Characterizing the Responses of sorghum and Camelina to Environmental Stress through a Multi-Modal Approach

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ABSTRACT

Due to their sessile nature, plants are unable to escape environmental stressors such as drought and salinity that can pose a risk to plant health and agricultural productivity. Rapid, non-invasive tools for detecting plant stress responses are essential for optimizing resource efficiency and mitigating the effects of undue stress due to extreme environmental pressures. In this study, we assessed the physiological responses of four-week-old camelina (*Camelina sativa*) and sorghum (*sorghum bicolor*) to chitosan, cold, drought, and salt treatments. Several physiological aspects were measured simultaneously in response to stress exposure, including fluorescence and gas exchange parameters (MultispeQ and LI-6800), tissue electrical impedance with wearable biosensors (Multi-PIP), and biochemical properties via Fourier-transform infrared (FTIR) spectroscopy. Using these tools, we compiled unique profiles for whole plant physiological changes in response to environmental stress, revealing that different parameters were altered on different temporal scales. This finding emphasizes the need for a comprehensive multi-modal approach for rapid and accurate remote sensing of plant health in the field. Within that multi-modal approach, we observed that leaf impedance was rapidly altered in response to treatment and could therefore be leveraged to detect very early signs of plant perturbation. This research demonstrates the utility of a holistic phenotyping approach to inform agricultural strategies aimed at enhancing crop resilience under changing environmental conditions.

Introduction

Abiotic stressors such as drought, salinity, and extreme temperatures are major limiting factors for plant growth and agricultural productivity globally, a challenge that will only intensify with the progression of climate change (Zhu, 2016). These environmental conditions negatively impact physiological processes, ultimately reducing crop yields and increasing plant mortality (Valliyodan and Nguyen, 2006). The detection and management of plant stress are critical to improving agricultural productivity and sustainability through mitigation of environmental damage to preserve crop yields. (Boyer, 1982; Mittler, 2006), but traditional methods of measuring plant stress, such as biomass analysis or biochemical assays, are often time-consuming, invasive, and provide limited insight into dynamic physiological processes (Araus and Cairns, 2014), making them ill-suited for field-based monitoring.

The need for real-time, non-destructive monitoring of plant stress is becoming increasingly relevant with the rise of smart agriculture—a farming approach that leverages sensor technologies, data analytics, and automation to optimize resource use and enhance crop productivity. This has in turn resulted in a range of plant phenotyping methods being developed for rapid, continuous and accurate assessment of plant properties related to health and yield in the field (Jonest and Vaughan, 2010; Zarco-Tejada et al., 2013; Ray et al., 2017). These technologies allow farmers to make informed decisions about irrigation, fertilization, and pest management. However, but many are susceptible to environmental influences due to measuring indirect soil or atmosphere parameters, lack the sensitivity to detect early stages or different types of stress response, or fail to complement other methods, resulting in incomplete assessments of plant physiology (Chaves et al., 2009). Therefore, continuous development, refining, and testing of more sensitive, selective, and compatible smart devices is critical for improving targeted palliative measures to reduce the negative impacts of environmental stress on plant growth and productivity.

Camelina (*Camelina sativa*) and sorghum (*sorghum bicolor*) are two crop species of emerging importance for biofuel production and global food security with relevant to both traditional and novel cropping systems (Kershen, 2013; Li et al., 2019). Camelina, a C3 oilseed crop, is frost-tolerant and possesses a high tolerance to drought, resulting in adaptability to a wide range of environments (Moser et al., 2010; Obour et al., 2015; Berti et al., 2016). Conversely, sorghum, a C4 plant, can grow in adverse environmental conditions due in part to efficient water use and maintained photosynthetic activity under drought or high salinity conditions (Ratnavathi et al., 2011; Dar et al., 2018; Choudhary et al., 2021). This inherent resilience to a wide range of environmental conditions and differing stress tolerance mechanisms makes them ideal candidates for comparative studies on environmental stress

Drought, salinity and cold stress were selected as the abiotic factors for study, as their relevance and impact on biofuel and agricultural crops has been well-characterized within the current literature (Cramer et al., 2011; Koryo et al., 2012; Zhu, 2016; Zhang et al., 2022). However, there is some evidence in the literature that type of salinity exposure results in differential plant stress response (Gupta and Huang, 2014). Considering this, two types of salt treatments were employed: salt stress, whereby concentration was gradually increased over time, and salt shock, wherein plants were immediately exposed to high concentrations. In addition, select plants were also exposed to chitosan, a biopolymer obtained from the fungal cell wall derivative chitin (Zheng et al., 2018). While chitosan nominally falls within the abiotic stress category due to triggering an immune response within plants (Chandra et al., 2015; Iriti and Varoni, 2015), application of this polysaccharide has in some cases been shown to enhance defense mechanisms and improve stress tolerance (Walker-Simmons et al., 1983; Hadwiger, 2013; Katiyar et al., 2015). On the other hand, other research suggests chitosan can have a debilitating impact on growth or microbe interactions, especially at higher concentrations (Calenn et al., 2018; Stasinka-Jakubas & Hawrylak-Nowak, 2022). We therefore applied chitosan to plants at a relatively high concentration (5 mg/ml) to observe if it acted as a source of biotic stress, a bio stimulant, or induced a mixed response in camelina and sorghum. Including this mixture of abiotic and potential biotic stressors enabled a detailed assessment of how sorghum and camelina respond and adapt to different types of environmental conditions over a short or long-term period.

In this study, we employ a multi-modal approach to capture a wide range of physiological and biochemical responses of camelina and sorghum in response to environmental stress. The MultispeQ device provided measurements of photosynthetic efficiency and leaf health using parameters such as chlorophyll fluorescence and photosystem II quantum yield (Kuhlgert et al., 2016), while the LI-6800 portable photosynthesis system captured gas exchange and water-use efficiency (Long and Bernacchi, 2003). The Multi-PIP biosensor measured tissue electrical impedance as an indicator of water transport efficiency and cellular damage (Flores et al., 2019). Finally, Fourier transform infrared (FTIR) reflectance spectroscopy was utilized to detect biochemical shifts in plant tissues indicative of stress responses, such as relative changes in cell wall composition and secondary metabolites (Lu and Rasco, 2010). These tools were chosen for their capacity to non-invasively capture key physiological and biochemical markers of stress in living plants in real-time, facilitating the identification of stress signatures with high temporal resolution. Together, this multi-modal approach provides a multi-layered dataset that comprehensively multiple aspects of plant responses to environmental stress, offering avenues by which to detect unique and early signs of stress that may inform future breeding programs and agronomic strategies aimed at improving crop tolerance in a rapidly changing climate.

Materials and Methods

2.1 Plant Material and Growing Conditions

sorghum bicolor (Silage Master) and *Camelina sativa* (spring variety) seeds were obtained from the New Mexico State University Agricultural Science Center (Los Lunas, New Mexico, USA). Seeds were sown in 2.5-inch pots containing Metro-Mix 820 potting soil (Sun Gro Horticulture, Seba Beach, AB, Canada) and grown under full-spectrum LED grow lights (Honeywell International, Inc., Charlotte, NC, USA) on a 16-hour light and 8-hour dark cycle. Plants were watered regularly with deionized (DI) water and kept at a constant temperature of approximately 24°C with controlled humidity.

After four weeks of growth, sorghum had between 3 and 4 leaves, and camelina had between 8 and 9 leaves. At this point, the plants were divided into six treatment groups, with four plants in each group.

2.2 Treatment Conditions

The experimental treatments included: Control: Plants watered with 500 mL of standard DI water. Chitosan treatment: Plants received 500 mL of a 2 mg/mL solution of chitosan (Thermo-Scientific, Fischer). Drought treatment: Plants were watered with 500 mL of 10% polyethylene glycol (PEG) 8000 solution (Fischer Scientific) to induce osmotic stress. Salt stress: Plants were initially treated with 50 mM NaCl, and the salt concentration was gradually increased to 150 mM in increments of 50 mM every 12 hours. Salt shock: Plants were exposed directly to 150 mM NaCl and treated every two days. Cold treatment: Plants were placed in a 4°C cold room and watered with 4°C DI water. The total experimental duration was 14 days, with two days of acclimation followed by 12 days of treatment. Treatment solutions were replaced every 2 days. The experiment was repeated for an overall total of n=8 plants for each treatment group.

2.3 Phenotypic Measurements

Photographs of the plants were taken at a standardized distance using a web camera. ImageJ software was used to quantify changes in leaf area, leaf length, shoot length, and leaf pigmentation over time. At the end of the experiment, plants were removed from soil and both the weight and length of the roots and shoots were measured. Plants were then individually wrapped in foil and dried in an oven for 80° C for ~48 hours. Plants were removed from oven and the roots and shoots were reweighed.

Relative Leaf Water Content was quantified from select using the formula:

$$Relative\ Water\ Content\ (\%) = \frac{(Fresh\ Weight\ - Dry\ Weight)}{(Turgid\ Weight - Dry\ Weight)} \times\ 100$$

as described by Pieczynski et al. (2013).

2.4 Gas Exchange Measurements

Changes to gas exchange within plant leaves was quantified using two analytical tools: MultispeQ (PhotoSynQ Inc., East Lansing, MI, USA) v 2.0 and the LI-6800 Portable Photosynthesis System (LI-COR Biosciences, Lincoln, NE, USA) equipped with a 6800-01A leaf chamber fluorometers and 6 cm2 apertures.

The methodology for using the MultispeQ has been previously described in (Kuhlgert et al, 2016). Briefly, the MultispeQ was outfitted with a “mask” (a small piece of black construction paper) following the instructions listed on the PhotosynQ website, as the sorghum leaves were narrower than the original sensor window. After activating the MultispeQ through the PhotoSynQ app, the device was gently clamped onto a leaf. While gently holding the MultispeQ still, a panel of environmental conditions and photosynthetic parameters were automatically measured over an ~20 second period by the Photosynthesis RIDES 2.0 open-close protocol. Measured parameters include Phi2: the ratio of PSII quantum yield (Φ_{PSII}) over ratio of incoming light; linear electron flow (LEF); and non-photochemical quenching (NPQt).

Methods for using and applying the LI-6800 have been previously described in (Riches et al., 2020). Briefly, system warmup tests were always run before each day’s experiments per the manufacturer’s recommendations. After the warmup tests were completed, IRGAs were matched using the range match feature. Survey measurements were taken by clamping the LI-6800 leaf chamber onto a leaf, waiting for the leaf to acclimate to the leaf chamber conditions, collecting gas measurements, and then removing the sample from the chamber and moving on to another leaf. Leaves measured were the youngest, fully expanded, intact leaf for each plant, generally the 3rd leaf for each sorghum plant and the 4th leaf for each camelina plant. Measurements were made at constant chamber air flow (600 µmol s-1), “auto” pump settings, chamber fan mixing speed (10,000 rpm), chamber air temperature (T_{air}) (27 °C, save for the cold-treated plants which were kept at 4°C), constant relative humidity (60%), PAR level (1500 µmol m-2 s-1), and carbon dioxide concentration (420 ppm).

Both tools were used daily between 9 a.m. and 1 p.m., measuring two fully expanded leaves per plant across three plants per treatment group.

2.5 Impedance Measurements

Two Multi-PIP (Growvera, Albuquerque NM) devices were programmed to measure impedance every 15 minutes at frequencies of 1000 or 100,000 Hz using the Default, Low, and High Modes. The Multi-PIP devices were attached to 6 inch plastic clamps, by 3 meter SMA male to SMA male cables. Each clamp contained two probes, separated by ~6 inch of space. The plant probes consisted of six 0.25 inch metal prongs, overlayed in a grid-like pattern on one side of a green circuit chip. For measuring

impedance, clamps were gently clamped onto leaves (3rd for sorghum, 6th for camelina) such that each metal prong was inserted into (and in most cases through) the tissue. For camelina, clamp was attached at the bottom right edge of the leaf from the stem. For sorghum, due to the size of the leaves, microneedles were inserted through the center of the leaf for maximum stability, although care was taken not to puncture the midrib. Once attached, the SMA cables were taped down to prevent unwanted twisting and minimize the amount of weight being placed on the leaves from the clamps. Once affixed, the Multi-PIP devices were affixed to a power source and left to run. Multi-PIP data-collecting was reset every day to check for any potential issues. One sorghum and one camelina plant from each treatment group was measured using the Multi-PIP devices for each experimental run.

2.6 FTIR Spectroscopy

The spectroscopic signature of plant leaves was quantified with a Bruker HYPERION 2000 Microscope (Bruker Corporation, Billerica, MA) using an MCT-FPA detector. As living, intact plant leaves were too thick for transmission mode, the reflectance of whole, intact leaves were measured at 36x background settings with 16 coadded scans. Each measurement took ~6 minutes. One leaf per plant was measured each day. After the experiment had concluded, three leaves and three roots of each plant were measured using reflectance. After whole plants were dried, the reflectance measurements were repeated. FTIR spectra were visualized using the open-source program Spectragryph. Data processing was performed by converting reflectance to 1/log(reflectance), baseline corrected using Spectragryph's "Advanced Baseline" adaptive method with 15 coarseness and 0 offset, and then smoothed using Savitzsky-Golay with an interval of 10 and a polynomial order of 3. Data was then normalized by dividing values by the maximum value within the spectra. Significant outliers were identified using the Mahalabinois distance and removed. Principal Component Analysis was performed in Python using the "sklearn" package.

2.7 Statistical Analysis

One-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) post hoc tests were used to determine significant differences ($\alpha = 0.05$) among the treatments. Normality and homogeneity of variances were checked using the Kolmogorov-Smirnov and Levene's tests, respectively. All code for fluorometric and gas exchange data analysis was developed in Python V 5.1 Version 1.4.02 of the instrument software were used for the work reported here. Impedance data was graphed and analyzed using the "Multi-PIP Analysis Suite" an open-source python module developed and provided by Growvera. Any FTIR spectra outliers were identified using the Mahalabinois distance and removed. Principal Component Analysis was performed in Python using the "sklearn" package.

Results

3.1 Growth and Morphological Responses to Stress

Twenty-four camelina and sorghum plants were organized into six groups, four plants per group. Each group was exposed to a different type of stress (control, chitosan, cold, drought, salt stress, or salt shock) for a period of twelve days, with the overall experiment being repeated twice. At the end of the treatment period, visual assessment indicated drought and salinity triggered drastic morphological changes within camelina seedlings, while cold and chitosan seemingly reduced or increased shoot length, respectively (S.Fig. 1). For sorghum, (S.Fig. 2), cold triggered severe morphological changes, while drought and salinity had a reduced effect, and chitosan may have increased leaf length. When the seedling growth traits were quantified, data regarding camelina seedling growth traits (Table 1) indicated significant differences between un-stressed and stressed plants. Shoot length (SL) and fresh shoot mass (FSM) were significantly reduced in response to drought, salinity, or cold imposition, while dry shoot mass (DSM) was only reduced in response to drought and salt stress, and leaf water content (LWC) decreased in response to both drought and salinity. In contrast, chitosan treatment triggered an increase in both (SL) and fresh root mass (FRM).

Table 1: Growth Indices of camelina seedings exposed to environmental stress.

Camelina	Length (mm)		Fresh Mass (mg)		Dry Mass (mg)			Leaf Water Content (%)
	Shoot	Root	Shoot	Root	Shoot	Root	Root:Shoot	
Control	233±29	172±31	861±209	30±13	109±24	22±10	0.20±0.07	86±2
Chitosan	283±35*	188±49	1102±253	53±14**	133±34	31±12	0.24±0.07	85±1
Cold	175±25**	201±45	563±236*	47±15	108±54	33±8.7	0.36±0.15	81±4
Drought	134±39**	116±43	71±52**	13±7.1	40±25**	12±6.8	0.40±0.33	25±7**
Salt Stress	163±17**	140±41	129±20**	20±12	51±30*	17±12	0.36±0.22	41±9**
Salt Shock	183±21*	163±21	107±34**	24±7.1	58±17	17±5.7	0.28±0.06	37±19**

± =SD, * = p<.05, ** = p<.01, n = 7 plants

In contrast, the sorghum seedling growth traits (Table 2) were less distinct. FSM was only reduced in cold-treated plants, with SL or SDM not approaching the conventional limits of significant for any stress group. However, LWC did show significant differences, exhibiting high reduction in response to cold, drought, or either salinity treatment as compared to normally grown plants.

Table 2: Growth Indices of sorghum seedings exposed to environmental stress.

Sorghum	Length (mm)		Fresh Mass (mg)		Dry Mass (mg)			Leaf Water Content (%)
	Shoot	Root	Shoot	Root	Shoot	Root	Root:Shoot	
Control	250±79	216±42	393±270	35±20	54±38	22±17	0.42±0.19	93±5
Chitosan	302±28	232±56	587±94	58±16	71±9.1	31±9.6	0.43±0.10	97±2
Cold	199±50	207±62	144±116*	23±15	31±19	12±6.9	0.44±0.23	18±10**
Drought	232±43	208±77	195±108	65±43	47±20	41±23	0.86±0.27**	24±19**
Salt Stress	234±36	175±42	228±74	49±6.1	36±14	17±8.2	0.48±0.12	38±27**
Salt Shock	264±40	204±34	170±93	51±23	49±23	23±8.2	0.52±0.12	31±24**

± =SD, *= p<.05, ** = p<.01, n = 7 plants

When SL, leaf length (LL), and leaf color (LC) of the seedlings were measured over time (Fig. 1), the SL of camelina seedlings exposed to abiotic stress (Fig. 1A) began to deviate at ~day 7, becoming significantly distinct by day 9. For LL (Fig. 1B), cold and chitosan did not significantly differ from control plants, while the drought-treated plants became significantly reduced by day 3 and the salt-treated plants by day 4. For LC (Fig. 1C), the stressed plants had a slight decrease in overall pigmentation but was not significant. However, when the green color intensity (GC) was quantified (S.Fig. 3A), drought and salt significantly reduced green pigmentation by day 6 and cold by day 10. For sorghum, results were once again less prominent. SL (Fig. 1D) and LL (Fig. 1E) never reached significance thresholds. LC (Fig. 1F) did significantly decrease in abiotically-stressed plants by day 10, however, this reduction was not observed in GC (S.Fig. 3B).

3.2 Fluorometric and Gas Exchange Responses

Highly significant variations ((P≤0.01) were recorded when the gas exchange characteristics of abiotically-stressed camelina and sorghum seedlings when compared to normally grown plants (Fig. 2). The parameters for chitosan and cold-treated plants remained stable and displayed little deviation from non-stressed plants. In contrast, drought and salt conditions caused major alterations to all measured variables. Drought imposition caused changes in non-photochemical quenching (NPQt) (Fig. 2A), chlorophyll fluorescence (ΦPSII) (Fig. 2B), linear electron flow (LEF) (Fig. 2C), photosynthetic rate (A) (Fig. 2D), transpiration (E) (Fig. 2E), and stomatal conductance (gs) (Fig. 2F), all becoming significantly different from control plants 2 or 3 days posttreatment. Salt stress and salt shock-treated plants exhibited similar phenotypes, although they only became significant 4 or 5 days posttreatment.

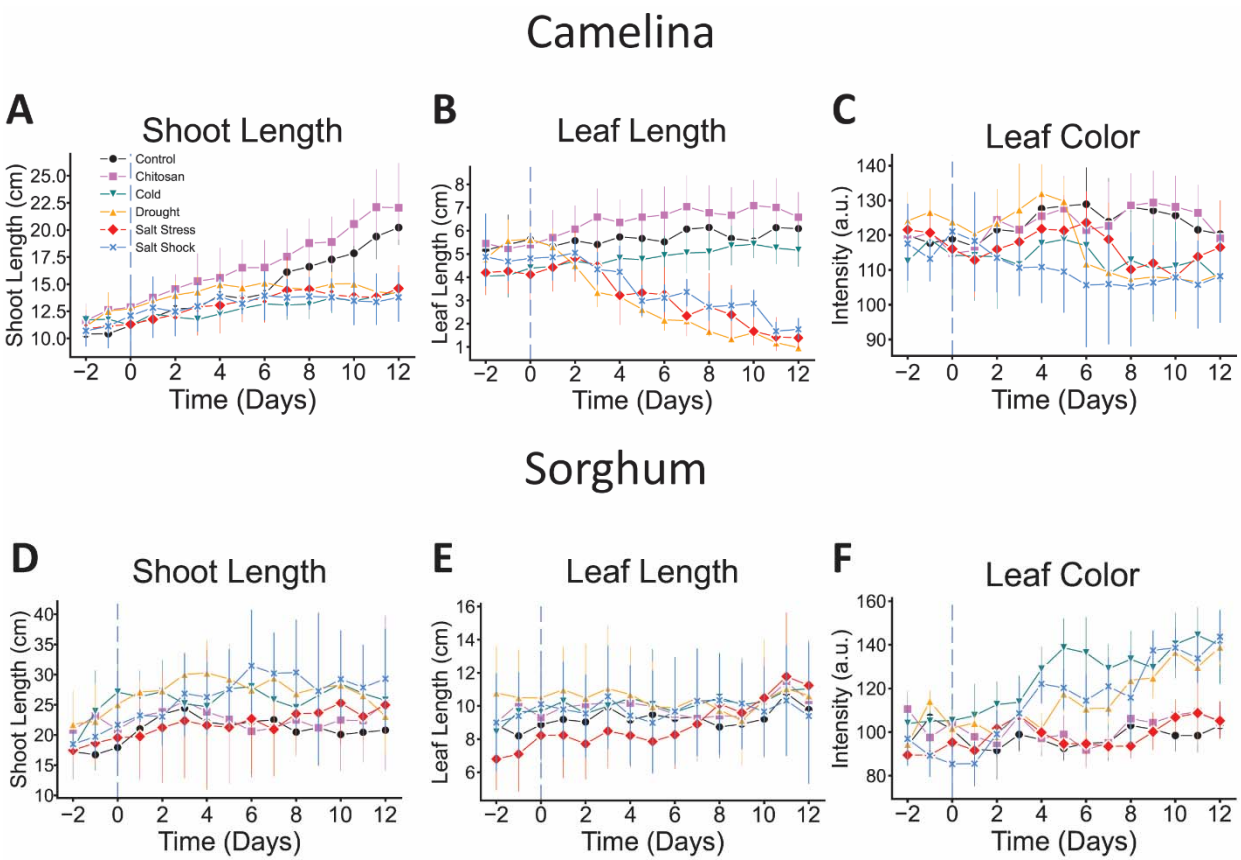

Figure 1. Growth Indices of camelina and sorghum seedlings exposed to environmental stress over time. Camelina and sorghum seedlings were exposed to either no treatment, chitosan, cold, drought, salt stress or salt shock stress over a 12 day period. Shoot length (A and B), leaf length (B and E), and the color intensity (C and F) of the leaves were measured each day, with results being plotted in line graphs. Vertical blue dashed line indicates when treatment exposure occured. n=7 plants (shoot length) or 12 leaves (leaf length and leaf color).

Chitosan-treated sorghum plants maintained stable values across all parameters throughout the experimental period and differed little from the control. However, plants subjected to cold stress showed a rapid and significant decline in all measured gas exchange parameters, starting 1 to 2 days after the initiation of the cold treatment. Drought and salinity resulted in slower, more gradual deviations from the control. For these stress treatments, ΦPSII, LEF and A began to decline after several days of exposure, with the most pronounced effects seen after day 7, while non-photochemical quenching (NPQt) exhibited the opposite response. Gs and E also showed gradual reductions under both drought and salt treatments, with salt shock-treated plants showing a slightly more rapid decline than salt stress-treated plants.

3.3 Impedance Measurements

Electrochemical impedance spectroscopy has recently emerged as a rapid, non-destructive method to directly quantify plant parameters related to health and stress in real-time (Lichtenthaler, 1998; Jócsák et al., 2019; Haeverbeke et al., 2023). Several groups have developed modalities for measuring plant impedance, but tend to be

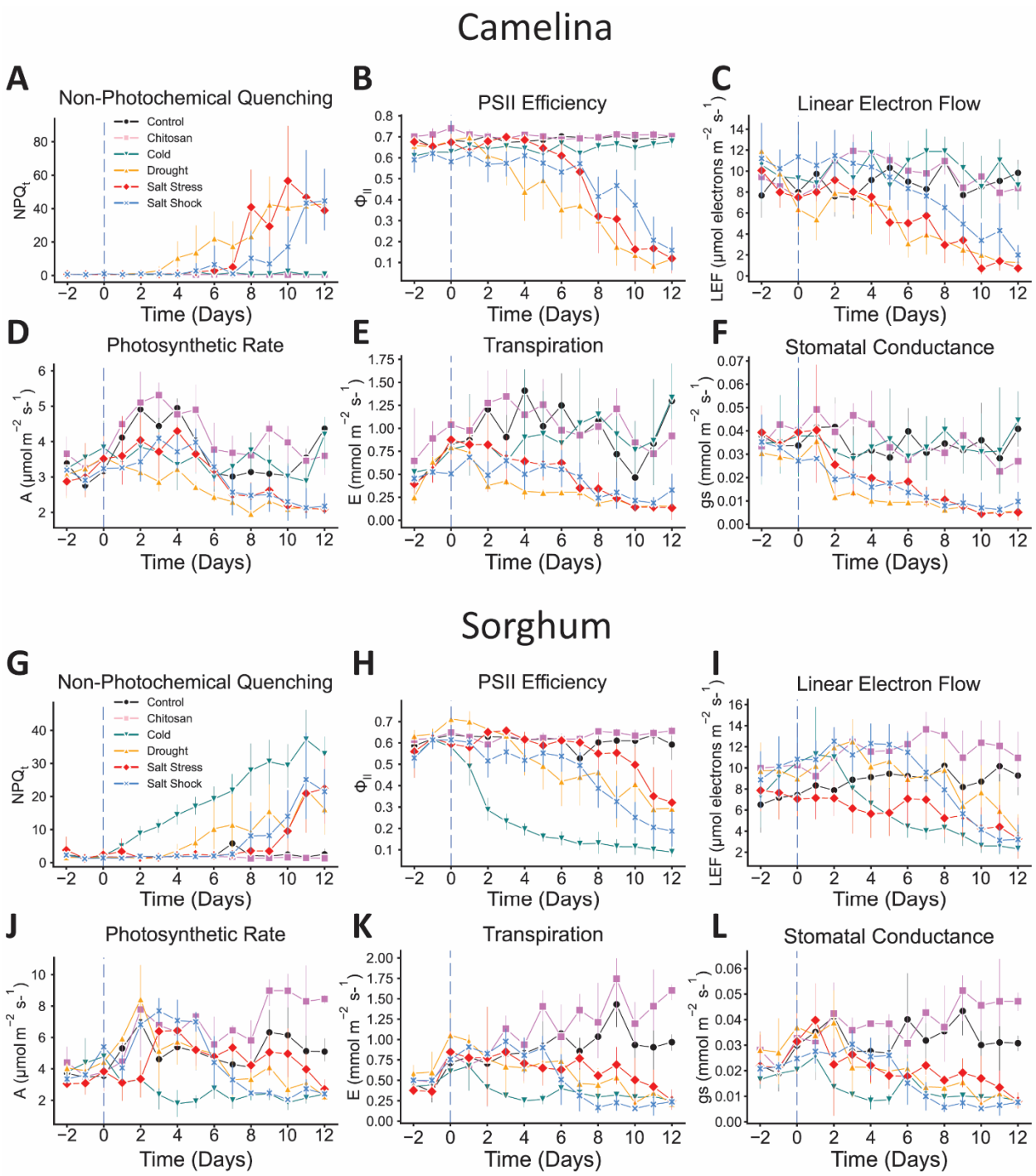

Figure 2. Fluorometric and gas exchange parameters of camelina and sorghum seedlings exposed to environmental stress over time. Camelina and sorghum seedlings were exposed to either no treatment, chitosan, cold, drought, salt stress or salt shock stress over a 12 day period. Non-photochemical quenching (A and G) PSII efficiency (B and H), and linear electron flow (C and I) were measured using a MultispeQ, while photosynthetic rate (D and J), transpiration (E and K), and stomatal conduncance (F and L) were measured using a LI-6800 each day, with results being plotted in line graphs. Vertical blue dashed line indicates when treatment exposure occured. n= 12 leaves.

limited by invasive probes, restrictions to woody tissues in mature plants, or an inability to differentiate between internal structures (El Khaled et al., 2017; Ibba et al., 2020,

Basak et al., 2020a; Garlando et al., 2022; Reynolds et al., 2023). Recently, Reed et al. published a method for quantifying impedance in plants via wearable biosensors consisting of <1 mm microneedles (Reed et al., 2024), which were both capable of quantifying impedance in a variety of tissues (stem, lamella, petiole), but also detected unique impedance patterns with tissue specificity. The setup used in that paper was further developed into a small, low-cost, smart device for continuously monitoring plants in the field, known as the Multi-PIP (S.Fig. 4). When impedance measurements obtained using the Multi-PIP device were compared with concurrent water potential measurements from a psychrometer, (S.Fig. 5), the data demonstrated a clear correlation between impedance and water potential, supporting the reliability of the Multi-PIP system in capturing plant water status over time. In addition, when the Multi-PIP was used to quantify impedance in vineyard grapes over an entire growing period, key physiological events, such as bud break and the start of watering, were reflected in impedance fluctuations (S.Fig. 6), demonstrating that impedance measurements reflect the physiological changes in live plant tissue in response to alterations in environmental conditions. As such, we used the Multi-PIP to examine plant impedance response to controlled environmental stress within our experimental methodology high temporal resolution. Fig. 3 presents the changes in impedance over the 12-day experimental period for both camelina and sorghum across all treatments.

3.3.1 Camelina Impedance Responses

For non-stressed plants (Fig. 3A), impedance measurements stayed relatively consistent throughout the measurement period, gradually increasing over time, only to begin to rapidly increase in value after day 10. While chitosan-treated plants (Fig. 3C) also exhibited a gradual increase in impedance over time, readings stayed below 3000 kOhms for the entirety of the experiment. Cold-treated camelina (Fig. 3E) displayed a notable increase in variability after one day of treatment, as evidenced by the scatterplot shifting from a nearly linear configuration to a thicker band, but otherwise exhibited little change in value beyond the dips associated with diurnal changes to water dynamics. Drought and salt-treated plants (Fig. 3G, 3I, 3K) reached maximum impedance values one order of magnitude higher than those displayed by the previous three treatment groups. Drought induced increases in impedance on day 4, day 7, and one large spike on 10; salt stress induced a gradual increase starting on day 5; and salt shock induced an increase on day 7 that spiked on day 9.

When the early response was examined (S.Fig. 7), drought induced extremely rapid increases in impedance a few hours after initial treatment, while salt shock and salt stress triggered increases a few days after exposure when compared to non-stressed plants. The same patterns and trends were observed when the experiment was repeated at a higher frequency (100k versus 1000) (S.Fig. 8).

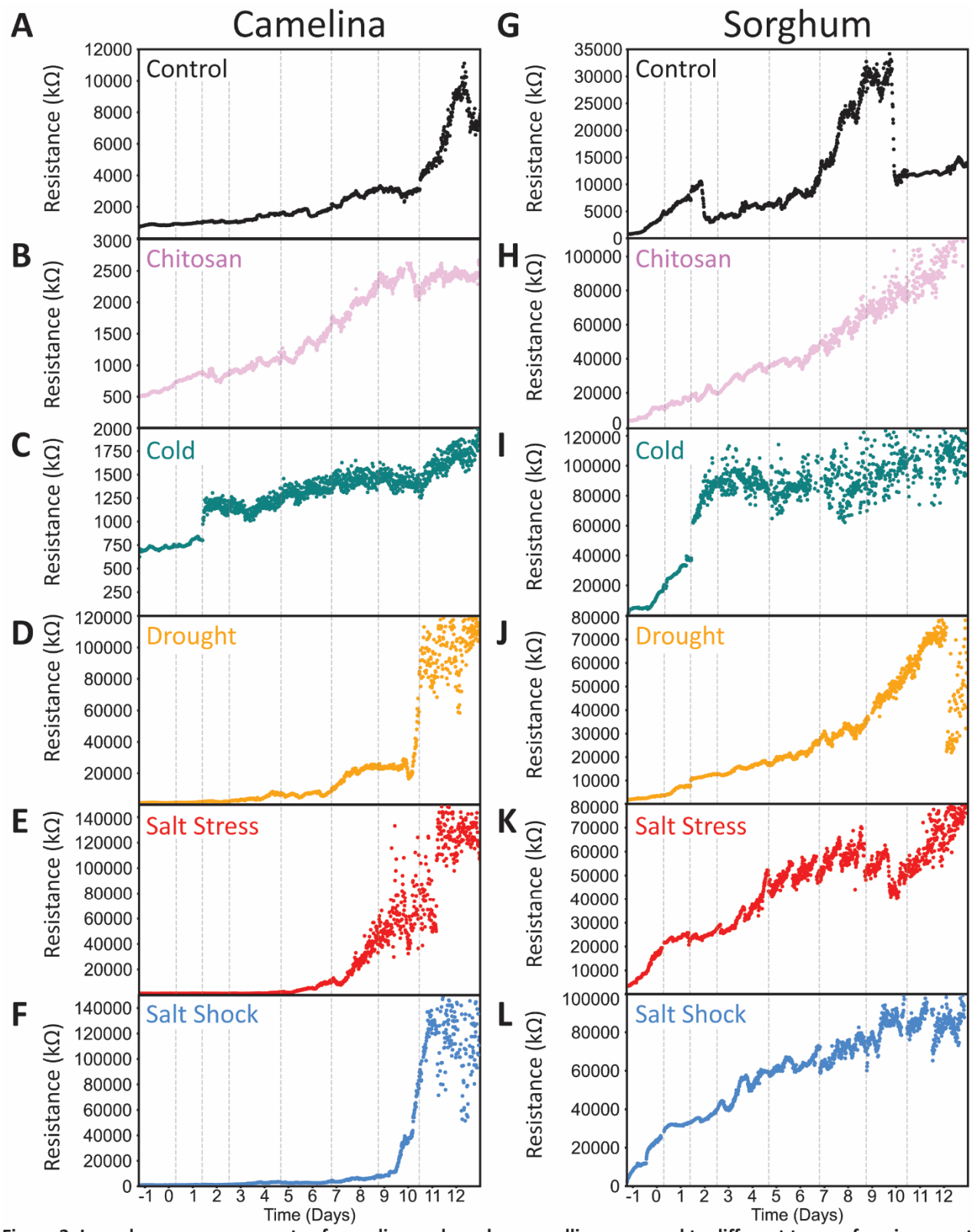

Figure 3. Impedance measurements of camelina and sorghum seedlings exposed to different types of environmental stress. Camelina and sorghum seedlings were exposed to either no treatment (A and G), chitosan (B and H), cold (C and I), drought (D and J), salt stress (E and K) or salt shock (F and L) stress over a 12 day period, and impedance of select leaves were measured using the Multi-PIP biosensor. Y-axis values indicate resistance measurements at 1000 frequency. Vertical dashed lines indicate when treatment exposure occured.

3.3.2 sorghum Impedance Responses

In sorghum, the control (Fig. 3B) and chitosan-treated (Fig. 3D) plants exhibited relatively stable impedance values over the course of the experiment, with only a gradual increase for chitosan exposure and a spike in the control plan that returned to baseline in the final days. Cold-treated sorghum (Fig. 3F), however, displayed a rapid escalation in impedance beginning almost immediately after treatment began. sorghum plants exposed to drought and salt treatments (both salt stress and salt shock) (Fig. 3H, 3J, 3L) exhibited more gradual increases in impedance over time. Drought-treated plants showed a slow but steady rise in impedance starting around day 3, whereas salt stress and salt shock treatments caused a more noticeable increase in impedance beginning after day 6. Notably, the diurnal changes in impedance were retained within the salt stressed and salt shocked plants, suggesting they may have undergone a lesser form of cellular damage or reduced water transport efficiency in comparison to plants exposed to drought.

However, unlike the camelina plants, which maintained relatively stable impedance values for the two days before treatment, sorghum plants exhibited sharp increases pre-treatment (S.Fig. 7), which unfortunately obscured how impedance values were altered in response to abiotic or biotic stress. Regardless, when the experiments were repeated with a frequency of 100k Hz (Supplemental Fig. 8), the same general trends in response to each type of stress were observed, once again suggesting that the impedance of plant leaves is altered in response to environmental stress, highlighting a potential novel monitoring method for field-based assessment.

3.4 FTIR Spectral Analysis and Principal Component Analysis (PCA)

To further investigate potential biochemical changes occurring in sorghum and camelina in response to stress treatments, Fourier-transform infrared (FTIR) spectroscopy was conducted. Previous application of FTIR to plant leaves mainly utilized dried and homogenized tissue (Naumann et al., 2010; Lu & Rasco, 2010; Cen & He, 2007), limiting non-invasive field application. To establish a baseline reflectance FTIR spectra of camelina and sorghum seedling leaves, reflectance measurements were conducted on non-treated intact leaves of both camelina and sorghum (S.Fig. 9). Peak location and amplitude were highly similar between both species, with 15 distinct peaks identified and annotated. These peaks were then correlated to specific functional groups and compounds commonly found in plant tissues (Supplementary Table 3). Some key peak assignments include 3488/3519 cm^{-1} (OH/NH stretching): associated with alcohols and various polysaccharides (e.g., cellulose) as well as water and proteins, 1744/1753 cm^{-1} (C=O stretching): found in ester groups and linked to compounds such as pectin, lignin, suberin, and cutin, indicating the presence of lipids and structural components in the plant cell wall, and 816/820 cm^{-1} (ring bending): Representing aromatic rings, these

peaks indicate the presence of crystallized cellulose within the sample. This representative spectral analysis established the biochemical baseline for sorghum and camelina under control conditions. The peak assignments provide insight into the functional groups and compounds present in the leaf tissues, which could then be further analyzed for changes under stress treatments.

After establishing the baseline spectra, FTIR spectroscopy was then conducted on leaf samples at end of the 12-day experimental period (Fig. 4). Fig. 4A shows the FTIR spectra for camelina plants across all treatments. Camelina exhibited substantial spectral shifts in response to salt and drought, particularly in the lipid and carbohydrate regions. Cold treatment had less of an impact on camelina's spectra, consistent with its greater tolerance to cold stress, but there were still significant shifts, most notably in the 3500 cm^{-1} and 1200 cm^{-1} region. For sorghum (Fig. 4B), the most significant changes in the spectra were observed under cold and drought treatments, with notable alterations in the lipid (2800-3000 cm^{-1}) and carbohydrate (1000-1200 cm^{-1}) regions. In contrast, while both salt shock and salt stress exhibited alterations in the same region, they were lesser in nature, with salt stress more closely resembling the control plants overall. When Principal Component Analysis was performed (Fig. 4C and D), the PCA plots reveal clear separations between the different treatments. For camelina (Fig. 4C), drought and salt led to the most significant divergence from the control, with cold-treated plants showing minimal but significant separation. For sorghum (Fig. 4D), drought and cold treatments resulted in the greatest divergence from the control, with distinct clustering observed along PC1. In contrast, salt shock displayed more intermediate clustering, while some of the salt stress spectra clustered with control, suggesting some heterogeneity within the biochemical profile. For both plants, chitosan-treated plants clustered closely with the control, suggesting minimal biochemical alterations. When examining which wavenumbers contribute the most to the variance observed in the PCA (Fig. 4E and F), for both sorghum and camelina, the carbohydrate region (1000-1200 cm^{-1}) and lipid region (2800-3000 cm^{-1}) were the primary drivers of the variance, especially under salt shock and drought conditions. This indicates that these biochemical components are particularly responsive to abiotic stress in both species.

When these leaves were dried (S.Fig. 11), the spectral shifts and PCA grouping separation disappeared, suggesting that water content of leaves may be one of the biomolecular components most impacted by environmental stress. Fresh roots were also analyzed (S.Fig. 12), however, the only shifts here were observed in the drought-treated plants. As the spectral shifts within these peaks exhibited similarities to the reflectance spectra of PEG alone (data not shown), it is likely that the separation was due to trace amounts of PEG in the sample rather than a natural biological response. Overall, these results suggest that not only are intact, unperturbed plants a viable target

for spectroscopic detection of plant stress, but they may also in some ways be favorable compared to dried samples or other types of plant tissues as an indicator of stress response.

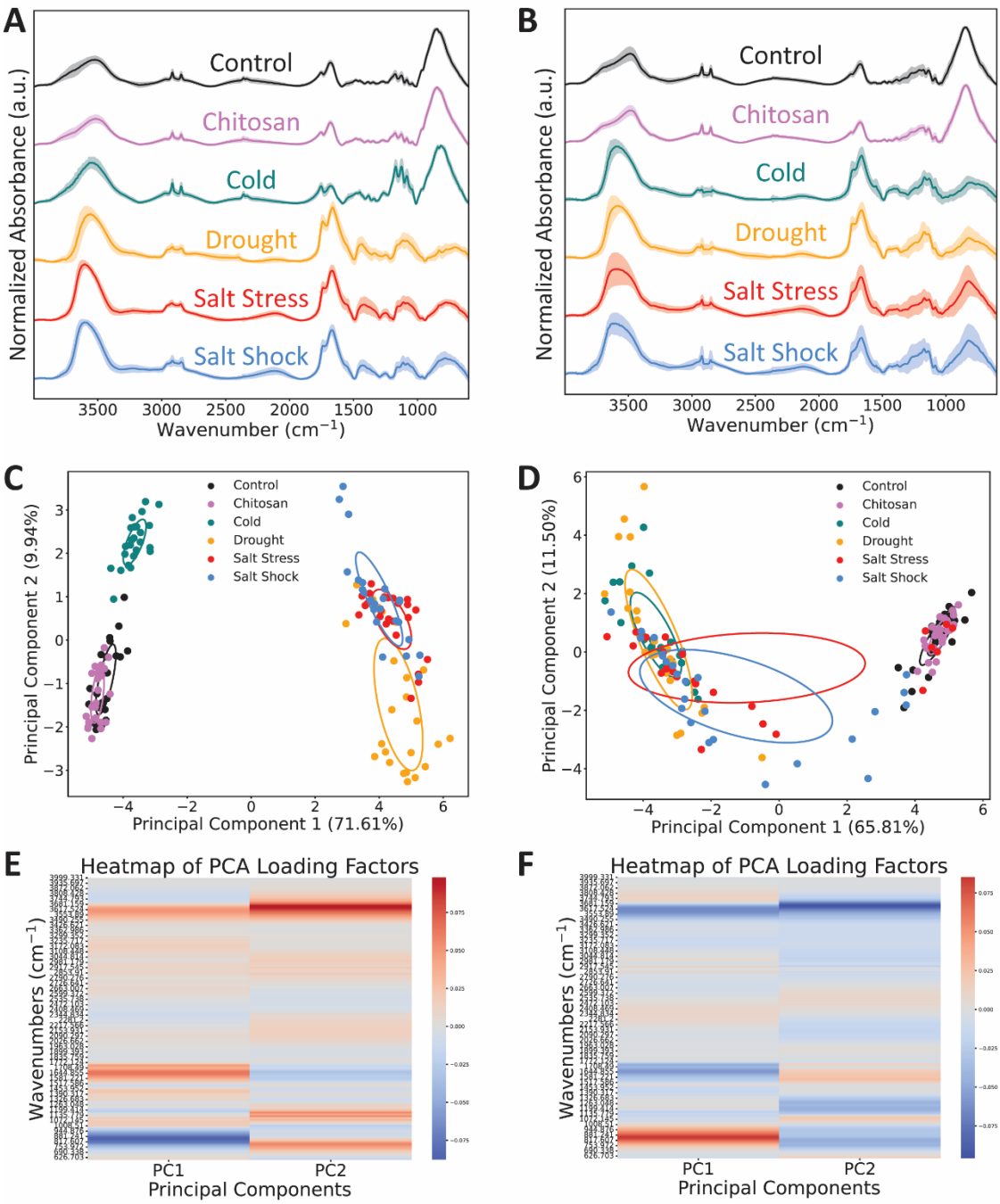

Figure 4. FTIR spectra of camelina and sorghum fresh leaves exposed to environmental stress. The reflectance spectra of stressed or non-stressed camelina (A) and sorghum (B) seedlings were measured after the 12 day experimental period. Principal Component Analysis (C and D) and Heatmaps of PCA Loading Factors (E and F) were also performed. n=24.

3.4.1 Changes in FTIR Spectra in Response to Stress Over Time

After establishing that FTIR spectroscopy could detect spectral shifts in response to abiotic stress, the next step was to determine if these changes could be detected over time. As such, FTIR measurements were conducted on leaves for every day of the 12 day-experiment period (S.Fig. 11). For camelina, the control and chitosan-treated camelina plants exhibited stable FTIR spectra throughout the experimental period. The PCA plots for these treatments showed tightly clustered data points, indicating minimal changes in the biochemical composition of the leaves. Camelina plants subjected to cold stress showed relatively minor changes in their FTIR spectra compared to sorghum, although these changes were distinct from control and chitosan. The PCA plot for cold-treated camelina showed only slight deviations from the control. Drought and salt shock treatments caused significant spectral shifts in camelina, particularly in the carbohydrate and lipid regions. The PCA plots revealed a clear separation between the salt shock and drought-treated samples and the control. Salt shock-treated camelina showed the most extreme divergence in the PCA, with samples clustering away from all other treatments, indicating a pronounced biochemical response. Salt stress-treated plants showed more moderate shifts, with PCA clustering like that observed in the drought treatment. The FTIR spectra of control and chitosan-treated sorghum plants exhibited stable profiles throughout the experiment, with only minor variations in spectral intensity over time. This stability was further supported by the PCA plots, which showed tight clustering of data points, indicating minimal biochemical changes under these conditions. sorghum plants subjected to cold stress displayed significant changes in their FTIR spectra over time. This divergence is indicative of substantial biochemical alterations, particularly in the regions associated with lipids and proteins, which are likely involved in the plant's cold stress response. However, these changes only became significant by day 12, suggesting that such changes may be delayed in comparison to other treatments. Drought and salt-treated sorghum plants exhibited distinct spectral shifts, particularly in the regions corresponding to carbohydrate and protein bands. Drought-treated plants showed the most pronounced changes in spectral intensity, especially after day 7. This was reflected in the PCA. Salt stress-treated plants exhibited a more gradual divergence in the PCA, suggesting a slower accumulation of stress-related changes in the biochemical composition. Overall, the FTIR and PCA analyses highlight the distinct biochemical responses of sorghum and camelina to various abiotic stress treatments and emphasized that such a change is slow enough that it can be captured with daily monitoring.

When the loading plots were examined (S.Fig. 12), in both sorghum and camelina, the control and chitosan treatments displayed loading plots with relatively evenly distributed contributions across the wavenumber range. This indicates that under non-stress conditions, or with chitosan treatment, no single functional group or compound dominated the biochemical variance, suggesting stable biochemical profiles for these plants. The cold-treated sorghum and camelina plants showed distinct shifts in the

wavenumber regions associated with proteins (around 1600 cm^{-1} to 1700 cm^{-1}) and lipids (around 2800 cm^{-1} to 3000 cm^{-1}), indicating that these functional groups contributed significantly to the variance observed in the FTIR spectra under cold stress. This suggests that cold treatment heavily impacted the protein and lipid composition of both species. Drought and salt stress (both gradual and shock treatments) exhibited strong contributions from wavenumbers associated with carbohydrates (around 1000 cm^{-1} to 1200 cm^{-1}) and lipids, with more pronounced peaks in the salt shock treatment. The loading plots for salt shock-treated plants showed particularly high variance driven by the 1000 cm^{-1} to 1200 cm^{-1} range, suggesting significant shifts in the carbohydrate content of these plants under acute salt stress. Overall, the loading plots reveal that different wavenumber regions dominated the spectral variance depending on the specific treatment. Salt shock and drought had the greatest influence on carbohydrate-related regions, while cold treatment primarily affected the protein and lipid bands.

Finally, when the change in slopes was measured over time (S.Fig. 13), it was shown that the changes in control and chitosan-treated plants were noticeably different from those of abiotically stressed plants for both camelina and chitosan, demonstrating the rates of change, how quickly they occur, and how they could be utilized as ways to detect early signs of stress in field-based applications.

Discussion

This study aimed to assess the physiological and biochemical responses of *Camelina sativa* and sorghum *bicolor* seedlings to chitosan, cold, drought, salt stress, and salt shock treatments using a range of non-invasive measurement tools. Our findings reveal distinct stress response mechanisms between the two species, highlighting sorghum's stronger response to cold stress and Camelina's pronounced sensitivity to drought and salinity, and how those differential stress responses could be quantified as unique physiological profiles born of a multi-modal approach.

Camelina was highly sensitive to both drought and salinity. Under these conditions, reductions in growth indices and photosynthetic activity were rapid. FTIR and PCA analyses revealed significant biochemical changes, especially in the water, protein, and glycosidic bond regions, suggesting that camelina's cellular integrity and water transport mechanisms are being severely in response to these types of environmental stress. In contrast, cold had little impact on gas exchange measurements but did deviate from control plants in growth indices, impedance readings, and biochemical makeup, suggesting that while day-to-day functions of these plants are not interrupted the mechanisms required to adapt to cold environments consume resources that would otherwise be allocated towards growth.

In sorghum, cold stress induced significant reductions in key growth metrics such as leaf area and photosynthetic efficiency. This was coupled with pronounced changes in impedance, suggesting impaired water transport efficiency and increased cellular damage. By contrast, responses to drought and salinity were more gradual, with observable changes in gas exchange and FTIR spectra only emerging later in the experiment. The alterations in the lipid and protein regions observed in FTIR data further suggest the metabolic shifts required for sorghum to manage these stressors.

Of the treatments, chitosan had little to no detectable negative effects. Rather, application of chitosan seemed to boost biomass accumulation and some aspects of photosynthetic activity. These results are quite like previous findings regarding the beneficial impact of chitosan on plants despite our use of higher concentrations than what is usually tested (5 mg/ml). As the negative impact of this concentration of chitosan was observed in Arabidopsis, a much smaller and less stress-tolerant plant, it is possible that higher concentrations of chitosan are required to produce deleterious effects in camelina or sorghum. Regardless, this work reaffirms that chitosan is a helpful supplement for bolstering overall plant growth and resistance to stress with minimal side effects.

In addition, our findings on the differences between salt stress and salt shock exposure was mixed, however, the general trend was such that plants seemed to develop a more rapid and robust tolerance to gradual salt exposure rather than acute salt exposure. More research is needed, but this emphasizes a promising avenue regarding how application of environmental stress may adjust plant stress response.

One of the central objectives of this study was to evaluate the efficacy of rapid, non-invasive, and non-destructive tools for monitoring plant stress responses. The use of technologies such as Multi-PIP impedance sensors, MultispeQ, and FTIR spectroscopy provided real-time insights into plant physiological changes, allowing for the early detection of stress before visible symptoms became apparent.

MultispeQ and LI-6800 gas exchange measurements provided detailed data on photosynthetic performance under different stress conditions. The sharp declines in net CO_2 assimilation, stomatal conductance, and chlorophyll fluorescence under drought and salt shock in camelina, and cold stress in sorghum, highlight the utility of gas exchange parameters in detecting stress-induced disruptions in photosynthesis. The correlation between these measurements and the FTIR spectra provides a comprehensive view of how stress affects both plant physiology and biochemistry.

Multi-PIP impedance sensors proved to be highly effective in capturing real-time changes in tissue water content and cellular integrity. The rapid increase in impedance observed under salt shock and cold treatments in sorghum, and under drought and salt shock in camelina, suggests that impedance measurements could be a reliable indicator

of early stress response, particularly under extreme environmental conditions. This is supported by the clear separation between treatments in the PCA analysis, further demonstrating that electrical impedance can be linked to specific stressors in both species.

FTIR spectroscopy allowed for the non-invasive analysis of biochemical changes in both fresh and dried tissues. The spectral shifts observed under salt shock, drought, and cold treatments revealed alterations in carbohydrates, lipids, and proteins, which are known to play critical roles in plant stress adaptation. Importantly, the FTIR analysis of dried leaves and roots confirmed the lasting impact of stress on plant biochemical composition, making it a valuable tool for post-stress analysis.

While this study provides valuable insights into plant stress responses, several limitations must be acknowledged. First, the use of polyethylene glycol (PEG) to simulate drought conditions may have introduced artifacts that do not perfectly replicate field drought scenarios, potentially skewing the results. Future studies should consider using water deprivation methods that better reflect natural drought conditions. Additionally, while the selected tools captured physiological changes effectively, the lack of spatial resolution limited our ability to observe localized stress responses within individual plants. Another limitation is the controlled environment of the study, which may not fully represent the complex interactions that occur in field conditions, such as fluctuating environmental factors or pest pressures. Scaling these non-invasive tools for large-scale field applications will also require improvements in sensor robustness, data integration, and deployment across larger agricultural landscapes.

Conclusion

This study demonstrates the potential of non-invasive tools for early detection of plant stress and highlights the divergent strategies employed by sorghum and camelina to cope with abiotic stress. As climate change continues to challenge global food security, adopting smart technologies for crop monitoring will be critical for enhancing resilience and maintaining productivity in a sustainable manner.

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Disclosures

The authors declare no conflicts of interest.

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