Terahertz spectroscopy and imaging as a tool to unlock physiological and molecular mechanisms for drought resistance of agaves

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Abstract: This article summarizes the findings of two of our own contributions and presents additional measurements that makes us reach a conjecture about the physiological and molecular mechanisms that confer Agaves their extraordinary capacity to withstand drought conditions. In these studies we used terahertz spectroscopy and imaging to investigate water retention mechanisms of Agaves as well as the hydration dynamics of agave fructans, which are a peculiar type of carbohydrate produced by these plants. THz imaging was applied to map water distribution across different tissue regions, revealing a highly hydrated region in the core of the leaves and a less hydrated layer in the outside. Additionally, THz spectroscopy was used to study the hydration behavior of agave fructans in aqueous solutions. The hydration number and absorption coefficient increased non-linearly with decreasing solute concentrations, reflecting the formation of complex hydration layers around these carbohydrates with an outstandingly large number of water molecules (~ 320) which is two to four times larger than that of other carbohydrates such as Inuliun or Maltodextrin. The findings underscore the importance of fructans in stabilizing membranes and enhancing drought tolerance by managing water at both tissue and molecular levels. This study demonstrates the versatility of THz technologies in plant science, offering a comprehensive approach to understanding water retention and hydration dynamics, with potential applications in improving agricultural practices for water-scarce environments.

1. Introduction

With increasing environmental challenges such as climate change and water scarcity, understanding plant water dynamics has become essential for sustainable agriculture and environmental management. Plant hydration directly influences physiological processes, including photosynthesis, transpiration, and nutrient transport, which are critical for growth and survival under fluctuating environmental conditions. As a result, precise tools to monitor water content and distribution within plant tissues are necessary for advancing plant science and developing strategies to enhance crop resilience.

Terahertz (THz) spectroscopy and imaging are gaining relevance as tools in plant science, providing non-invasive techniques to analyze water dynamics and tissue hydration [1–3]. THz radiation, with frequencies between 0.1 and 10 THz, interacts sensitively with polar molecules

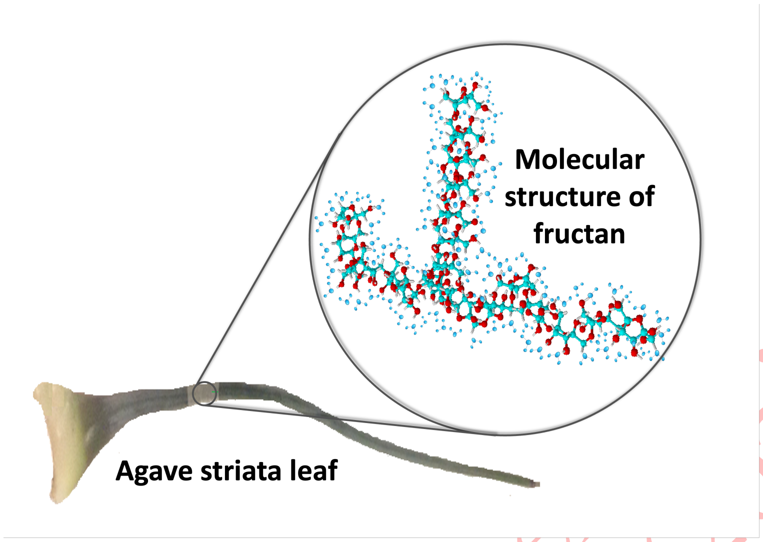

Fig. 1. Structure and water-binding dynamics in *Agave striata*. The illustration shows an *Agave striata* leaf alongside the molecular structure of its fructans, with water molecules bound around the fructan chains.

like water, allowing it to detect subtle variations in water content and molecular hydration states. This makes it a powerful tool for real-time monitoring of hydration changes at the tissue, cellular, and molecular levels. Traditional methods, such as gravimetric analysis, are labor-intensive, time-consuming, and relatively inaccurate, making THz-based techniques more practical and efficient for real-time monitoring [4, 5]. These capabilities are particularly valuable for studying plants adapted to arid conditions, where understanding water retention mechanisms is critical.

In arid and semi-arid regions, drought-tolerant plants like *Agave striata* have evolved complex mechanisms to retain water. Native to regions with low precipitation, *Agave striata* exhibits a range of physiological and biochemical adaptations, including the presence of specialized tissues that store water and the production of fructans—polysaccharides that serve as both energy reserves and hydration buffers [6, 7].These fructans help stabilize cellular membranes by forming hydration layers around macromolecules, enhancing the plant's ability to withstand extreme environmental stress. Monitoring these dynamics at both the tissue and molecular levels requires non-destructive, and water-dynamics-sensitive tools, making THz time-domain spectroscopy (THz-TDS) ideal for this purpose [8–10]. Research using THz spectroscopy has demonstrated its capability to track water dynamics during drought stress and rehydration phases in several plant species, including barley and silver fir [11–14]. This has shown the high sensitivity of the technique to subtle changes in water content across biological tissues [6, 15–18].

Our research explores the internal water distribution within *Agave striata* tissues and the hydration dynamics of fructans in aqueous solutions using THz spectroscopy. The study integrates macroscopic imaging with molecular spectroscopy to provide a comprehensive understanding of water retention strategies at multiple scales. Figure 1 illustrates an *Agave striata* leaf, the molecular structure of its fructans, and the arrangement of water molecules bound around them. By examining how water is distributed within tissues and how fructans behave under different hydration conditions, this research offers new insights into the adaptive mechanisms of drought-tolerant plants. The findings of this study have broader implications beyond plant physiology. Understanding the hydration properties of fructans could inform the development of functional food ingredients with enhanced water-binding capacities, contributing to innovations in food science.

2. Materials and Methods

2.1. Plant material preparation

The *Agave striata* specimens utilized in this study were cultivated under controlled greenhouse conditions at CINVESTAV, Unidad Irapuato. The plants, approximately 4 years old, were originally obtained from the Botanical Garden "El Charco del Ingenio" in San Miguel de Allende, Guanajuato, Mexico. They were maintained in $3500cm^3$ pots containing a soil mixture composed of fine tezontle and leaf litter $(50 : 50v/v)$ to simulate their natural substrate. The plants were watered once a week with approximately $500cm^3$ to ensure adequate hydration while maintaining conditions that reflect the arid environments to which the species is adapted.

To obtain tissue samples, individual leaves were carefully detached, following the natural spiral arrangement characteristic of the species. Freshly collected leaves were rinsed with deionized water to remove surface impurities and promptly chilled to $4^\circ C$ to preserve their physiological properties. Thin sections, each $600\mu m$ thick, were meticulously prepared by hand using a razor blade to maintain precision and avoid mechanical damage. These sections were immediately used for THz imaging to capture their native hydration state and internal water distribution accurately.

2.2. Preparation of saccharide solutions

Commercial agave fructans (FAC) with an average degree of polymerization (DP) of approximately 18 were obtained from Inufib, located in Jalisco, Mexico. The original fructans contained around 6% free fructose, with less than 1% glucose and sucrose present as minor impurities. To enhance purity, these fructans were further refined following a previously established protocol [19], yielding samples with less than 0.5% residual mono- and disaccharides. After purification, the average DP increased to approximately 22, reflecting the removal of smaller sugar units.

For experimental purposes, 5 mL aqueous solutions were prepared using purified fructans, ensuring an excess concentration of solutes to maintain saturated conditions. The preparation method closely followed the protocol described in [19], ensuring consistency with prior studies. The solubility limit of the purified agave fructans is 31.4 g of solute per 100 g of water at 25°C. To thoroughly explore water dynamics in saccharides under THz spectroscopy, the solutions were prepared at two concentrations well below the solubility threshold and two concentrations near the solubility limit. This approach ensures that the study captures hydration behavior across a range of concentrations, from dilute to near-saturated conditions, highlighting any nonlinearities in the interaction between fructan molecules and surrounding water.

2.3. Terahertz spectroscopy setup

The time-domain terahertz imaging measurements were conducted using an API TeraGauge spectrometer, equipped with a femtosecond fiber laser. This laser was coupled to photoconductive switches that served as both the emitter and detector of terahertz pulses, covering a frequency range of 0.1 THz to 2 THz. The terahertz radiation was focused and collected by high density polyethylene lenses. The imaging experiments were carried out in a transmission configuration, as illustrated in Figure 2 a).

To enhance resolution, a $1.5mm$ pinhole was mounted at the focal plane of the terahertz transmitter, which was aligned with the receiver on a common optical axis. The measurements were performed with a spatial resolution defined by a pixel size of $0.5mm$, and the scanning speed was maintained at $0.5mm/s$. Measurements were conducted on $0.6mm$ thin transverse sections of *Agave striata* leaves, as these leaves are significantly thicker than those from typical

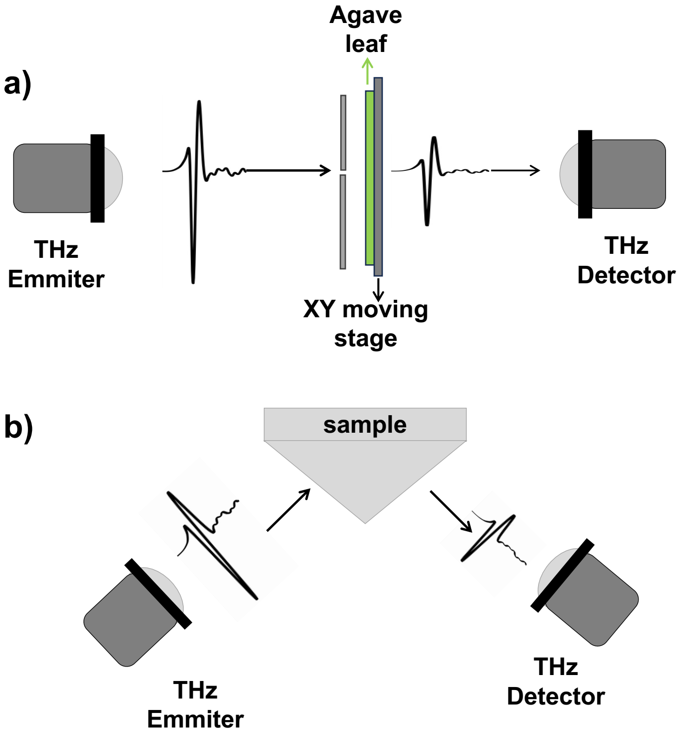

Fig. 2. Terahertz time-domain setup in transmission configuration. **a)** Imaging setup for agave leaves, the emmiter and detector has the same optical axis. The agave leaves were placed on a XY moving stage. **b)** ATR configuration. A Si prism was placed at focal position of the emmiter THz wave. The incidence angle of the THz pulse at the prism surface is 45 degrees.

plants. The samples were placed between two thin polyethylene films and held by a frame on the XY stage.

To analyze the terahertz images and estimate water content at a pixel-by-pixel level, an effective medium theory model was used. This model was fit to the transmission spectra for each individual pixel, allowing for the quantification of water distribution within the plant tissues, following the method described in [20].

For the study of the aqueous saccharide solutions, the same API TeraGauge terahertz time-domain spectrometer was employed, but the system was reconfigured to an attenuated total reflection (ATR) geometry. A silicon prism was incorporated into the setup, positioned at the focal point of the terahertz waves emitted from the source, as depicted in the corresponding Figure 2 b). The terahertz pulses were directed to the prism surface at an incidence angle of ~45°. In this configuration, TE polarization was used to investigate hydration-induced changes in the complex refractive index of the agave fructan solutions. This setup allowed measurement of the interaction between terahertz waves and the saccharide solutions, facilitating the study of their hydration dynamics.

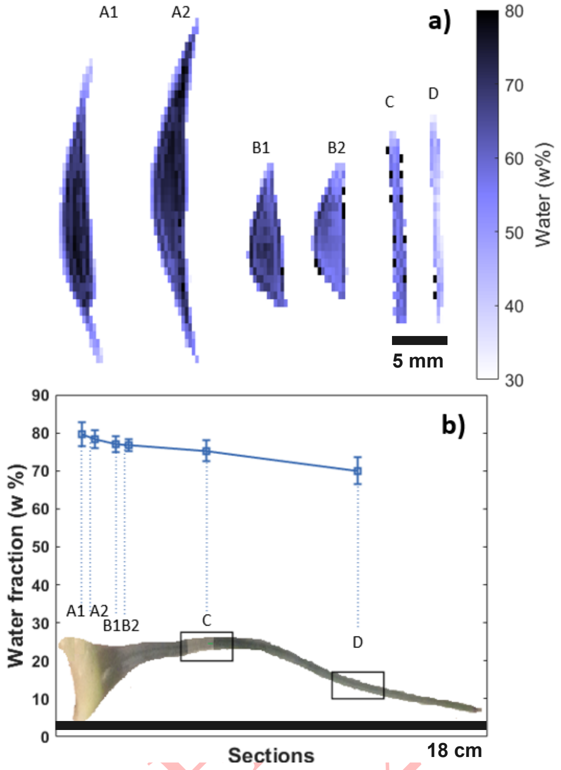

Fig. 3. **a)** Terahertz images of tissue cross sections from the Agave leaves and **b)** their average water content is shown. A photograph of the leaf before sectioning is shown at the bottom indicating the positions of the sections. Sections A1, A2, B1 and B2 are transverse, while C and D are longitudinal.

3. Results

3.1. Water mapping

The tomographic water content images presented in Figure 3 a) provide a detailed visualization of the internal water distribution within *Agave striata* leaves. The weight percentage (w%) that water represents in the tissue was obtained from the THz measurements as described in [21]. These images reveal two distinct regions: an outer, less hydrated layer, and an inner, more water-rich core. The outer tissue layer, identified as the chlorenchyma containing chloroplasts, displays hydration levels ranging between 30w% and 40w%. In contrast, the internal portion of the leaf exhibits more significant variation, with water content exceeding 70w%, peaking towards the central sections. The spectra acquired for the different pixels shows a power SNR ranging between 10^2 and 10^4 for each pixel of the images presented later depending on the hydration level. Figure 3 b) shows the average water content along different cross-sections of the leaf, we obtained a positional profile of succulence along the leaf axis.

3.2. Hydration dynamics of saccharide solutions

Figure 4 a), b) presents the real (n) and imaginary (κ) parts of the complex refractive index for agave fructan solutions across a frequency range between 0.3 THz and 1.2 THz. Both components show a steady decline with increasing frequency, indicating frequency-dependent hydration behavior. To analyze the hydration effect, Figure 4 c) displays the absorption coefficient (α)

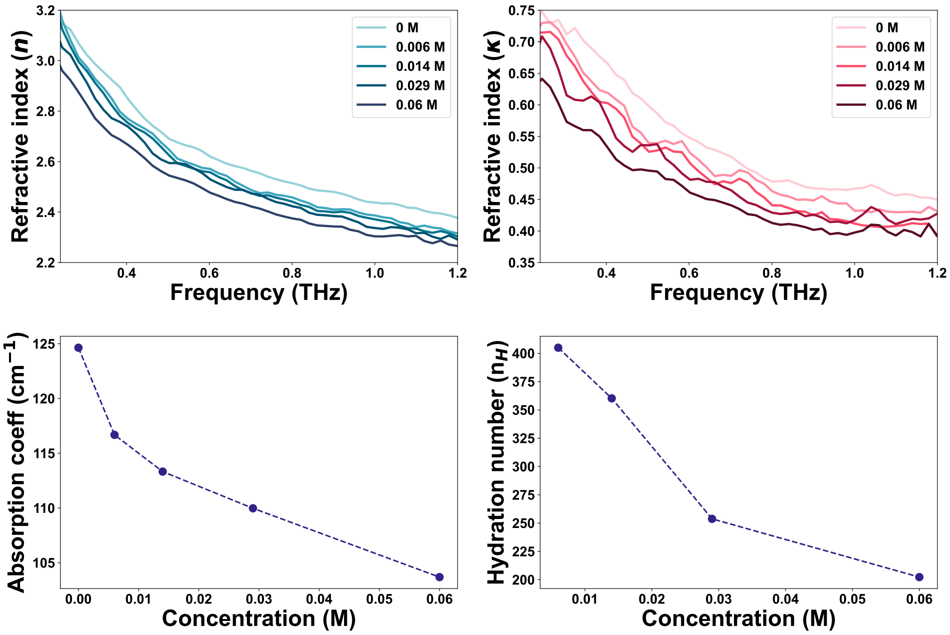

Fig. 4. **a)** and **b)** Variations of the complex refractive index as a function of frequency at different concentrations. **c)** Variation of absorption coefficient at 0.5 THz and **d)** hydration number at different concentrations.

at a test frequency of 0.5 THz for varying solute concentrations. As shown, higher solute concentrations result in a lower absorption coefficient, reflecting a reduction in bulk water due to an increase in hydration water. Using the absorption data at 0.5 THz, we calculated the hydration number of the fructans

$$n_h = N \frac{\epsilon''_{water}(0.5THz) - \epsilon''_{solution}(0.5THz)}{\epsilon''_{water}(0.5THz) - \epsilon''_{background}(0.5THz)}, \tag{1}$$

where N is the total number of water molecules per agave fructan molecule, $\epsilon''_{water}(0.5THz)$ is the imaginary part of the dielectric constant of deionized water, $\epsilon''_{solution}(0.5THz)$ corresponds to the dielectric constant of the fructan solution, and $\epsilon''_{background}(0.5THz)$ represents the fast relaxation component of liquid water [22–24]. Figure 4 d) shows the relationship between hydration number and solute concentration of purified fructan solutions.

The results indicate that as solute concentration decreases, both the absorption coefficient and hydration number increase, reflecting more extensive hydration layers surrounding the macromolecules. These trends suggest that the hydration spheres of neighboring fructans molecules overlap at high concentrations enhancing interactions between water molecules and the solutes. The hydration shells of fructants are rather large in comparison to other carbohydrates as we have shown in a previous contribution [19]. The observed hydration dynamics align with previous studies on polysaccharides in solution, highlighting the complex organization of hydration water in the vicinity of the macromolecules [25–27].

4. Discussion

The terahertz imaging results provided valuable insights into the water distribution within *Agave striata* leaves, revealing distinct differences between the outer and inner regions. Water content was relatively stable along the length of the leaf, with a noticeable drop near the tip, aligning with the diminishing size of internal tissues towards the apex. This finding highlights the spatial variability in water storage within the leaf, a crucial feature for plants adapted to arid environments. The presence of fructans in the succulent tissues likely contributes to the plant's resilience under low water conditions, as fructans are known to stabilize cellular membranes, preventing damage during dehydration and rehydration cycles [28–30].

A direct comparison between the water content measured using THz imaging and traditional gravimetric methods highlights the distinctive advantages of THz imaging. Traditional methods require samples to be weighed, dried and reweighed, making the process destructive and time-consuming, while THz imaging only involves cutting sections and immediately subjecting them to analysis. This non-invasive, and rapid approach significantly enhances efficiency, making it particularly well-suited for high-throughput studies of water content in plant tissues. Beyond its practicality, THz imaging offers a unique advantage in detecting water content with higher precision, thanks to the large number of OH groups in fructans that interact through hydrogen bonding. The particularly branched structure of agave enhances hydration levels, an effect that THz imaging can capture more effectively than traditional methods.

In addition to tissue analysis, terahertz spectroscopy was used to investigate the hydration dynamics of agave fructans in aqueous solutions. Both the absorption coefficient and hydration number were calculated, with results showing a non-linear increase as solute concentration decreased. This behavior is attributed to the aggregation of water molecules within hydration layers, forming complex and unusually large bound-water-structures around the fructans which, owing to the formation of fructan-water hydrogen bonds, are expected to require higher energy investment to evaporate in comparison to free water.

These findings demonstrate the versatility of terahertz spectroscopy for both tissue imaging and molecular hydration studies. The ability to quantify water content and explore hydration dynamics at the molecular level underscores the utility of terahertz technology for plant science research. This dual approach provides a comprehensive understanding of water retention within agave plants, from large-scale tissue hydration to the microscopic behavior of water around macromolecules. The integration of these insights reveals how water-retention strategies, aided by fructans, enhance the drought tolerance of agave, offering promising implications for agricultural practices in arid climates.

5. Conclusions

This study demonstrates the utility of terahertz spectroscopy and imaging in exploring water retention strategies and hydration dynamics in *Agave striata*. Terahertz imaging enabled non-invasive, high-resolution mapping of water distribution within leaves, providing insights into how hydration varies along the tissue structure.

The role of fructans in drought resistance was further elucidated through terahertz spectroscopy, revealing complex solvation dynamics of these particular carbohydrates in aqueous solutions. The non-linear relationship between solute concentration and hydration number underscores the importance of molecular crowding and hydration layer formation in water retention. The purified fructans showed higher hydration levels, confirming that reducing impurities enhances their interaction with water molecules.

By combining tissue-level imaging with molecular hydration analysis, this research offers a new approach to studying plant water management strategies. These findings contribute to our understanding of drought tolerance mechanisms and open avenues for future applications in agriculture, particularly for enhancing crop resilience in arid environments. Terahertz imaging opens the possibility of resolving the water distribution spatially and its temporal evolution in almost any plant species. Yet the uniqueness of the role played by fructans as water retention agents in agaves and similar species opens the question of the existence of other biomolecules with large hydration shells as a mechanism to counter act drought, this is an aspect in which terahertz spectroscopy could become a key tool in the future to obtain an answer.

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Data Availability Statement. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

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