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Wearable device for in-situ plant sap analysis: Electrochemical lateral flow (eLF) for stress monitoring in living plants

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ABSTRACT

Smart agriculture and environmental monitoring claim innovative wearable sensing technologies suitable for real-time, *in-situ* biochemical analysis for non-specialized users in plants. Current strategies measure physical parameters, ions or hormones by amperometry or potentiometry. Among these, plant hormones serve as stress biomarkers due to their role in stress response mechanisms. While electrocatalysis has been explored for their detection, early-stage stress monitoring at low concentrations demands higher selectivity and specificity. Therefore, new strategies integrating biorecognition elements, such as antibodies, with autonomous sample collection and bioassay performance are required. In this regard, this work proposes a novel wearable immunosensor device based on an electrochemical lateral flow assay (eLF) that includes an autonomous microsampling technology for minimally invasive *in-situ* sap extraction and abscisic acid (ABA) detection. This sap device collects, processes and analyzes plant sap with low sample volume (<10 μL) and short assay time (9min) using immunosensing for the first time in ABA wearable detection. Validation in drought-stressed cucumber plants demonstrated 78 % sensitivity and 71 % specificity in detecting subtle water stress with 77 % accuracy. These findings highlight the potential of this plant-wearable biosensor for early stress detection and its versatility to be adapted for the detection of other relevant molecules (proteins or DNA), key for smart agriculture and environmental monitoring.

1. Introduction

Smart agriculture or environmental monitoring fields demand the development of wearable sensing technologies capable of being adapted to real-time and *in-situ* biochemical analysis in any plant by non-specialized users. Wearable sensors have emerged as invaluable tools for personal monitoring, offering significant benefits due to their high sensitivity, specificity, and rapid response. Combining point-of-care (POC) devices with mobile connectivity in autonomous operation, they provide real-time data to enable early-stage actions (Ates et al., 2022). In this field, physical activity monitoring has reached society with several examples such as heart rate or temperature sensing using smartwatches. Minimal or non-invasive monitoring of biochemical parameters in biofluids constitutes the challenge in the generation of wearable devices (Ates et al., 2022). Some examples in the clinical field are facing this challenge successfully, such as the FreeStyle sensors for real-time glucose measurement in interstitial fluid (Jennewine, 2024). However, for vegetal organisms, very few options have arisen for biochemical analysis so far.

Currently, plant phenotyping is the most common approach to detect plant issues in precision agriculture. This involves mostly the use of images treated by AI, piezoresistive or capacitance sensors placed in the leaves or fruits to detect elongation, bioelectric potential, water content, or temperature (Chen et al., 2023; Zhang et al., 2023). These approaches are used to detect stress, growth or microclimate conditions in the plant and/or the environment, to indirectly inform about plant health by using first generation of wearable sensors (Li et al., 2024). However, to truly benefit smart agriculture, advances in wearable plant sensors require efficient and proactive farming practices (Zhang et al., 2023) that enable the detection of stress factors before visible effects appear,

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reducing the negative consequences in agri-food industry and human health (Li et al., 2021).

In this regard, the second generation of wearable sensors for bio-based analysis of the biochemical components directly in sap emerge as a solution. Several sap molecules have been depicted as stress biomarkers, such as reactive oxygen species (ROS) or phytohormones (Coatsworth et al., 2023). Among those, the phytohormone abscisic acid (ABA) is a key stress biomarker, since it is involved in multiple biochemical regulation routes against abiotic and biotic stresses that trigger physiological modifications in plants (Jones, 2016). For this reason, monitoring directly ABA *in-vivo* and real-time constitutes a relevant aspect to achieve a radically new generation of wearable sensing tools for environmental intelligence and smart farming. Nonetheless, despite the valuable information provided, access to sap in a non-invasive way remains challenging and requires the development of new wearable biosensors (Bukhamsin et al., 2022; Lee et al., 2024).

Current methods to analyze sap content are based on ELISA or mass spectrometry. Those analytical techniques are robust and reliable, but require high-size equipment, specialized personnel and sample processing. These features hinder their delocalization from specialized laboratories. As portable and easy-to-use alternatives, biosensors for ABA analysis have appeared, such as the one based on fluorescence developed by Waadt et al. and Jones et al., designed to analyze ABA from plant's extracts or *in-vivo* by using labeled proteins in the plant, transgenic plants or radioactive chemicals (Jones et al., 2014; Waadt et al., 2014). Nonetheless, although those strategies are very interesting to understand ABA mechanisms, they are far from being suitable for wearable sensing in a crop, contrary to electrochemical biosensors. Electrochemical techniques are commonly used for the development of POC or wearable biosensors due to their sensitivity, low-cost and portable equipment, easy-to-use protocols and their compatibility with micro and nanotechnologies. These features make electrochemical biosensors a suitable option for ABA wearable sensors.

In this sense, Yardım, 2011 developed a sensor based on a bismuth-film electrode for the direct detection of ABA by voltammetry stripping analysis with a limit-of-detection (LOD) of 0.209 μM. Likewise, Wang et al., 2021 developed a direct biosensor for ABA by using microelectrodes modified with Au@SnO_2 and vertical graphene almost 100 times more sensitive than the biosensor created by Yardim et al. Although direct detection methods are convenient for continuous monitoring of ABA, specificity could be compromised by interferents in untreated sap samples. Moreover, those assays are only suitable for the detection of electroactive molecules within a specific potential range, so they cannot be adapted to any target of interest. For these reasons, immunosensing assays appear as a more convenient alternative for phytohormone portable sensing, due to its higher specificity sensitivity and selectivity (Li et al., 2003, 2021), crucial features for precision agriculture. However, all these POC require several steps of human intervention, either in sample extraction, treatment or assay performance, that hinder their applicability since it could lead to false results and increase the duration of the assay.

To address these limitations, paper-based needles combined with electrochemical lateral flow assay (eLF) offers a promising solution. Lateral flow assays (LFAs) can incorporate the additional reagents necessary for specific target detection, enhancing the functionality of the biosensors in terms of sample treatment, sensitivity and selectivity (Brazaca et al., 2024; Hasan et al., 2023) whilst electrochemistry provides higher sensitivity than optical LFAs. There are many examples of eLF that have successfully proven detection of key organic molecules, proteins or RNA analysis in the security, environmental and clinical fields, showing promising results for portable electrochemical biosensors (Deenin et al., 2023; Dortez et al., 2024; Nandhakumar et al., 2023; Raucci et al., 2024). Recent works also include electrophoretic approaches into the paper to provide specific detection in eLF devices (Panferov et al., 2023). Nonetheless, those electrochemical lateral flow devices are generally non-autonomous in both sample extraction and assay performance, so sample handling and addition is still required by the end user. This could be overcome by integrating automatic sampling mechanisms into the eLF assay.

Sampling techniques have been mainly explored in medical applications for POC or patient-centric settings (Li et al., 2015; Ribet et al., 2023). Such technologies have been improved by using low sample volume and extraction methods that do not require specialized personnel and can therefore be conducted in hard-to-reach areas and with none, or very little, special equipment. Moreover, most of the devices sample in a manner that causes very little or no pain to the individual (Faraji Rad, 2023; Faraji Rad et al., 2021; Lei and Prow, 2019). Recent studies have emerged to apply these technologies for plant and crop health evaluations to achieve the requirements of precise and smart agriculture (Faraji Rad, 2023). One such technology describing a system for plant sap sampling is a dragonfly-like micro-sampling device presented by Gao, 2019. The device has the potential to extract xylem sap from plant stems to enable nutritional monitoring. Another technology focusing on microneedle-based systems for plant sap extraction is the phloem/xylem extraction and storage device presented by Shikata et al. that use microprobes combined with sensors to locate phloem and xylem vessels respectively using electrical conductivity (Shikata et al., 2019). Building from these technologies presented in both medical and agricultural applications, it is possible to design a sample extraction strategy with the capability of extracting plant sap in a minimally invasive and autonomous manner through capillary action, feasible to be combined with an eLF device.

Therefore, in response to the above, this work proposes a wearable sensor for the biochemical *in-situ* analysis of sap in living plants using antibodies-based assay for the first time in a wearable biosensor for plants. This includes both autonomous sample collection and assay performance using eLF immunosensing for ABA detection. Due to its compatibility with wireless technology, it can be easily connected to other components, thus enabling it to be integrated into the IoT trend and adapted to any other molecule such as proteins or DNA/RNA (Hasan et al., 2023). As far as we know, it represents the first immunosensor wearable device for plants capable of *in-situ* analysis of the biochemical content of the plant. In this work, ABA detection was addressed due to its relevance for smart agriculture using an eLF combined with a microsampling device for sap extraction which led to minimal human intervention for *in-situ* biochemical analysis of sap using low sample volumes (<10 μL) with a very short analysis time (9 min) for non-specialized users. This technology is easily adapted to the specific monitoring of other complex molecules autonomously and compatible with multiplexing. Thus, the eLF proposed enhances the ability to monitor plant health and contributes to the development of smart agricultural practices and environmental monitoring, ultimately leading to improved crop management, productivity and air quality monitoring.

2. Experimental

2.1. Materials

Aniline and N-Hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. N-Etil-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) was purchased from Iris Biotech GMBH. Mouse anti-abscisic acid monoclonal antibody (Cat. No. MBS7041609) was purchased from bioNova científica S.L. (+)-cis, trans-Abscisic acid (Cat. No. A-1103) was purchased from AG Scientific. HRP Conjugation Kit was purchased from Abcam. Bovine Serum Albumin (BSA) was purchased from Fisher Scientific. Hydrogen peroxide (H_2O_2) was purchased from Labkem. Hydroquinone (HQ) was purchased from Merck. Sulfuric acid (H_2SO_4) was purchased from Sigma-Aldrich. All the solutions were prepared in Milli-Q water (Millipore system with 18.2 MΩ cm at 25 °C).

2.2. Screen printed carbon electrode (SPCE) fabrication

SPCE were fabricated by using a semi-automatic screen-printed equipment from CROMA. Electrodes were prepared layer-by-layer by using silver paste for connections; carbon paste for the working and counter electrodes; Ag/AgCl paste for the reference electrode; and dielectric ink for insulation. All the inks were purchased from Sun-Chemical. The electrodes were printed on flexible PET from Puetz Folien.

2.3. Microsampling device fabrication

The microsampling device was fabricated from hydrophilic plastic sheets (Xerox type C laser printing transparency, Xerox, Elmstock UK), double sided adhesive (3 M, VWR, Spaånga, Sweden) with thickness 50 μm and 601/N filter paper (Qualitative Grade 601/N Filter Papers, Sartorius, Germany). The components were designed in Adobe Illustrator and cut from the corresponding materials using a CO_2 laser (VSL 2.3, Universal Laser Inc.).

2.4. Sap device

The sap device comprised a competitive ABA assay based on electrochemical lateral flow (eLF). The lateral flow assay was comprised of glass fiber conjugate pad (CP, GFCP103000) and cellulose fiber sample pad (SP, CFSP173000) from Merck. The latter was only included in the assays where the sap samples were added manually. CP was modified by adding 175 μg/mL Ab anti-ABA modified with HRP (modification was performed following the protocol from the HRP conjugation kit from Abcam). SP was blocked by adding 10 mg/mL BSA. The sap devices with autonomous sample extraction did not include SP, but the microsampling device coupled with the chromatography paper. The chromatography paper grade 238 from Ahlstrom was modified with 45 mM HQ and dried at room temperature. Then, it was protected by a thin layer of soluble wax barrier.

SPCEs for the electrochemical assay were modified with polyaniline (PANI) by electropolymerization of 0.1 M aniline in 0.5 M H_2SO_4 by applying 10 μA/cm^2 for 10 min. Then, the electrodes were washed with ultrapure water and the PANI-SPCE were incubated in an aqueous solution containing 32 μg/mL ABA, 0.2 M EDC and 0.05 M NHS for 2 h at room temperature. The electrodes were washed with ultrapure water and dried. After the immobilization of ABA, the exposed polymeric surface was blocked by the incubation of the electrodes in 1 mg/mL BSA for 30 min at room temperature. After that, the electrodes were washed with ultrapure water and assembled with the microsampling device, SP, CP and chromatography paper to obtain the eLF strip, as depicted in Fig. 1, for its use.

2.5. Electrochemical measurements

Electrochemical measurements were performed on a PalmSens 4 and Sensit BT portable potentiostat from Palmsens. Modification of SPCE was characterized by electrochemical impedance spectroscopy (EIS) from 100 kHz to 0.1 Hz in 5 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M KCl at the formal potential of the redox couple (~ 0.17 V) and 10 mV amplitude. ABA detection was performed by chronoamperometry measurements applying −0.2 V for 400 s in 1 mM HQ and adding 0.1 M H_2O_2 after 30 s. Variation in those concentrations are depicted throughout the text. OCP value was verified to be stable (0.05 V–0.09 V) before initiating the measurement at the PSTrace 5.9 Palmsens software display.

2.6. UHPLC-MS/MS analysis

Stem samples from cucumber plants were collected during the drought experiment. Vegetal tissue was frozen. The protocol adapted from Perin et al. (2018) was used. Briefly, 100 mg of frozen sample was used and extracted by adding 1 mL of methanol (80 % v/v). Then, the sample was vortexed (30 s) and sonicated (15 min). Afterwards, samples were centrifuged at 12,000 rpm for 5 min and the supernatant was filtered through a nylon membrane filter (0.22 μm) before injection in

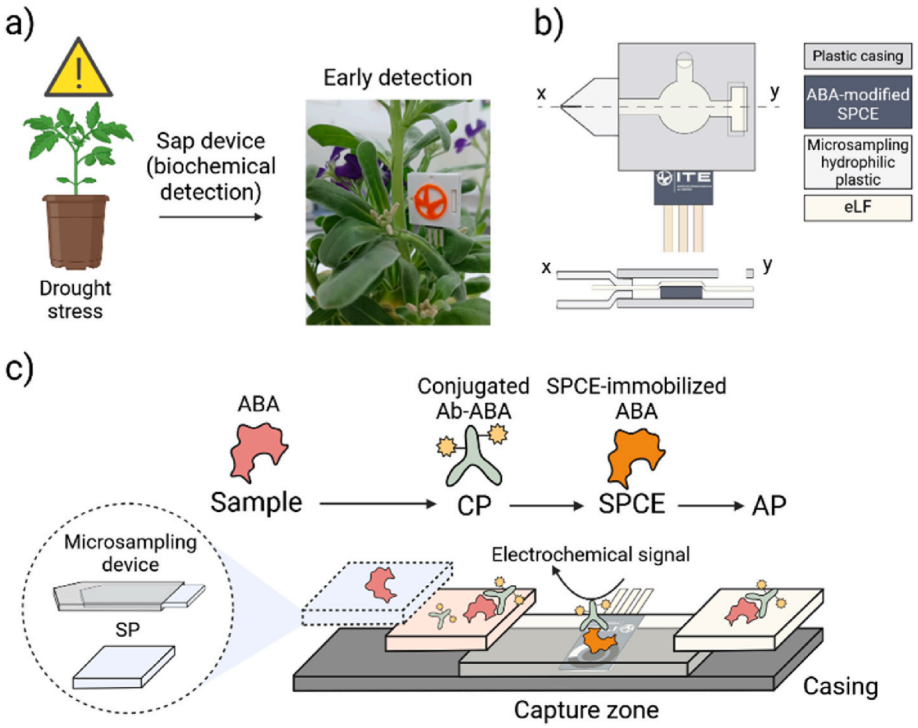

Fig. 1. a) Sap device inserted into a plant stem. b) Scheme of the interior of the sap device (top) and cross-section of the device (bottom). c) Schematic representation of the early-stage stress detection by the competitive eLF ABA assay. On the right side it is depicted the components of the eLF assay, wherein SP is sample pad; CP is conjugate pad; SPCE is screen-printed carbon electrode; and AP is absorption pad.

the UHPLC-MS/MS.

For the ABA detection, a UHPLC-MS/MS analysis was performed using a QTrap 6500 plus system (Sciex, USA) with a C18 Kinetex column (2.1 × 100 mm, 1.6 μm) at 35 °C and a 0.4 mL/min flow rate. The mobile phase was a gradient of water and acetonitrile (both with 0.1 % formic acid). Electrospray ionization (ESI) was used in positive and negative modes at 400 °C and 5000 V (−4500 V), with a cycle time of 1 s. Transitions were monitored in MRM mode with compound-specific parameters.

3. Results and discussion

3.1. In-situ sap extraction

The microsampling device is created from sequential stacking and layering of hydrophilic plastic sheets with a double-sided adhesive in between (Fig. 1b). The tape layer determines the height of the capillary channel (50 nm) as well as the inlet needle and collection chamber, which are all patterned onto the tape layer. The device inlet needle channel has dimensions (1 mm × 1.2 mm), with a curved tip with a radius of (0.5 mm). The curved tip enables puncturing of the plant stem but reduces the risk of clogging of the inlet channel with plant tissue remnants. The collection chamber is a paper matrix of size (2 mm × 3 mm) that connects the microsampling device to the ABA sensor components and enables sap flow to the corresponding areas (Fig. 1b). The paper ensured that the sap would have a clear flow path and allows for wetting of the matrix and flow to the downstream components.

3.2. Electrochemical ABA POC biosensing assay

The ABA assay consisted of a competitive assay based on the use of HRP-labeled ABA antibody as redox reporter added to the sample and an ABA-modified SPCE. Thus, the amount of ABA in the sample is reciprocal to the current generated due to HRP redox reaction. SPCE was modified as described in the experimental section and characterized by EIS (Fig. 2a). The polymerization with PANI decreased the impedance of the electrode almost 20 times. Compared to PANI electrode, ABA immobilization decreases impedance too due to the charge variation of the electrode surface. Then, the modified electrode was incubated with the blocking solution that increased the impedance of the surface. After those steps, the ABA electrode for the assay was obtained.

To analyze the sensitivity of the assay, ABA samples containing 1, 10 and 1000 ng mL^{-1} ABA in PBS pH 6.5 were prepared. Prior to the incubation on the modified SPCE, 50 μg/mL of HRP-Ab ABA was added to the samples and incubated for 30 min at room temperature. After this pre-incubation, modified SPCEs were incubated with the samples for 30 min at room temperature, wherein the remaining free HRP-Ab ABA molecules would interact with the ABA molecules immobilized on the electrode surface. After the incubation, electrodes were washed with ultrapure water and the redox reaction from HRP immobilized on the surface was measured. The current difference within the initial value and after the reported reaction was used as an analytical signal, wherein the higher the difference, the lower the ABA concentration in the sample, as observed in the results from Fig. 2b.

After the sensitiveness characterization, the specificity and the potential of the assay to discern between healthy and stressed conditions was analyzed in sap samples from control and stressed plants (Fig. 2c). It was measured 6 control and 4 stressed samples and the results evidenced higher variation in the control than in the stressed ones (Fig. 2d), suggesting lower ABA concentration in the control plants than in the stressed ones, as expected. Thus, the results demonstrated the success of the qualitative ABA assay developed.

3.3. eLF ABA assay

After the development of the ABA POC assay, the electrochemical assay was assembled to the chromatography paper to reduce the steps and the human interventions. Electrochemical ABA assay and

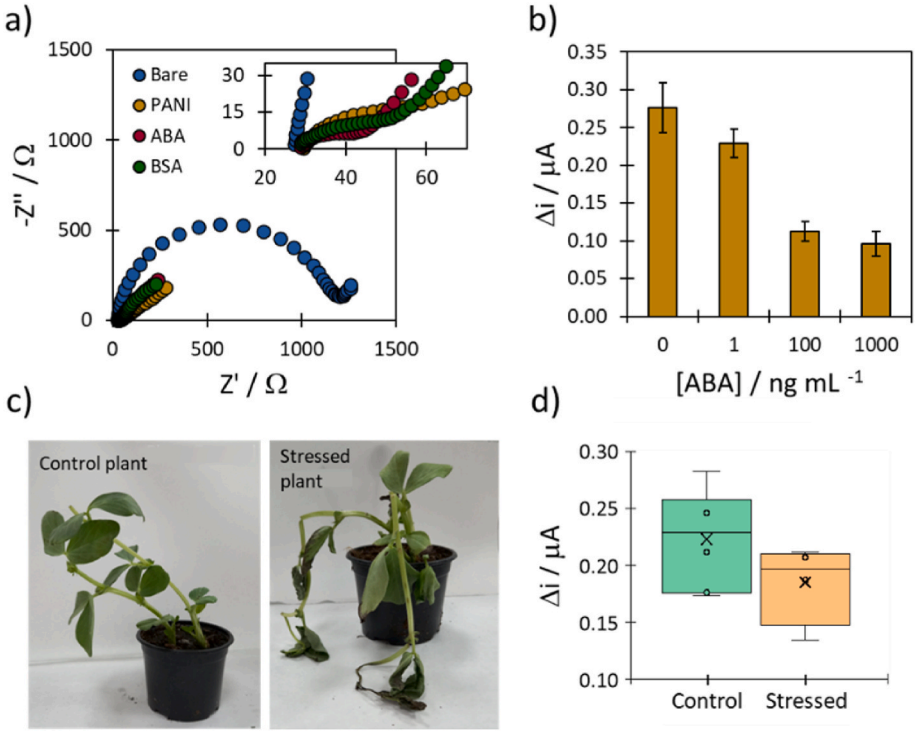

Fig. 2. Electrochemical competitive ABA assay. a) EIS measurements for the characterization of SPCE modifications. b) Standard curve in the 1–1000 ng mL^{-1} ABA concentration range. The error bars represent the standard error obtained from independent assays (n > 3). c) Example of a control (on the left) and stressed plant (on the right) from which the sap sample were collected. b) Box plot of the current variation of the Ab-HRP ABA assay from control (n = 6) and stressed (n = 4) plant samples.

chromatography paper composed the electrochemical lateral flow (eLF) assay, wherein modified SPCE was used for detecting electrochemically ABA and lateral flow was to automatize the biosensing procedure by sample and electrochemical reagents transportation to the electrode surface for the electrochemical reaction to occur.

SP and CP were modified as depicted in the experimental section. Chromatography paper was modified with the HQ close to the location of the SPCE (capture zone in Fig. 1c) and protected with a soluble wax barrier. Modified SPCE was assembled underneath the chromatography paper and the device was sealed to avoid sample evaporation throughout the strip. Once the sample is added to the SP, it passes through the CP where the HRP-Ab ABA interacts with the ABA molecules present in the sample. The sample/HRP-Ab ABA solution reaches the capture zone where the free HRP-Ab ABA molecules interact with the ABA on the SPCE surface. The solution dissolves the wax barrier, releasing the HQ. After that, the H_2O_2 is added to the device triggering the redox reaction of the HRP molecules immobilized on the SPCE surface.

The experimental procedure of the eLF ABA assay was optimized by analyzing the minimal sample volume, H_2O_2 volume and timing of the steps involved in the electrochemical reaction. It was determined that 10 μL of sample was enough to cover the three electrodes from the modified SPCE and to achieve a stable OCP value and starting the measurement (~ 1.5 min). The H_2O_2 volume established to obtain the redox reaction was 3 μL and it was added to the device 30 s after starting the measurement. After the optimization of the protocol, the analytical features of the qualitative eLF ABA assay were analyzed by measuring sap samples from control (n = 6) and stressed (n = 9) plants (Fig. 3). The current variation of the control group was 6.2 ± 1.9 μA, while the stressed sample group's current variation was 1.8 ± 1.0 μA. Moreover, the p-value was <0.001, denoting a statistically different response of the eLF ABA assay within the control and the stressed samples. The cut-off (CO) value to discerned within sample groups was 3.4 μA, determined by $CO = \overline{\Delta i} - 1.5 \cdot SD$, wherein $\overline{\Delta i}$ is the current variation average value of the control samples, and SD is the standard deviation of the control samples. Those results demonstrated the success of the electrochemical lateral flow assay in the analysis of ABA in real samples from control and stress plants.

After the validation of the ABA assay with real sap samples, the long-term performance of the ABA biosensor was analyzed. Biosensors were fabricated, stored at 4 °C and measured several days during storage. The results demonstrated a stable response in terms of the current value of the biosensor 3 days after the fabrication, denoting its viability in real-world applications for agricultural monitoring. Reusability is another important aspect of the applicability of the biosensor, due to its environmental impact. ABA biosensor was designed as a disposable unit due to the nature of the immunosensing assay, which limits the sensor to a single use, as the antibody-analyte interaction is almost irreversible. Nonetheless, the assay was designed to minimize material consumption and residue production by reducing the number of disposable materials, such as the SPCE electrode and the chromatography papers, while enabling the reuse of the plastic-based casing components to produce new biosensors. For future improvements of the device, the possibility of including recycled materials to decrease the footprint of the production and usage of the biosensor developed will be explored.

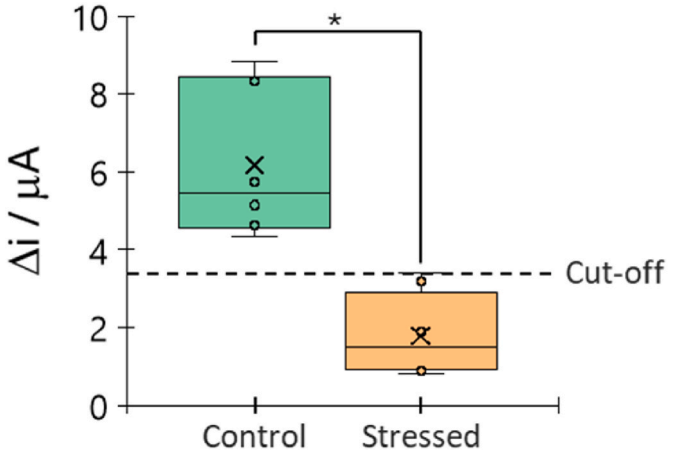

Fig. 3. Box plot of control and stressed samples analyzed by the eLF ABA assay. * denotes p-value <0.001.

3.4. Sap device validation by drought stress detection in cucumber plants

The integration of the eLF qualitative ABA assay with the micro-sampling device developed for sap extraction resulted in the sap device that enabled *in-situ* sap extraction without sample pre-treatment and semi-autonomous ABA detection in the same device, with just one step of human intervention (H_2O_2 addition). The sap device was validated in 5 control and 5 cucumber plants under drought stress (Fig. 4a) and measurements were performed once per week for 3 weeks. The stressed plants were subjected to a three-week period with reduced irrigation in plant growth chambers, receiving only 50 % of the regular watering that the control group received. This approach was designed to induce mild water stress conditions, allowing for the assessment of the wearable biosensor's ability to detect early-stage drought stress through the *in-situ* analysis of ABA levels in plant sap which constitutes one of the most innovative features of the system. Note that few phenotypical differences are observed between the two groups, mostly based on decreased leaf production due to the water deficit.

The data was analyzed by ROC analysis that measures the accuracy of qualitative assays through their sensitivity (Se) and specificity (Sp). Those values are given by $Se = TP/(TP+FN)$ and $Sp = TN/(TN + FP)$, wherein TP is true positive, FN is false negative, TN is true negative, and FP is false positive. The Se and Sp of the assay are calculated in a range of CO values, that results in different numbers of TP, FN, TN and FP. ROC analysis results in a curve that provides the optimal CO value, the Se, the Sp and the accuracy of the assay. The latter is obtained by the area under the curve (AUC), wherein 50 % is related to a random assay, while 100 % represents a perfect assay. The ROC analysis of the Sap device demonstrated 78 % and 71 % of sensitivity and specificity, respectively; and 77 % of accuracy (Fig. 4b). These data denoted that the Sap device discerned the stressed plants with 78 % of accuracy and control plants with 71 % of accuracy. Moreover, ROC analysis determined the optimal CO value to distinguish control and stressed plants, being $CO = \overline{\Delta i} - 0.45 \cdot SD$. Assuming this CO value, the Sap device discriminated against stressed from control plants with statistical relevance (p-value <0.005) with 4 false positive and 3 false negative results (Fig. 4c). Those false results could be a consequence of other factors related to the real environment, such as the influence of other stressful factors, in the case of the false positive results; or the adaptation capacity of the plants to drought, in the case of the false negatives results. Moreover, false negative data could be related to a lower amount of sap, which affected the sap extraction process and the sample volume obtained. Nonetheless, the results obtained in the demonstration in real plants under control stress demonstrated reliable performance due to the high accuracy, sensitivity and specificity obtained.

Moreover, the results from the sap device were compared to a gold-standard analytical technique. The ABA variation in the cucumber plants during the 3 weeks was confirmed by mass spectroscopy by measuring one control and one stressed plant (Table 1). ABA content in the control plant was high at the beginning of the assay but it decreased in the following weeks, denoting a healthy status. Contrary, the ABA levels increased in the plant under drought stress, reaching the highest level in the last week of measurement. This data was compared to the results obtained from the sap device in each sampling week. As it is

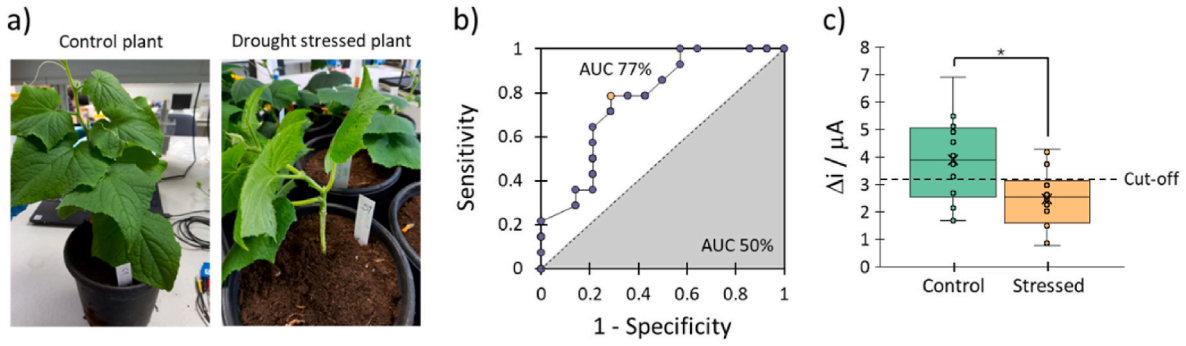

Fig. 4. Sap device validation. a) Example of control and drought-stressed cucumber plants. b) ROC analysis, wherein the grey area denotes the area under the curve (AUC) of a random assay. The orange point represents the optimal CO value and the point to measure the sensitivity and specificity of the assay. c) Box plot of control and stressed cucumber plants analyzed by the sap device. * denotes p-value <0.005. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
ABA concentration from mass spectroscopy analysis compared to the sap device results from one control and one drought stressed plant during the 3 weeks analysis. * denotes a false negative result of the sap device.

Technique	Plant	Week 0	Week 1	Week 2
Mass spectroscopy (ppb)	Control	1.59	N/A	N/A
	Drought stressed	N/A	1.56	5.31
Sap device	Control	Stressed	Healthy	Healthy
	Drought stressed	Healthy	Stressed	**Healthy***

observed in Table 1, the sap device identified correctly the stressed status of the control plant at the beginning of the assay and in the first week. However, it failed in the identification of the stressed plant in week 2, which resulted in a false negative result. Nonetheless, despite this last result, the sap device presented 83 % of correlation with the mass spectroscopy data, demonstrating its capability in measuring ABA and detecting stress in plants.

4. Conclusions

This study reports for the first time *in-situ* analysis of ABA by a novel wearable biosensor integrating an electrochemical lateral flow (eLF) assay with an autonomous microsampling device. The biosensor successfully discerned within healthy and drought-stressed cucumber plants by detecting ABA as a stress biomarker in <10 μL of sap in <10mins. The sap device demonstrated 78 % sensitivity, 71 % specificity, and 77 % accuracy, confirming its potential for early-stage drought stress detection in smart agriculture and environmental monitoring. Building on these promising results, future work will focus on expanding the capabilities of the biosensor prototype to detect other types of relevant molecules (e.g., proteins or nucleic acids) or even, to obtain a multiplex biosensor. Moreover, the use of biodegradable materials for the device casing will be explored to enhance the sustainability of the biosensors. Therefore, these improvements will further position the sap device as a versatile and sustainable tool for precision agriculture and plant health monitoring.

CRediT authorship contribution statement

Beatriz Lucas Garrote: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marta Vegas-García:** Writing – review & editing, Validation, Methodology, Investigation. **Ellinor Hedberg:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Federico Ribet:** Supervision, Methodology, Investigation. **Niclas Roxhed:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Laura García-Carmona:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation. **Alfredo Quijano-López:** Writing – review & editing, Funding acquisition. **Marta García-Pellicer:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare no conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Data availability

The data that has been used is confidential.

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