, Fig. 1B. The storage paper is placed between the layers and not attached, which enables easy removal of the paper through delamination of the plastic and subsequent analysis of the collected sap. The section of the paper closest to the inlet channel has a needle-like shape that protrudes 0.4 mm into the microfluidic channel to ensure that low volume samples reach the collection chamber *via* the channel and thereby enables filling of the storage paper, Fig. 1C. The inlet needle has a width of 6.5 mm and a curved tip with a radius of 0.6 mm, this to ensure puncturing of the plant stem but also avoiding clogging of the inlet channel with plant tissue, creating a biopsy-punch effect and preventing liquid flow, Fig. 1A–C. All components were designed in Adobe Illustrator and cut from the corresponding materials using a CO₂ laser cutter (VLS 2.3, Universal Laser Systems).

Phytohormonal stability in different storage conditions

To evaluate the effect of drying plant sap samples in a paper matrix, an experiment was conducted with both artificial sap samples with a mixture of phytohormones of known concentrations (PHM) and samples collected from tomato plants (*Solanum lycopersicum*, Tres Cantos) grown from seeds. The tomatoes were grown to approximately 30 cm in a sunny location in a south facing window and outdoors when good weather conditions allowed for it. Phytohormones: indole-3-acetic acid (IAA) (I3750 Sigma-Aldrich), abscisic acid (ABA) (A4906 Sigma-Aldrich), salicylic acid (SA) (247 588 Sigma-Aldrich), *trans*-zeatin riboside (tZR) (6025-53-2 Olchemim) and jasmonic acid (JA) (3572-66-5 Olchemim) were used to evaluate the PHM stability upon drying. The phytohormones were mixed to make a solution with concentrations determined from a preliminary experiment. In the preliminary experiments the microfluidic device was evaluated to determine if phytohormones could be detected in a sap sample stored in a paper matrix, and in what concentrations. From the evaluated phytohormones, IAA, SA, ABA, tZR and JA were determined to be the ones with the most relevance for these experiments as well as showing good detectability. The concentrations for each of the phytohormones were selected based on the concentration they had in the preliminary study multiplied with a factor of five to ensure good readings away from limit-of-detection.

The selected phytohormones were mixed to their determined concentrations (IAA 0.05 pmol μL^{-1} , ABA 0.5 pmol μL^{-1} , SA 0.5 pmol μL^{-1} , tZR 0.5 pmol μL^{-1} and JA 0.5

pmol μL^{-1}), and 7 μL of the PHM was pipetted onto Sartorius 601-N filter paper with dimensions (5.5 mm, 8.5 mm, 0.19 mm), same as the papers in the microfluidic device. Five of the paper pieces were immediately placed in Eppendorf tubes and stored at $-80\text{ }^\circ\text{C}$ until analysis. The rest were left in room temperature for drying for 20 min, 140 min, 5 h, 24 h and 7 days. The PHM and the individual phytohormone components were stored in $-80\text{ }^\circ\text{C}$ and $4\text{ }^\circ\text{C}$ for 10 days to compare their stability in liquid form over time, Fig. S1.

As a biological comparison to the PHM samples, the cultivated tomato plants were sampled by insertion of the microfluidic device into the stem. The sampling was made at the fourth node from the top of the plant, and this was the same for all the plants where possible. If not possible, for example due to the shape of the plant and the size of the stem at that location, sampling was done as close to that point as possible. Once filled, the devices were removed from the stems and left at room temperature for the same time intervals as the artificial phytohormone samples. The storage paper was removed from the device and placed in an Eppendorf tube prior to storage at $-80\text{ }^\circ\text{C}$ until further analysis. Three tomato plants were also sampled using a stem severing method in which a piece of the stem was cut and placed in an Eppendorf tube followed by centrifugation in a table top centrifuge to extract sap. The collected sap was stored in liquid form at $-80\text{ }^\circ\text{C}$ until analysis.

Device accuracy measurements

To evaluate the accuracy of the sampling approach an experimental setup was established with paired sampling on the same plant using both the microfluidic device and a stem severing approach to directly compare the methods.

36 tomato plants (*Solanum lycopersicum*, Tres Cantos) were placed in a greenhouse with temperature and humidity controls (HC2S3, Campbell Scientific) and plant lights (The Jackson Nemesis 250 W, The Jungle) to ensure good growth conditions. Plants were placed in methacrylate boxes with dimensions $34 \times 37 \times 57\text{ cm}^3$ to ensure controlled environments. The plants were sampled at three different time points with five replicates over four days (exception at three time points where three or four plants were sampled instead of five), creating a total of 72 samples. The sampling was carried out by first using the microfluidic sampling device followed by destructive sampling of cutting a piece of the plant for sap extraction close to the sampling site from the device. This to keep the samples as similar as possible. Approximately 8 cm of the stem was cut from the plant close to the incision site of the device. The plant section was placed in liquid nitrogen and the frozen plant samples were placed in Eppendorf tubes and spun in a tabletop centrifuge (for 60–75 seconds) at 12 000 rpm. The liquid obtained was aspirated and 7 μL placed on the same paper matrix as in the devices used for the microfluidic sampling. These samples were then placed in $-80\text{ }^\circ\text{C}$. The microfluidic samples were collected by inserting the needle at the second node of the



plant from the top, and once the devices had filled with liquid they were opened and the storage paper was placed in an Eppendorf tube and stored in $-80\text{ }^{\circ}\text{C}$ until analysis. Fig. 1G shows a schematic overview of the sampling process.

The volumes extracted with the microfluidic sampling devices varied as indicated in the metadata, (SI), where the collected volume was determined by visual inspection and comparison to the calibration experiment with devices filled with known volumes to indicate the filling, Fig. 1D. The volumes have been accounted for in the analysis by normalizing to the collected volume range.

The drying times for the storage paper in the microfluidic devices when evaluated with DI water are presented in Fig. 1F and S4. The average evaporation rate is $0.05\text{ }\mu\text{L min}^{-1}$ for all devices. The first 20 minutes show a clear non-linear behaviour, this corresponds to evaporation of water that has adhered to the surface of the device when filling and potentially evaporation from the inlet channel. The linear range, where the evaporation rate is calculated, corresponds to evaporation from the storage paper matrix. This means that on average it takes approximately 2 hours for the devices to dry.

Mass spectrometry analysis

All samples (artificial phytohormones and PHM in liquid form stored in fridge and freezer as controls; PHM dried in paper in a time series; sap samples dried in paper in a time series; destructive sap samples stored in liquid form; and sap samples collected using paired sampling with a destructive method and the sampling device stored on paper) were analyzed using UHPLC-QQQ-MS/MS.

Extraction of the sap samples from the storage paper was done using the following protocol. $400\text{ }\mu\text{L}$ ice cold 60% acetonitrile (ACN) and $2\text{ }\mu\text{L}$ of isotopically labeled internal standard mix was added to the samples followed by shaking in mixer mill at 30 Hz for 10 min. The samples were then centrifuged at 14 000 rpm for 10 min at $4\text{ }^{\circ}\text{C}$. After centrifugation, $350\text{ }\mu\text{L}$ of the supernatant was transferred to new vials and dried using speedvac at $40\text{ }^{\circ}\text{C}$ for 1 h, followed by room temperature for 1 h and storage overnight at $-70\text{ }^{\circ}\text{C}$. The samples were dissolved in $43\text{ }\mu\text{L}$ 20% acetonitrile and $10\text{ }\mu\text{L}$ was analyzed with UHPLC-QQQ-MS/MS, a 1290 Infinity Binary LC System coupled to a 6495 Triple Quad LC/MS System with Jet Stream and Dual Ion Funnel technologies (Agilent Technologies). Metabolite separation was performed with a Acquity UPLC CSH C18 RP column ($150 \times 2.1\text{ mm}$, particle size of $1.7\text{ }\mu\text{m}$), with a column temperature of $40\text{ }^{\circ}\text{C}$, and a flow rate of 0.25 mL min^{-1} . The mobile phases were A: 0.01% formic acid in Milli-Q H₂O, and B: 0.01% formic acid in 100% acetonitrile. The quantification was carried out in Agilent MassHunter Workstation Software Quantitative (Agilent Technologies). Solvents used: methanol, HPLC-grade was obtained from Fischer Scientific, and acetonitrile,

hypergrade for LC-MS LiChrosolv was obtained from Merck.²⁸

Results and discussion

Rapid low-invasive microsampling

The microfluidic sampling device, Fig. 1A, enables easy and rapid collection of plant sap directly from the stem of tomato plants. We collected samples using more than 50 devices in total, with a collection time $<1\text{ min}$ per device. The device is designed to collect a total of $8\text{ }\mu\text{L}$ of liquid, determined by the geometry of the device and absorbance of the selected paper matrix, Fig. 1B and C shows the device layers and 3D view of the geometries. The filling time and collected volume can vary depending on several factors, for example: plant size, texture, and the selected sampling location.

The storage paper holds a maximum of $7\text{ }\mu\text{L}$, the remaining $0.5\text{ }\mu\text{L}$ is the volume of the needle/capillary channel and an additional $0.5\text{ }\mu\text{L}$ which collects around the edges of the storage paper in the collection chamber. The volume collected in the storage paper can be visually determined by observing the liquid filling and the corresponding colour shift in the wetted paper matrix. If the sampled plant does not have a lot of readily available sap, enabling a fully filled device, the visual indication of the colour shift can be used to determine the collected volume. The accuracy of the device was determined using gravimetric measurements combined with visual observation of volume filling, Fig. 1D and E. At volumes above $5\text{ }\mu\text{L}$ the device reaches saturation, meaning that the collected volumes cannot be reliably separated from one another. At lower volumes the spread through wetting of the paper can cause greater spans of visually observed filling for the same volume. To account for these variations, a min/max approach has been taken for the analysis where the curve presented in Fig. 1D has been used to define a max, min, and average volume collected, Fig. S2 presents the detected phytohormonal concentrations with error bars indicating the max and min concentrations in this range. Additionally, a gravimetric evaluation was conducted through collection of sap visually indicating 100% filling, *i.e.* a volume range of $4.5\text{--}8\text{ }\mu\text{L}$. By gravimetric evaluation of the collected mass, it could be determined that the average mass collected was 5.4 mg , with a standard deviation of 0.87 mg . This corresponds to a coefficient of variation of approximately 16%, Fig. 1E. For the pipetted volumes the CV% varies from approximately 30% for the greatest distributions at $1\text{--}2\text{ }\mu\text{L}$ down to 0% for the largest volumes that are always accurately recorded as fully filled, Fig. S3.

Phytohormones are stable when dried in paper

To evaluate the phytohormonal stability over time, artificial phytohormones of known concentrations were evaluated side by side with plant sap samples collected using the microfluidic device. Three tomato plants were also sampled using a destructive stem severing method as a control, stored



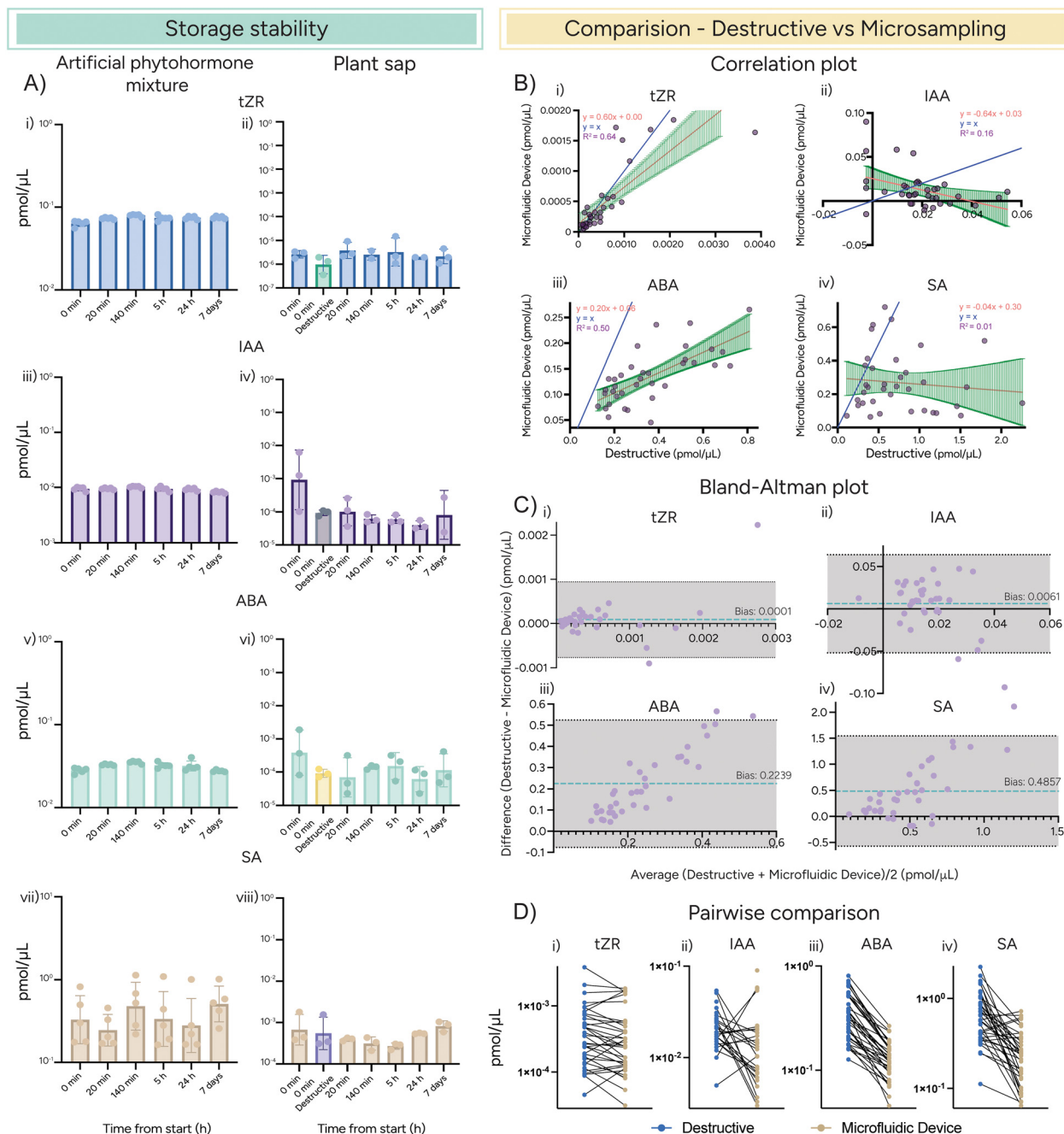


Fig. 2 Phytohormonal storage stability on paper and sample concentrations. A) Phytohormone concentrations over time in the artificial PHM (i, iii, v, vii) and in plant sap samples extracted with the microfluidic device as well as destructive samples collected at time point 0 min using a stem severing method and stored in liquid form (ii, iv, vi, viii). B) Correlation plots showing the linear regression and 95% CI (in green) for the paired microfluidic device and destructive samples. C) A difference vs. average Bland-Altman analysis plot for each of the evaluated phytohormones for microfluidic vs. destructive samples. D) Paired destructive and microfluidic samples, the lines indicate the destructive and device sample originating from the same plant.

in liquid form. The collection papers in which the phytohormone mixture (PHM) or plant sap from the device sampling was collected, were left at room temperature for drying for 0 min, 20 min, 140 min, 5 h, 24 h and 7 days. Fig. 2A shows the detected concentrations over time. The phytohormone JA could be detected in the PHM but was below limit of detection in the device plant sap samples and

was thus excluded from further analysis. The analytes extracted and evaluated using the device have differing hydrophilicities. However, a more directed evaluation is required to determine matrix effects and potential binding selectivity to certain compounds in the device and how elution protocols could affect the detected concentrations. The individually detected phytohormones in the PHM,



Fig. 2A (left), are all stable over time, with no major concentration reductions seen even after 7 days. MS/MS analysis enabled reliable detection of phytohormones in sap samples at low attomole concentrations, Fig. 2A (right). These samples generally presented more variability, both over time and between replicates compared to the PHM samples, thus pointing to variations between plant individuals and/or displayed variations in the accuracy of the microfluidic sampler.

The destructive samples collected from tomato plants through centrifugation at time point 0 min presented concentrations similar to the device samples. Although the 0 min destructive samples do not always display similar concentrations as the 0 min device sample counterpart for all the phytohormones, the destructive samples remain within the expected range when looking at all samples combined.

The phytohormone tZR, Fig. 2Ai and ii, is relatively stable over time, with no major deviations observed over the evaluated days, indicating that the phytohormone can be reliably dried and stored in paper.

IAA present a large drop in concentration after the first 20 min in the plant sap sample, Fig. 2Aiv, this can in part be attributed to the high variability of the concentrations at sampling time 0 min. The distribution of IAA concentrations are similar from 140 min to 24 h for all triplicates. However, the greater variability compared at time points 0 min and 20 min, indicates that the IAA concentrations vary quite a lot between individual plant samples. Since the same effect cannot be seen in the artificial PHM, Fig. 2Aiii, this could be attributed to individual differences between plants or potentially enzymatic effects in the plant sap, degrading the endogenous plant sample while not showing the same effects in the artificial counterpart. Since the drying time for the devices is approximately 2 hours, this correlate with the observation that IAA concentrations vary more before this timepoint and is reduced after when the enzymatic effect should be lower.

ABA concentrations show a main deviating sample at time point 0 min for the plant sap samples, Fig. 2Avi. There is variability seen in most ABA sap samples, but they are quite consistent although more dispersed than in the PHM samples.

SA shows more fluctuating concentrations in the sap samples, and the increase at 24 h and 7 days indicates variability in the stability of the phytohormone and/or individual differences between plants, Fig. 2Aviii. In the PHM, differences can be observed for SA that indicate issues with fully dissolving the phytohormone, leading to greater variations between samples, Fig. 2Avii.

Device accuracy – comparing destructive and device sampling

To evaluate the accuracy of the device, an experimental setup was established with paired sampling of the same plant using both the device and a destructive centrifugation approach, Fig. 1G.

36 plants were sampled over four days. First, the microfluidic sampling device was used, followed by cutting a piece of the plant close to the microdevice sampling site to keep the samples as similar as possible. By obtaining a paired measurement from the same plant using both these methods, we could compare the sampling methodologies and evaluate the accuracy of the sampler. In the pairwise comparison, Fig. 2D, the destructive samples are generally higher in concentration compared to the microfluidic device samples.

For tZR, high concentrations of the destructive samples correspond with high concentrations from the microsampler and *vice versa*, with similar values across both methods, Fig. 2Di. IAA show greater variability for the sampling methods and not a clear pattern between the high vs. low measurements from the two approaches, Fig. 2Dii. ABA display a clear difference where destructive samples are consistently higher in concentration than their microfluidic counterpart, Fig. 2Diii. However, the pairs correlate with high destructive sample concentrations pairing with the higher device concentrations. The variability of the SA samples is greater than that of the ABA and tZR samples, and more similar to IAA. The destructive samples are generally higher in concentration than the microfluidic device samples but the correlation between high and low values between the sampling approaches is not as prominent as for ABA, Fig. 2Div.

To further compare the two sampling procedures, a Bland Altman analysis and a linear regression correlation analysis were conducted, Fig. 2B and C. The Bland–Altman analysis was conducted through a pairwise comparison of the destructive method as control with the microfluidic device sampling approach as the comparative method. The destructive and device samples were taken as pairs, therefore the comparison reduce potential errors that can arise from two different individuals and rather only evaluates the sampling approach, Fig. 2C.

The Bland–Altman analysis clearly display that both ABA and SA show a bias in favour of the destructive method, as was also observed in Fig. 2D where the concentrations from the destructive sampling are generally higher than the device sample concentrations. However, the samples (except for two points for ABA and one for SA) are within the 95% CI in Fig. 2Ciii and iv. With the knowledge of the under-representation of the device for these phytohormones, appropriate adjustments could be made to create a standard curve for determination of the concentrations from the microfluidic device. In Fig. 2B, the correlation analysis show ABA samples following a linear distribution ($p < 0.0001$), with no evidence of deviation from linearity (runs test $p = 0.44$), compared to SA where no clear correlation ($p = 0.545$) can be seen. For tZR the linear regression analysis revealed a linear relationship between destructive and device samples ($p < 0.0001$), and no evidence of deviation from linearity (runs test $p = 0.21$). IAA show a negative linear relationship ($p = 0.014$), however a low R^2 indicates weak associations,



this introduces complexity in attempting to create a conversion factor between the two approaches, Fig. 2Ci and ii. Thus, ABA and tZR appear to be the two phytohormones that provide the most accurate concentrations from the microfluidic sampler when compared to the destructive sampling approach.

The degree of similarity when comparing the destructive stem severing approach to the microfluidic sampler, varies between the tested phytohormones. Some phytohormonal concentrations are underrepresented in the sampling device (ABA and SA) whereas others show similar concentrations using both approaches (tZR and IAA) but with no clear pattern between high and low concentrations (IAA and SA). The trend observed for all phytohormones excluding IAA, is that their concentrations can be translated between the sampler and the control method to some degree, where tZR and ABA present the most consistent relationships to enable such conversions. To enable a good use of the microfluidic sampler, a conversion factor or standard curve should be developed that can be used to translate a detected concentration in the microfluidic sampler to the concentration as detected in the destructive samples.

Conclusions

We present a microfluidic sampling device that, in a low-invasive manner, can be used to collect and store a sap sample for seven days with minimal phytohormone degradation. The results indicate a possibility of implementing the method of collecting samples in analytical grade paper and drying them as an on-site sampling approach, removing the need for freezing and the specialized shipping associated with such procedures.

The main limitation of the microfluidic sampler is the variability in the collected volume. To address this, modifications to the design are required to increase accuracy in the collected volume and thus the concentrations in the dried paper matrix. For example, a different storage paper could be used that can absorb larger quantities of sap and thereby make clearer distinctions about visual filling of different volumes. The length of the device can be extended to allow for a larger wicking area and thus provide the same benefit as a different material selection. Introducing a metering capability to ensure that a specific volume is always collected could be implemented, with the limitation of biological variance between different plant individuals. Some plant individuals allow for the extraction of large volumes (up to 15 μL) whereas others only allow for 1–2 μL . By having a set metering capability, the variability would be significantly reduced, but the number of samples collected would be reduced as well. Potentially a combination could be envisioned as the best approach where volumes below a certain limit can be visually determined and a metering capability that has a cut-off at the defined highest volume extraction.

Future evaluations should also consider the aspect of in-field sampling in different environments, such as tropical settings or greenhouses with high humidity that would affect the drying process and thereby potentially cause degradation of analytes. The drying time in a laboratory environment is observed to be on average 2 hours. With increasing humidity this can be expected to increase as well. The drying speed of DBS has been shown to depend on the relative humidity (RH) of the surrounding environment, and the analyte distribution is altered based on RH, leading to a reduced homogeneity.²⁹ It can therefore be expected that a similar effect would occur for the sap samples drying in environments with increased RHs. To what extent and what analytes are affected is a highly relevant and natural extension for future validations of this device for usability in real life environments.

The device presents new opportunities for longitudinal monitoring of plants over time, an approach that has had major successes in both health care and patient centric sampling settings for humans. Underlying patterns of disease have been discovered, and new understandings of human physiology and potential inter-individual variability has been gained.^{30,31} Having the same opportunities for measurements in plants could enable a better understanding of temporal changes in the phytohormonal fingerprint, provide a means of environmental monitoring and allow for easy and rapid sample collection in the field for a variety of experimental evaluations.

Author contributions

Ellinor Hedberg: formal analysis, investigation, methodology, validation, visualization, writing – original draft, writing – review & editing. Jaime Sebastián-Azcona: investigation, validation. Virginia Hernandez-Santana: investigation. Federico Ribet: methodology. Antonio Diaz Espejo: resources, investigation. Göran Stemme: conceptualization, supervision. Niclas Roxhed: conceptualization, supervision, writing – review & editing.

Conflicts of interest

EH, FR, GS, NR are inventors of pending patent applications on plant microsampling devices.

Data availability

The data supporting this article has been included as part of the supplementary information (SI). See DOI: <https://doi.org/10.1039/d6lc00201c>.

Acknowledgements

This work was funded by the European Union Horizon 2020 project WATCHPLANT (grant agreement no. 101017899). The authors would like to thank the Swedish national



infrastructure SciLifeLab and access to Swedish Metabolomics Centre, Umeå, Sweden, for carrying out the phytohormonal analyses presented in this paper, and collaborators at Instituto Tecnológico de la Energía (ITE).

Notes and references

- W. H. O. Ernst, *Sci. Total Environ.*, 1995, **176**, 15–24.
- E. Eymar, C. Cadahia, A. Sanchez and D. Lopez-Vela, *Commun. Soil Sci. Plant Anal.*, 2000, **31**, 1225–1248.
- N. Killiny, *Plant Signaling Behav.*, 2019, **14**(8), DOI: [10.1080/15592324.2019.1618181](https://doi.org/10.1080/15592324.2019.1618181).
- A. Santner, L. I. A. Calderon-Villalobos and M. Estelle, *Nat. Chem. Biol.*, 2009, **5**, 301–307.
- H. K. Kim and R. Verpoorte, *Phytochem. Anal.*, 2009, **21**, 4–13.
- M. Farneselli, E. H. Simonne, D. W. Studstill and F. Tei, *J. Plant Nutr.*, 2006, **29**, 1975–1982.
- W. F. Tjallingii, *J. Exp. Bot.*, 2006, **57**, 739–745.
- Q. Yu, C. Tang, Z. Chen and J. Kuo, *New Phytol.*, 1999, **143**, 299–304.
- M. Flajšman, S. Mandelc, S. Radišek and B. Javornik, *Bio-Protoc.*, 2017, **7**, e2172.
- J. López-Portillo, F. W. Ewers, R. Méndez-Alonzo, C. L. Paredes López, G. Angeles, A. L. Alarcón Jiménez, A. L. Lara Domínguez and M. del Carmen Torres Barrera, *Am. J. Bot.*, 2014, **101**, 1013–1022.
- N. A. ElNaker, M. Daou, M. A. Ochsenkühn, S. A. Amin, A. F. Yousef and L. F. Yousef, *Sci. Rep.*, 2021, **11**, 22679.
- E. Karakas, P. Ferrante, R. Schafleitner, G. Giuliano, A. R. Fernie and S. Alseekh, *Curr. Protoc.*, 2023, **3**, e952.
- R. Powers and E. Riekeberg, *F1000Research*, 2017, **6**, 1148.
- M. A. Salem, L. P. D. Souza, A. Serag, A. R. Fernie, M. A. Farag, S. M. Ezzat and S. Alseekh, *Metabolites*, 2020, **10**(1), 37.
- L. Fernandes, S. Casal, J. A. Pereira, J. A. Saraiva and E. Ramalhosa, *Braz. J. Food Technol.*, 2018, **21**, e2017211.
- R. Drapron, *Properties of Water in Foods: in Relation to Quality and Stability*, Springer, 1985, pp. 171–190.
- C. P. Sherwin and T. P. Labuza, *Water properties of food, pharmaceutical, and biological materials*, 2006, pp. 343–362.
- N. Grüner, O. Stambouli and R. S. Ross, *J. Visualized Exp.*, 2015, (97), 52619.
- B. S. Frey, D. E. Damon and A. K. Badu-Tawiah, *Mass Spectrom. Rev.*, 2020, **39**, 336–370.
- J. Björkesten, S. Enroth, Q. Shen, L. Wik, D. M. Hougaard, A. S. Cohen, L. Sörensen, V. Giedraitis, M. Ingelsson, A. Larsson, M. Kamali-Moghaddam and U. Landegren, *Mol. Cell. Proteomics*, 2017, **16**, 1286–1296.
- N. Grecsó, A. Zádori, I. Szécsi, Á. Baráth, Z. Galla, C. Bereczki and P. Monostori, *PLoS One*, 2021, **16**(5), e0252091.
- K. Li, J. C. Naviaux, J. M. Monk, L. Wang and R. K. Naviaux, *Metabolites*, 2020, **10**(3), 82.
- G. Lenk, S. Sandkvist, A. Pohanka, G. Stemme, O. Beck and N. Roxhed, *Bioanalysis*, 2015, **7**, 2085–2094.
- J. Hauser, G. Lenk, J. Hansson, O. Beck, G. Stemme and N. Roxhed, *Anal. Chem.*, 2018, **90**, 13393–13399.
- J. Hauser, G. Lenk, S. Ullah, O. Beck, G. Stemme and N. Roxhed, *Anal. Chem.*, 2019, **91**, 7125–7130.
- J. Hauser, G. Kylberg, M. Colomb-Delsuc, G. Stemme, I.-M. Sintorn and N. Roxhed, *Lab Chip*, 2020, **20**, 4186–4193.
- J. Hauser, M. Dale, O. Beck, J. M. Schwenk, G. Stemme, C. Fredolini and N. Roxhed, *Anal. Chem.*, 2022, **95**, 1350–1358.
- J. Šimura, I. Antoniadi, J. Široká, D. e. Tarkowská, M. Strnad, K. Ljung and O. Novák, *Plant Physiol.*, 2018, **177**, 476–489.
- G. Lenk, J. Hansson, O. Beck and N. Roxhed, *Bioanalysis*, 2015, **7**, 1977–1985.
- X. Shen, R. Kellogg, D. J. Panyard, N. Bararpour, K. E. Castillo, B. Lee-McMullen, A. Delfarah, J. Ubellacker, S. Ahadi and Y. Rosenberg-Hasson, *et al.*, *Nat. Biomed. Eng.*, 2024, **8**, 11–29.
- S. M. Schüssler-Fiorenza Rose, K. Contrepolis, K. J. Moneghetti, W. Zhou, T. Mishra, S. Mataraso, O. Dagan-Rosenfeld, A. B. Ganz, J. Dunn and D. Hornburg, *et al.*, *Nat. Med.*, 2019, **25**, 792–804.

