Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Non-Resonant Air-Coupled Ultrasound for Quantifying Leaf Hydration Dynamics and Turgor Loss in Potato Leaves

Muhammad Awais Sattar

muhammad.awais.sattar@associated.ltu.se

Luleå University of Technology

Tomas E. Gomez Alvarez-Arenas

Instituto de Tecnologías Físicas y de la Información, Spanish National Research Council (CSIC)

Eustaquio Gil-Pelegrín

Estación Experimental de Aula Dei

Domingo Sancho-Knapik

Agri-Food Research and Technology Centre of Aragon

Velemir Ninkovic

Swedish University of Agricultural Sciences

Dina Shona Laila

Luleå University of Technology

Article

Keywords:

Posted Date: February 25th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8355261/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on September 27th, 2026. See the published version at https://doi.org/10.1038/s41598-026-72465-3.

Non-Resonant Air-Coupled Ultrasound for Quantifying Leaf Hydration Dynamics and Turgor Loss in Potato Leaves

Muhammad Awais Sattar[1,*], Tomas E. Gomez Alvarez-Arenas[2], Eustaquio Gil-Pelegrín[3], Domingo Sancho-Knapik[4], Velemir Ninkovic[5], and Dina Shona Laila[1]

[1]Automatic Control, Department of Computer Science, Electrical and Space Engineering, Luleå University of Technology, 971 87, Luleå, Sweden
[2]Instituto de Tecnologías Físicas y de la Información, Spanish National Research Council (CSIC), Serrano 144, Madrid 28006, Spain
[3]Estación Experimental Aula Dei (EEAD-CSIC), 50059 Zaragoza, Spain
[4]Agri-Food Research and Technology Centre of Aragon (CITA), Zaragoza, Spain
[5]Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden
[*]Corresponding author: muhammad.awais.sattar@associated.ltu.se

ABSTRACT

This study investigates the use of non-resonant air-coupled ultrasound technique as a non-destructive tool for quantifying hydration dynamics in potato (*Solanum tuberosum*) leaves. Conventional methods for measuring leaf water status, such as relative water content or pressure–volume (P–V) curves, are destructive and labor-intensive, whereas ultrasound can probe internal tissue structure through mechanical wave propagation. In this work, freshly detached potato leaves were gradually dehydrated under controlled laboratory conditions while ultrasonic transmission signals were recorded using broadband air-coupled transducers operating between 250 kHz and 900 kHz. Time-domain and frequency-domain features, including peak-to-peak amplitude, time-of-flight (ToF), spectral amplitude, and transmission coefficient, were extracted and analyzed as a function of relative water content (RWC). The normalized peak-to-peak (pk–pk) amplitude curve exhibited a clear correlation to the P–V curve, with ultrasonic turgor loss point (TLP) candidate ($\mathrm{TLP_c} = 0.973$) that closely matched the physiological turgor loss point (TLP) obtained from P–V analysis ($\mathrm{RCW_{TLP}} = 0.978$). ToF showed a characteristic biphasic response with a slight initial decrease at high RWC followed by a sharp decrease around the dehydration threshold ($\mathrm{TLP_c} \approx 0.956$), reflecting the progressive loss of cell-wall tension and increasing mesophyll deformability that reduce acoustic velocity during dehydration. Spectral amplitude and transmission coefficient exhibited frequency-dependent sensitivity that peaked near 650 kHz. All ultrasonic parameters produced TLP candidates within 2.5% of the physiological reference, confirming that acoustic propagation is governed by turgor-driven structural transitions in the mesophyll. These results establish that non-resonant ultrasound technique can characterize hydration status in leaves that lack thickness resonances, extending the scope of ultrasonic hydration sensing beyond resonant techniques. The high physiological TLP observed for potato confirms that turgor is lost very early during dehydration, reflecting the species limited osmotic adjustment and drought tolerance. The limitation of this study is that measurements were performed on detached leaves under controlled conditions; future in situ validation on intact plants under varying microclimates is required.Future work will also focus on deriving attenuation coefficients to separate intrinsic viscoelastic attenuation from absorption mechanisms. Overall in this study, pk–pk amplitude emerged as the most sensitive, computationally efficient, and practical parameter for real-time ultrasonic monitoring of leaf hydration and early stress detection.

Introduction

Monitoring plant hydration condition is of paramount importance in agriculture, as water availability directly affects plant growth, crop yields[1] and plant health and productivity in general. Water deficits (drought stress) can sharply reduce productivity. For example, severe drought events have been shown to reduce global cereal yields by about 10% on average[2]. Agriculture is the largest consumer of freshwater worldwide, accounting for roughly 70% of global water withdrawals[3]. Inefficient water use not only threatens farm sustainability but also exacerbates water scarcity under climate change, which is causing more frequent crop water stress and yield reductions in many regions[4]. In this context, reliable methods for assessing plant water status in real-time are crucial for optimizing irrigation and enhancing crop water productivity. A key physiological marker of leaf water status is the turgor loss point (TLP). Typically determined from pressure–volume (P–V) curves[5], TLP is defined as the leaf water potential at which cell turgor pressure becomes zero and tissues transition from an elastic to an inelastic state. The TLP marks the threshold at which loss of turgor triggers mechanical and metabolic limitations on photosynthesis, stomatal

conductance, and hydraulic function. It is therefore widely used as an indicator of species drought tolerance and survival capacity under water stress[6,7]. Species with low (more negative) TLP values can maintain turgor and metabolic activity under drier conditions, while species with high TLPs lose turgor early, reflecting limited osmotic adjustment and higher drought sensitivity. In crops such as potato, which exhibit relatively high TLP values, this transition occurs at very high hydration levels, making early detection of turgor loss essential for timely irrigation and stress management. Early detection of water stress in crops like potato is critical, as even moderate dehydration can impair physiological functions (e.g., stomatal conductance, photosynthesis) and ultimately diminish tuber yield and quality[8]. Potato (*Solanum tuberosum*) is a globally significant food crop ranked fourth in production after maize, wheat, and rice[9] and is known to be sensitive to water deficits[10]. Studies show that insufficient watering can dramatically curtail potato yields. For instance, reducing irrigation to about one-third of full requirements caused over a 35–40% drop in potato tuber yield in field trials[11]. These considerations underscore the need for practical, non-destructive tools to monitor plant water status and guide irrigation decisions in potato cultivation and beyond.

Conventional measures of plant water status (such as leaf water potential or relative water content) often involve destructive sampling or labor-intensive protocols, making them impractical for continuous monitoring in the field e.g, measuring relative water content (RWC) typically requires excising leaves for immediate weighing and drying, a cumbersome process, disrupts the plant, and cannot be repeated on the same tissue[12]. Likewise, the pressure chamber (pressure bomb) method for leaf water potential, while reliable, involves detaching leaves and is not amenable to high-frequency measurements on living plants[13].

Over the past decades, researchers have sought non-invasive techniques to track water status dynamics in situ. Several plant-based sensors and remote sensing approaches have been explored. Thermocouple psychrometers can continuously measure internal water potential, but their installation is complex and not well-suited to rapid leaf monitoring[14]. Infrared thermography and canopy reflectance indices allow repeated, non-contact estimation of water status by detecting plant temperature or color changes related to transpiration[15–17]. These remote sensing methods have shown promise for irrigation scheduling since a closed leaf stomata under water stress leads to leaf heating detectable via thermal imaging and changes in reflectance at certain wavelengths[18]. However, such optical techniques can be confounded by environmental factors and plant structure, such as variable leaf angles and backgrounds, which limit their accuracy in some conditions. Most of these approaches rely on optical or radiometric properties that can be affected by environmental conditions. Microwave and terahertz-wave sensors have also emerged as novel tools for assessing leaf water content from a distance[19,20]. For instance, terahertz time-domain spectroscopy has been used to monitor leaf water dynamics and was able to distinguish changes in leaf water content with high sensitivity[21,22]. Despite these advances in optical and radiometric methods, there remains a demand for alternative approaches that can directly and non-destructively probe plant water status, with minimal interference from external conditions. In this regard, ultrasound-based techniques are gaining attention as a complementary solution for sensing plant hydration. Ultrasound refers to mechanical sound waves at frequencies above the audible range. Ultrasonic waves are well known as a non-destructive tool for probing the internal structure and properties of materials, from engineering components to biological tissues[23,24].

In plant science, ultrasonic measurements offer a means to investigate the hydration status of tissues physically, since the propagation of sound waves in a plant is influenced by the tissue's water content and elasticity. Ultrasonic techniques allow probing the physical structure and water content of the tissue directly. Historically, most ultrasonic studies of leaves have employed resonant techniques, where the leaf behaves as an acoustic resonator and thickness resonances are excited and detected. Sound waves travel through plant organs by vibrating cell walls, water, and air spaces. Changes in hydration alter the density and modulus of the tissue, which in turn affect the speed and attenuation of acoustic waves[25]. Notably, water has a much higher acoustic impedance than air, so the presence of water in plant cells enables efficient transmission of ultrasound. However, as leaves or stems lose water, sound propagation becomes slower and more attenuated[26]. Prior studies on woody stems have found a strong inverse relationship between moisture content and ultrasonic velocity[27]. As stem water content increases, the velocity of an ultrasonic pulse decreases significantly[28]. For example, recent experiments on timber and tree trunks have shown that ultrasound velocity drops measurably with higher wood moisture. A study quantified this effect in tropical wood samples, providing calibration models for moisture estimation via acoustic speed[29]. Similarly, ultrasound signal attenuation tends to increase in wetter or more heterogeneous plant tissues, since more acoustic energy is dissipated or scattered when fluid-filled structures are abundant. Ultrasonic detection thus inherently offers a non-invasive way to sense internal hydration status. Unlike soil probes that must be inserted or leaf-clipping methods, an ultrasound transducer can be placed against the plant or even contactlessly coupled through air to send and receive sound waves, causing minimal disturbance[30], and it distinguishes itself from other sensing modalities by its ability to penetrate plant tissues and reveal internal structural and hydration changes that are invisible to optical methods, offering a uniquely non-destructive approach for real-time plant health assessment[31].

Over the last decade, a number of researchers have pioneered ultrasound-based measurements of water status in plants. Early work focused on tree trunks and large stems, using transit-time or echo analysis of ultrasonic pulses to detect changes in stem water content. They observed that the time-of-flight of the primary echo (related to sound velocity) lengthened as stems dehydrated, and they reported a near-linear correlation (average $r \approx 0.98$) between stem moisture content and ultrasonic velocity. In live sunflower plants under variable soil moisture, the ultrasonic echo position shifted consistently with diurnal changes in

water status, and the shifts were closely aligned with soil moisture fluctuations (correlation about 0.92). These results indicate that simple ultrasonic metrics can reflect dynamic water uptake or loss in stems with high fidelity. Beyond stems, ultrasound has also been applied to leaves, where water content can change rapidly and is directly tied to physiological function[25]. A notable initial approach was the work on the non-contact resonant ultrasound spectroscopy (NC-RUS) method[32,33], which enabled accurate measurements in leaves that exhibit distinct thickness resonances. In this configuration, broadband airborne ultrasound excites natural vibration modes within the leaf, and the resulting resonant frequencies depend on its elastic modulus, density, and thickness, all of which vary with water content. Resonant methods have therefore proven highly effective for species that display well-defined thickness modes, but they cannot be applied to leaves that do not resonate, which represents a significant limitation for many agronomically important crops[12,30].

In the non-contact resonant ultrasound spectroscopy (NC-RUS) technique, broadband airborne ultrasound in the MHz range is directed at the leaf to excite its natural vibration modes, or thickness resonances. The leaf behaves as an acoustic resonator in which standing waves are established across its thickness, producing peaks in the transmitted or reflected signals. These resonant frequencies depend on the elastic modulus, density, and thickness of the tissue, all of which are strongly influenced by water content. As a leaf dehydrates, it typically becomes thinner and more compliant, leading to an increase in resonance frequency. In contrast, a fully turgid leaf exhibits a lower resonance frequency because of its higher water mass and stiffness. The bandwidth or quality factor of the resonance, which is related to attenuation, also varies with hydration: drought-stressed leaves often show sharper, higher-Q resonant peaks, whereas hydrated leaves exhibit broader and more damped resonances. This behavior has been successfully used to estimate leaf hydration non-destructively in a variety of species. However, the applicability of resonant techniques remains limited to leaves that display distinct thickness modes. Many agronomically important species, including potato, do not exhibit clear resonances, which restricts the use of NC-RUS for their characterization. This limitation highlights the need for non-resonant ultrasonic methods capable of analyzing leaves with diverse structures under realistic agricultural conditions.

To overcome these limitations, Fariñas *et al.* (2022)[30] introduced a non-resonant, air-coupled ultrasonic transmission method capable of characterizing leaves that do not exhibit thickness resonances. That study demonstrated the equivalence between the parameters obtained with resonant and non-resonant measurements, confirming that prior findings from resonant approaches can be extended to this new configuration. However, it did not analyze how ultrasonic properties vary with hydration level. The present work addresses this gap by applying the non-resonant approach to potato leaves, marking both the first ultrasonic hydration study based on non-resonant transmission and the first ultrasonic characterization ever conducted on potato leaves. Unlike resonant methods, the non-resonant approach also enables direct examination of how ultrasonic attenuation varies with frequency, providing new insight into hydration-dependent acoustic loss mechanisms within the leaf.

To address this specific gap, the present study investigates how low-frequency ultrasound can be used to characterize water status in potato leaves by tracking changes in ultrasonic signal features during progressive leaf dehydration. The focus of this work is on developing a non-contact sensing framework tailored specifically for potato physiology, using frequency-domain ultrasonic techniques to probe internal leaf structure without physical disturbance. Potato (*Solanum tuberosum*) is chosen in this study for obvious reason because it is one of the most important staple crops in Europe. Although this study focuses on potato, the ultrasonic techniques can likely be applied also for real-time hydration monitoring of other crops, such as tomato and maize. This work extends previous resonant studies to non-resonant hydration analysis and is driven by the following objectives:

1. To characterize how multiple ultrasonic features, including peak-to-peak amplitude, time-of-flight delay, spectral amplitude, and transmission coefficient, change with relative water content (RWC) in potato leaves during controlled dehydration.
2. To determine which ultrasonic parameters provide the greatest sensitivity to water status and physiological transitions in leaf tissue.

Correspondingly, we aim to answer the following research questions (RQs):

RQ1: How do different ultrasonic features (peak-to-peak amplitude, time-of-flight, spectral amplitude, and transmission coefficient) respond across the complete dehydration profile of potato leaves, and are these responses consistent across multiple leaves and plants?

RQ2: Which frequency-dependent ultrasonic parameters provide the strongest contrast with relative water content (RWC), and can they serve as reliable, non-destructive indicators of leaf water status?

RQ3: Do amplitude-based ultrasonic features exhibit a characteristic curve dependence on RWC, and can this relationship be linked to the physiological turgor loss point (TLP) of the leaf?

To address these research questions, a series of controlled experiments using non-contact ultrasonic transmission measurements were conducted on freshly detached potato leaves undergoing natural drying. At defined time points, ultrasonic signals were recorded, and concurrent changes in leaf mass were tracked to calculate relative water content (RWC). Each signal was compared against a baseline reference to account for system variability and environmental drift. Time-domain features such as peak-to-peak amplitude and time-of-flight were extracted, and frequency-domain transmission coefficients were computed to

examine hydration-dependent trends. By relating these ultrasonic measurements directly to physiological metrics such as RWC, this study provides a dedicated, non-invasive approach for characterizing hydration status in potato leaves and contributes to the development of new ultrasonic tools for precision crop monitoring in water-sensitive systems.

Materials, Apparatuses and Methods

Experiments were conducted on potato plants cultivated under controlled indoor conditions beginning in May-2025. The plants were maintained in well-drained soil with regular irrigation and moderate light to ensure uniform growth. Ultrasonic measurements were performed in three experimental rounds between May-2025 and October-2025, corresponding to different stages of plant development (approximately two to four months after planting). From these plants, a total of seventeen fully expanded, healthy leaves were selected sequentially for dehydration experiments. Prior to each measurement, plants were kept in low-light conditions for 24 hours to ensure full leaf turgor (100% hydration at $t = 0$). Each selected leaf was excised at the petiole immediately before testing to preserve its natural hydration state. Leaf thickness at the mid-lamina was measured using a digital micrometer immediately after excision, yielding values in the range of approximately 140–320 μm. During ultrasonic testing, each detached leaf was mounted flat and held stationary in a custom frame positioned vertically between the air-coupled transmitter and receiver transducers. All measurements were conducted under ambient laboratory conditions (temperature 22–24 °C, relative humidity 35–45%). Although environmental factors such as humidity and air temperature can influence airborne ultrasound propagation, these effects were minimized by comparing each signal to its corresponding reference measurement under the same conditions. A representative example of the sampled plants and corresponding leaves used in the measurements is shown in Figure 1.

To characterize the water status of potato leaves using ultrasound, we employed a non-resonant, air-coupled ultrasonic transmission system developed by Fariñas *et al.*[30]. The system comprises four core components, i.e. a pair of wideband air-coupled ultrasonic transducers (Tx and Rx), analog pulser/receiver electronics, digitization and control software, and a mechanical holder to ensure proper leaf positioning between the transducers.



Figure 1. Representative potato leaves at full hydration and after drying. Top: two cultivated plants at the start of the measurement series (May 2025), showing selected leaves (labeled 1–4) at full hydration. Bottom: the same leaves following dehydration to the dry endpoint (post-drying), placed in Petri dishes for reference.

The sensors were 1–3 connectivity piezoelectric composite transducers made of PZT-5A fibers embedded in an epoxy matrix, with a ceramic volume fraction of approximately 65%. The central frequency range (250–900 kHz) was selected based on pilot measurements on potato leaves to ensure adequate penetration and signal-to-noise ratio across the frequency band of interest. The transducers exhibited piston-like behavior, producing a quasi-planar acoustic wavefront suitable for accurate through-transmission measurements. Ultrasonic pulses were generated using a commercial Olympus 5077 pulser,

delivering up to 400 V spike or square-wave excitation. The received signals were amplified by 40 dB and digitized using a Tektronix TBS2000 series oscilloscope. Time-domain signals were recorded and processed using Python-based scripts for spectral analysis and feature extraction.

Ultrasound Measurement Procedure

The measurements were performed by repeating a cycle of *weighing, recording ultrasound data, and drying* as each leaf dehydrated naturally under ambient conditions. Starting from a fully hydrated state, the leaf was first weighed and then an ultrasonic through-transmission waveform $x(t)$ was recorded with the leaf held in a fixed geometry between the air-coupled transducers. After the final measurement, the leaf was oven-dried to determine its dry mass.

The water status was quantified as relative water content (RWC) to enable comparison across leaves of different sizes, following

$$\mathrm{RWC}(t) \;=\; \frac{W_t - W_{\mathrm{dry}}}{W_0 - W_{\mathrm{dry}}} \times 100\ \%, \tag{1}$$

where W_t is the fresh mass at time t, W_0 is the fully hydrated mass at the start of the experiment, and W_{dry} is the oven-dry mass. By definition, $0 \le \mathrm{RWC} \le 100\,\%$.

Each recorded trace $x(t)$ was aligned to a reference trace $x_{\mathrm{ref}}(t)$ obtained under identical geometry (air path or an early hydrated baseline). A short tapered window $w(t)$ centered on the first arrival was applied to isolate the direct transmitted wave and minimize echoes and reverberations,

$$x_w(t) \;=\; x(t)\, w(t).$$

Using a consistent window across all time points ensured that extracted features corresponded to the same physical arrival.

The transmission amplitude in the time domain was quantified as the peak-to-peak value,

$$A_{\text{pk-pk}} \;=\; \max_t x_w(t) \;-\; \min_t x_w(t), \tag{2}$$

which increases when coupling through hydrated tissue is stronger. Plotting $A_{\text{pk-pk}}$ against RWC provides a direct measure of how transmission varies with hydration for each leaf.

The time-of-flight (ToF), defined as the relative delay of the ultrasonic wave through the leaf compared with a reference, was estimated by two complementary methods. The primary estimator was cross-correlation,

$$\Delta t_{\mathrm{xcorr}} \;=\; \arg\max_{\tau} \int x_w(t)\, x_{\mathrm{ref},w}(t+\tau)\, \mathrm{d}t, \tag{3}$$

which uses all samples within the analysis window. A secondary estimator based on edge detection was applied when waveform shapes changed significantly. After normalizing both signals to unit peak, the first threshold crossing at a fixed level θ defined the arrival indices T_{meas} and T_{ref}, and the delay was computed as

$$\Delta t_{\mathrm{edge}} \;=\; t_{\mathrm{meas}}(T_{\mathrm{meas}}) \;-\; t_{\mathrm{ref}}(T_{\mathrm{ref}}). \tag{4}$$

Using both approaches ($\Delta t_{\mathrm{xcorr}}$ and Δt_{edge}) provided cross-validation and reduced the risk of cycle skips when waveforms changed during dehydration. Throughout this work, ToF is expressed as the relative delay between each dehydrated waveform and its corresponding fully hydrated reference trace, computed using cross-correlation. Because this delay is defined relative to the hydrated signal, the resulting ToF values may take on negative values and may shift toward more negative delays as dehydration progresses (as shown in Figure 3). This behavior reflects changes in waveform shape, reduced similarity to the hydrated reference, and the position of the correlation peak not the absolute acoustic propagation time through the leaf. In physical terms, the absolute ToF is expected to increase with dehydration because ultrasound travels more slowly as the tissue loses turgor, becomes more deformable, and its effective elastic modulus decreases. The decreasing trend observed in the relative ToF therefore corresponds to correlation-based alignment effects rather than a true increase in acoustic velocity.

The frequency content of the gated signal was obtained via the discrete Fourier transform (DFT),

$$X(f) \;=\; \left| \sum_{n=0}^{N-1} x_w[n]\, e^{-j2\pi f n/N} \right|, \tag{5}$$

to examine how different frequency components were transmitted. Tracking $|X(f)|$ at selected frequencies as a function of RWC revealed how the spectral magnitude evolved with hydration.

The normalized transmission coefficient, removing effects of source level and gain, was defined as

$$\mathscr{T}(f) = \frac{|X(f)|}{|X_{\text{ref}}(f)|}, \tag{6}$$

$$\mathscr{T}_{\text{dB}}(f) = 20\log_{10}\big(\mathscr{T}(f)\big), \tag{7}$$

so that $\mathscr{T}(f)$ or a band-average of $\mathscr{T}_{\text{dB}}$ could be plotted against RWC to provide a frequency-resolved view of hydration effects.

All features, including $A_{\text{pk-pk}}$, Δt, spectra, and $\mathscr{T}(f)$, were computed using identical preprocessing to ensure that the observed trends reflected changes in the leaf rather than the measurement setup. To quantify variability across biological replicates, all hydration-response curves were averaged across the seventeen leaves measured in this study. For each ultrasonic feature, the mean value and the 95% confidence interval (CI) were computed at each RWC bin. This approach provides a robust non-parametric estimate of uncertainty and accounts for the uneven sampling density across the dehydration trajectory. All figures displaying hydration-response curves therefore show the mean trend with shaded 95% CIs.

Results

All ultrasonic hydration response curves exhibited a clearly similar trend. For each ultrasonic feature, the point of inflection (PoI) of the fitted sigmoid curve was extracted as the hydration level where the acoustic response changed most rapidly. Borrowing the notion of TLP, hereafter this PoI is denoted as the TLP candidate (TLP_c), which serves as a comparative marker for identifying the hydration transition detection.

Time-domain transmission (normalized pk–pk) as a function of hydration

The normalized peak-to-peak (pk–pk) amplitude, representing the relative change in transmitted voltage with respect to the fully hydrated state, showed a consistent and reproducible decrease with declining relative water content (RWC) across all measurements. Data from a total of seventeen leaves, collected over three experimental rounds, were averaged to obtain the mean hydration response. The resulting mean trend revealed a characteristic curve dependence on RWC (Figure 2). The amplitude decreased progressively with dehydration, showing the steepest decline at high RWC values near full hydration. This strong early reduction indicates that ultrasonic transmission is highly sensitive to the initial stages of water loss, and the gradient continues to decrease as the curve approaches the TLP_c, where the ultrasonic response changes most rapidly. This behavior reflects a progressive reduction in ultrasonic transmissivity as water is lost from the mesophyll, caused by reduced cell-wall tension, increased tissue deformability, and enhanced internal friction that increases ultrasonic attenuation. The fitted sigmoid model showed excellent agreement with the experimental data ($R^2 = 0.997$), with $\text{TLP}_\text{c} = 0.973$. These results demonstrate that normalized ultrasonic amplitude provides a reliable quantitative marker of leaf water status and turgor in potato tissue.

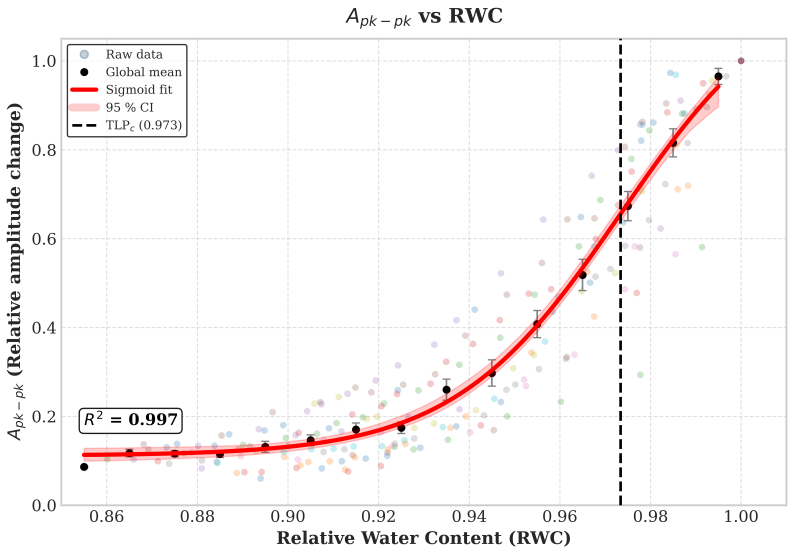

Figure 2. Normalized peak-to-peak amplitude ($A_{\text{pk}-\text{pk}}$) as a function of relative water content (RWC) for all measured potato leaves. Each point represents the relative amplitude change with respect to the fully hydrated state. The solid red line shows the fitted sigmoid model ($R^2 = 0.997$), and the dashed vertical line denotes the TLP candidate ($\text{TLP}_\text{c} = 0.973$).

Time-of-flight as a function of hydration

The mean time-of-flight (ToF) of the transmitted ultrasonic pulse, averaged across all seventeen leaves, showed a consistent and reproducible decrease with declining relative water content (RWC) (Figure 3). This decreasing trend reflects a shift in the correlation-based delay relative to the hydrated baseline, producing more negative relative ToF values as the waveform becomes increasingly distorted during dehydration. It does not indicate that ultrasound propagates faster in drier tissue; the absolute propagation time is still expected to lengthen as acoustic velocity decreases with water loss. The global mean trend revealed a gradual reduction in ToF across the full hydration range, with a steeper decline near higher RWC values, indicating that ultrasonic measurements are sensitive to early structural changes within the mesophyll. Across the full range, the mean ToF variation was approximately 0.4 μs, representing measurable mechanical changes accompanying dehydration. As RWC decreased further, the ToF decline intensified below 0.90, reflecting the progressive loss of cell-wall tension, increased mesophyll deformability, and enhanced viscoelastic damping within the tissue. The TLP_c derived from the ToF curve occurred at RWC = 0.956, demonstrating that ToF is highly sensitive to the mechanical relaxation processes accompanying dehydration. The ToF trend mirrors the behavior observed for the normalized amplitude, both exhibiting similar trend of dependencies on RWC but in opposite directions. Together, these results demonstrate that ToF provides a complementary metric to amplitude-based features, capturing correlation-based signatures of the structural dehydration of the leaf.

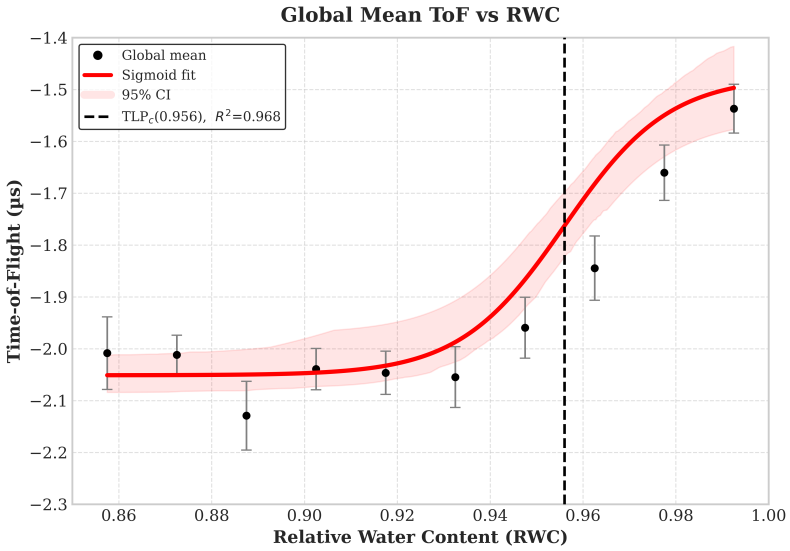

Figure 3. Global mean time-of-flight (ToF) as a function of relative water content (RWC) for all seventeen potato leaves. The averaged ToF decreases systematically with dehydration, reflecting correlation-based shifts rather than absolute acoustic velocity changes. The dashed line marks the TLP candidate ($TLP_c = 0.956$).

Spectral amplitude as a function of hydration

The normalized spectral amplitude, averaged across all seventeen potato leaves, showed a consistent and reproducible decrease with declining relative water content (RWC) across all analyzed frequency bands (Figure 4). The amplitude declined steeply at higher RWC values, indicating strong ultrasonic sensitivity to early dehydration, and then decreased more gradually toward lower hydration levels. The fitted sigmoid curves captured this behavior accurately across all frequencies, with the TLP_c shifting toward higher RWC as frequency increased from $TLP_c = 0.943$ at 350 kHz to $TLP_c = 0.964$ at 650 kHz. This frequency-dependent shift indicates that higher-frequency components respond earlier to dehydration, as they are preferentially attenuated because higher-frequency waves experience stronger viscoelastic losses as cell walls lose tension and mesophyll tissues become more deformable during dehydration. The upward shift in TLP_c with increasing frequency arises because higher-frequency waves experience stronger viscoelastic losses as cell walls lose tension and the mesophyll becomes more deformable during dehydration. As turgid cells lose wall tension during dehydration, their walls become more curved and deformable, leading to increased viscoelastic losses that disproportionately affect higher-frequency components such as those in the 550–650 kHz range. This makes these frequencies especially sensitive to early mechanical relaxation in the mesophyll. Lower-frequency waves, with longer wavelengths, are less affected by these initial disruptions and therefore register the transition at slightly lower RWC. Among the analyzed bands, 650 kHz exhibited the steepest decline and the strongest sensitivity to hydration, confirming it as the optimal frequency for detecting dehydration-induced changes in ultrasonic transmission. Overall, the spectral amplitude results highlight that frequency-resolved ultrasonic attenuation, particularly in the 450–650 kHz range, serves as a sensitive indicator of mesophyll integrity and water continuity during dehydration.

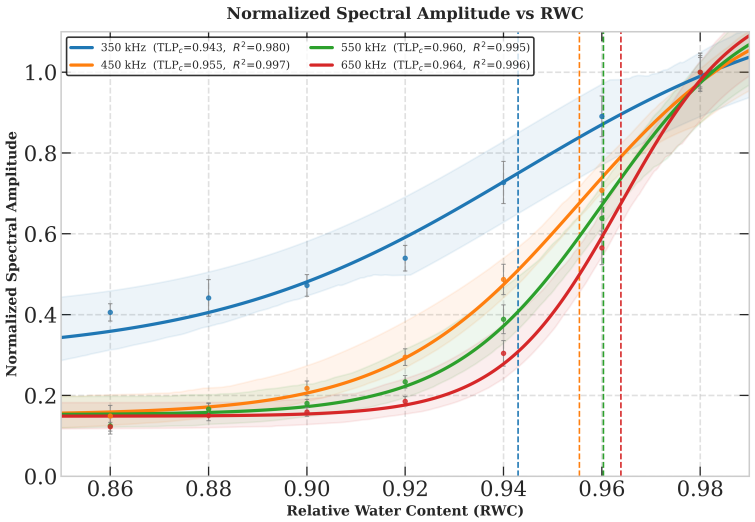

Figure 4. Normalized spectral amplitude as a function of relative water content (RWC), averaged over seventeen potato leaves for four frequency bands (350–650 kHz). Spectral amplitude decreases with dehydration, and the fitted TLP candidates (TLP_c) shift toward higher RWC with increasing frequency. The 650 kHz band shows the steepest decline and the strongest sensitivity to hydration changes, indicating that higher frequencies respond earlier to dehydration.

Transmission coefficient as a function of hydration

The normalized transmission coefficient, averaged across all seventeen leaves, exhibited a systematic decrease with declining relative water content (RWC) across all analyzed frequency bands (Figure 5). Transmission coefficient decreases progressively with dehydration, followed by a sharper drop at lower hydration levels. Sigmoid fits captured this behavior accurately at each frequency, with the TLP_c shifting toward higher RWC as frequency increased, from $TLP_c = 0.937$ at 350 kHz to $TLP_c = 0.965$ at 650 kHz. This frequency-dependent shift demonstrates that higher-frequency components respond earlier to dehydration, as they are more strongly affected by microstructural changes such as reduced cell-wall tautness, increased deformability, and higher internal friction within the mesophyll, all of which enhance viscoelastic attenuation. Among all frequencies, the 650 kHz band displayed the steepest decline and the strongest sensitivity to hydration changes, confirming it as the most responsive frequency for tracking ultrasonic transmission changes during leaf dehydration. Overall, these results show that the transmission coefficient provides a robust, frequency-resolved indicator of hydration state.

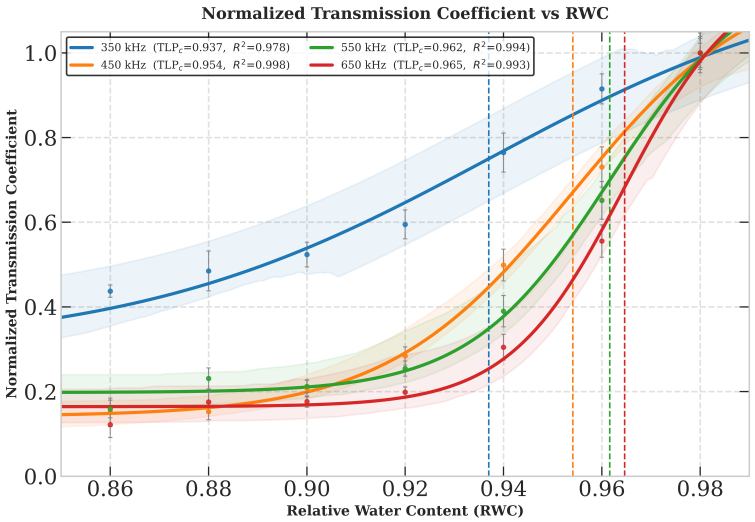

Figure 5. Normalized transmission coefficient as a function of relative water content (RWC), averaged over seventeen potato leaves for four frequency bands (350–650 kHz). Transmission decreases with dehydration, and the fitted TLP candidates (TLP_c) shift toward higher RWC with increasing frequency. The 650 kHz band shows the steepest decline and the strongest sensitivity to hydration changes.

Comparison of turgor loss points across methods

In the ultrasonic analyses, the hydration transition detected acoustically is quantified using the TLP candidate (TLP_c), which is the hydration level at which the fitted sigmoid curve changes most rapidly during dehydration. Although TLP_c often lies close to the physiological turgor loss point (TLP), it does not represent an independent TLP for each frequency; the physiological TLP is a single species-specific threshold at which leaf cells lose turgor and tissues transition from elastic to inelastic behavior due to water loss. The TLP is typically determined from pressure–volume (P–V) curves that describe the relationship between leaf water potential (Ψ) and relative water content (RWC)[5]. At high hydration, the P–V curve follows a nonlinear trajectory as turgor pressure buffers changes in Ψ, whereas at lower hydration it becomes linear once turgor is lost and only osmotic potential remains. The intersection of these regions defines the physiological TLP (RWC_{TLP}), marking the point below which mesophyll elasticity, hydraulic conductance, and gas exchange decline sharply. Note that we use the commonly used notation RWC_{TLP}[34], as we are looking at the symplastic RWC at turgor loss point rather than the water potential.

In this study, P–V curves were obtained using a Scholander pressure chamber following the free-transpiration methods described by Corcuera *et al.* (2002)[35] and Brodribb *et al.* (2003)[36]. Water-saturated leaves were dried gradually in darkness at room temperature, and paired measurements of fresh mass and water potential were recorded until Ψ approached approximately -0.7 MPa. The inverse of water potential ($1/\Psi$) was plotted against RWC, and the TLP was identified as the intersection of the nonlinear and linear regions of the curve (Figure 6). Across six replicates, the mean physiological TLP was $RWC_{TLP} = 0.978 \pm 0.003$ and $\Psi_{TLP} = -0.48 \pm 0.04$ MPa, consistent with values reported for other broadleaf species. The high physiological TLP indicates that potato leaves lose turgor at very high hydration levels, consistent with their known drought sensitivity and limited osmotic adjustment capacity. These physiological measurements provide a reference against which the TLP_c derived from ultrasonic parameters can be compared to assess how closely acoustic inflection points reflect the true turgor-loss transition.

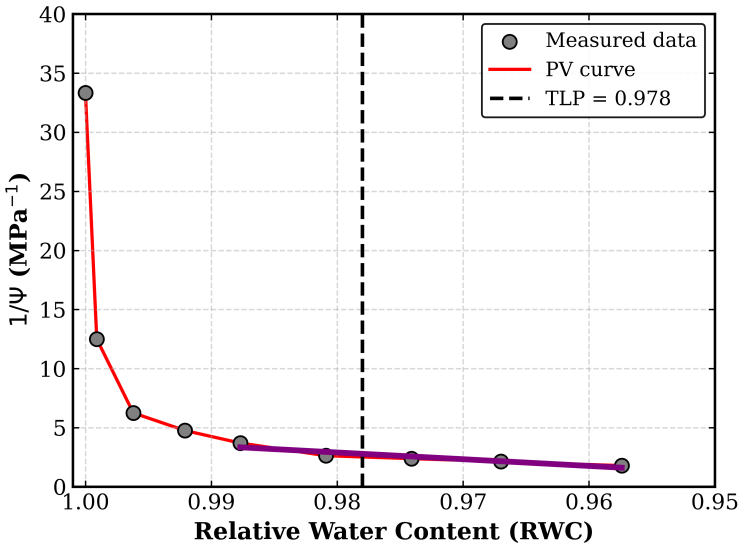

Figure 6. Pressure–volume (P–V) curve for a representative potato leaf showing the relationship between the inverse of water potential ($1/\Psi$) and relative water content (RWC). The nonlinear and linear regions correspond to the pre- and post-turgor phases, respectively. The intersection (dashed line) identifies the turgor loss point (TLP) at RWC = 0.978, confirming that potato leaves lose turgor at very high hydration levels.

To evaluate the correspondence between physiological and ultrasonic hydration thresholds, the TLP_c derived from ultrasonic parameters were compared with the physiological TLP obtained from P–V analysis (Table 1, Figure 7). All ultrasonic features produced TLP_c values within 2.5% of the physiological reference, demonstrating strong agreement between acoustic and physiological measurements. Among the ultrasonic features, the normalized amplitude ($A_{pk\text{-}pk}$) produced the closest TLP_c to the physiological TLP ($TLP_c = 0.973$, $\Delta RWC = -0.005$), followed by the frequency-domain spectral ($TLP_c = 0.964$, $\Delta RWC = -0.014$) and transmission ($TLP_c = 0.965$, $\Delta RWC = -0.013$) components at 650 kHz. The time-of-flight (ToF) exhibited a slightly lower TLP_c value ($TLP_c = 0.956$, $\Delta RWC = -0.022$),indicating that changes in propagation time occur earlier during dehydration when cell-wall tension declines and viscoelastic relaxation begins in the mesophyll. Despite these small differences, all ultrasonic measurements converged near the same physiological threshold, confirming that ultrasonic propagation is closely governed by turgor-driven structural transitions in the tissue. The agreement between the physiological TLP and the ultrasound-derived TLP_c values demonstrates that air-coupled ultrasound provides a reliable, non-destructive proxy for quantifying the turgor-loss transition and assessing leaf water status in real time.

Table 1. Comparison of TLP candidates (TLP_c) obtained using ultrasound techniques with the physiological turgor loss point (TLP) obtained from pressure–volume (P–V) analysis.

Method	Measurement Domain	TLP_c (RWC)	ΔRWC vs P–V
P–V (Pressure–Volume)	Physiological (Pressure–Volume)	0.978	+0.000
$A_{pk\text{-}pk}$ (Amplitude)	Ultrasound (Amplitude)	0.973	-0.005
ToF	Ultrasound (Time-of-Flight)	0.956	-0.022
Spectral (650 kHz)	Ultrasound (Frequency-domain)	0.964	-0.014
Transmission (650 kHz)	Ultrasound (Frequency-domain)	0.965	-0.013

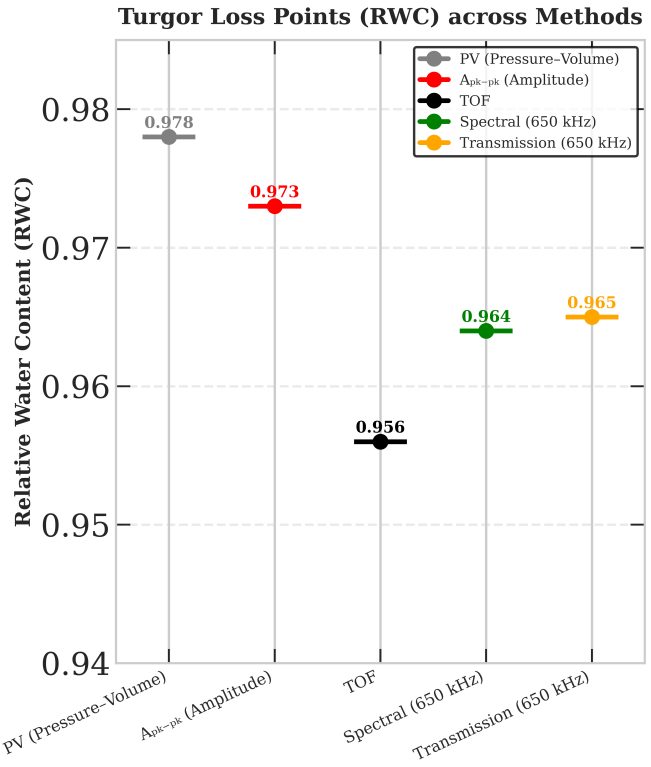

Figure 7. Pressure–volume (P–V) curve for a representative potato leaf showing the relationship between inverse water potential ($1/\Psi$) and relative water content (RWC). The nonlinear region at high hydration corresponds to turgid tissues, while the shallow linear segment (purple) represents the post-turgor phase governed by osmotic potential. The physiological turgor loss point (TLP) is indicated by the vertical dashed line at RWC = 0.978, confirming that potato leaves lose turgor at very high hydration levels.

Discussion

The results presented above directly address the three research questions (RQs) formulated in this study. Each question is considered in turn below in relation to the corresponding ultrasonic observations and physiological interpretations.

The normalized peak-to-peak (pk–pk) amplitude provided a clear and highly reproducible signature of hydration dynamics in potato leaves. The strong dependence between pk–pk amplitude and relative water content (RWC) reflects the close coupling between ultrasonic transmission and the mesophyll's mechanical and hydraulic state. As the leaf dehydrates, cell walls gradually lose turgor-derived tension, become more deformable, and develop increased curvature and wrinkling. These structural changes reduce the effective stiffness of the mesophyll and increase internal friction, leading to stronger ultrasonic attenuation. Consequently, the pk–pk amplitude decreases sharply as RWC falls below approximately 0.95, with the hydration threshold identified as the TLP candidate ($TLP_c = 0.973$). This tight correspondence shows that amplitude-based ultrasonic sensing effectively captures the transition from a mechanically turgid to a relaxed mesophyll structure. The pk–pk parameter is fundamentally sensitive to the continuity of the hydraulic pathway and provides a simple, single-measurement indicator of water status that requires minimal signal processing. In relation to RQ3, these findings confirm that amplitude-based ultrasonic

features not only exhibit a strong characteristic dependence on RWC but also reliably identify TLP_c, demonstrating that pk–pk amplitude is a robust, non-destructive indicator of leaf hydration state.

The temporal and spectral analyses complement the amplitude results and further confirm the biophysical basis of the ultrasonic response. The time-of-flight (ToF) also decreased systematically with dehydration, indicating a reduction in effective sound velocity as the bulk modulus of the tissue declined. The ToF response curve produced a TLP_c of 0.956, which parallels the pk–pk behavior but reflects an earlier stage of dehydration. This difference arises because ToF is sensitive to gradual reductions in elastic properties, whereas amplitude is influenced more strongly by increases in internal friction that develop when cell walls lose tension and become more deformable. Together, these two parameters provide a comprehensive description of the ultrasonic response to water loss: pk–pk amplitude captures the attenuation associated with increased viscoelastic losses in the mesophyll, while ToF reflects the concurrent mechanical relaxation of the tissue. It is important to note that the decreasing ToF trend represents a relative correlation shift rather than an actual increase in acoustic velocity. The absolute velocity still decreases during dehydration. The slightly lower TLP_c derived from ToF is consistent with its sensitivity to early biomechanical changes. Amplitude and frequency-domain metrics respond to increases in internal friction and viscoelastic losses that arise when cell walls lose tension, develop greater curvature, and form fine wrinkles. These changes begin early in the dehydration sequence, which explains why ToF detects an earlier stage of structural relaxation while amplitude-based parameters align more closely with the hydration transition represented by the ultrasonic TLP_c. In addressing RQ1, these observations demonstrate that multiple ultrasonic features, including amplitude and time-of-flight, respond consistently and reproducibly across the dehydration profile of potato leaves, confirming that ultrasonic propagation provides a robust and repeatable measure of hydration dynamics across biological replicates.

Frequency-domain measurements of spectral amplitude and transmission coefficient add further insight into the attenuation mechanisms that develop during dehydration. Both parameters decreased with decreasing RWC, and their response curves shifted toward higher hydration levels at higher frequencies. The strongest sensitivity occurred in the 450 to 650 kHz range, with TLP_c values between 0.943 and 0.965, indicating that high-frequency components respond earliest to dehydration. It is important to clarify that the observed increase in attenuation should not be interpreted as evidence that water is replaced by air inside the mesophyll. The present study did not include shrinkage measurements, and potato leaves are known to shrink substantially during drying, which can fully compensate for water loss and prevent the formation of internal air spaces. A more accurate explanation is based on biomechanical changes: as turgor decreases, cell walls lose tension, their curvature increases, and fine wrinkling develops. These structural modifications make the tissue more deformable, which reduces its effective stiffness and lowers acoustic velocity, and they also increase internal friction, which increases attenuation. These effects are more pronounced at higher frequencies, which explains why the 650 kHz band shows the strongest sensitivity to hydration changes. These results demonstrate the capability of non-resonant air-coupled ultrasound to resolve frequency-specific attenuation patterns, a feature not accessible through resonant techniques. While resonant methods are extremely accurate for leaves exhibiting thickness modes, they cannot probe non-resonant tissues such as potato, nor can they quantify continuous frequency-dependent attenuation. The present findings therefore extend the applicability of ultrasound to a wider class of plants, establishing a universal basis for hydration monitoring. In relation to RQ2, these results confirm that ultrasonic sensitivity is frequency-dependent, with the 650 kHz band providing the strongest contrast with hydration level and serving as the most reliable non-destructive indicator of leaf water status.

Comparison of the ultrasonic TLP candidates with the physiological reference obtained from pressure–volume (P–V) curves reinforces the accuracy of the acoustic approach. The mean physiological TLP derived from P–V analysis ($RWC_{TLP} = 0.978$) confirms that potato leaves lose turgor at an exceptionally high hydration level. Although this value is high relative to many woody or drought-adapted species, it is consistent with mesophytic crops that exhibit limited osmotic adjustment. It is also important to note that TLP values obtained under detached-leaf, free-transpiration protocols can be slightly elevated compared with in situ measurements, because apoplastic water is lost rapidly while symplastic solute concentrations remain relatively unadjusted during the early phases of dehydration. Thus, the high RWC_{TLP} reported here should be interpreted as a functional threshold under the specific experimental conditions rather than a species-invariant constant. In practical terms, this means that potato leaves begin to lose turgor very early during dehydration, even when the tissue remains above 97% of its full water content. Such an early turgor collapse indicates extremely limited osmotic adjustment and a strong predisposition to hydraulic failure under mild water deficits. All ultrasonic parameters closely reproduced this physiological threshold, with TLP_c deviations of less than 2.5% from the P–V reference. The pk–pk amplitude exhibited the closest agreement, followed by transmission and spectral parameters, while ToF indicated an earlier onset of change. The coherence among all acoustic indicators underscores the physical robustness of ultrasound as a method for detecting turgor loss and validates its use as a non-destructive alternative to traditional P–V measurements.

From a practical standpoint, the pk–pk amplitude emerges as the most efficient and reliable metric for assessing leaf hydration. Although frequency-domain and ToF analyses offer deeper mechanistic understanding, they require additional computation and spectral processing. In contrast, pk–pk amplitude can be obtained directly from the time-domain waveform

with minimal post-processing, yet it retains high sensitivity and strong physiological relevance. The simplicity of pk–pk extraction, combined with its close correspondence between TLP_c and the physiological TLP, makes it an ideal parameter for real-time field implementation. It provides a direct acoustic signature of leaf water status without the need for curve fitting, frequency-domain transformation, or calibration against reference media.

Overall, the integration of amplitude, ToF, and spectral data establishes that non-resonant air-coupled ultrasound accurately captures the mechanical and hydraulic transitions associated with turgor loss. The results confirm that ultrasonic propagation encodes both the continuity of water pathways and the stiffness of the mesophyll, linking these structural properties directly to hydration state. The ability of ultrasound to detect the hydration transition represented by TLP_c non-destructively, with frequency-dependent sensitivity and sub-millisecond temporal resolution, offers an unprecedented opportunity for in vivo water-status monitoring. By extending ultrasonic diagnostics beyond resonance-based methods, this work provides a framework for universal, non-contact assessment of plant hydration and stress physiology. The findings position pk–pk amplitude as the most practical and robust indicator of leaf water status, enabling future development of simple, cost-effective ultrasonic sensors for precision irrigation and drought management in agriculture. These results collectively fulfill the two objectives defined at the outset of the study: first, all major ultrasonic features, including peak-to-peak amplitude, time-of-flight, spectral amplitude, and transmission coefficient, were characterized across the full dehydration profile of potato leaves, revealing consistent and reproducible dependencies on relative water content; and second, the analyses identified the most sensitive ultrasonic parameters and operating conditions, with the normalized pk–pk amplitude emerging as the most reliable single indicator of water status and the 650 kHz band showing the greatest frequency-dependent contrast. Together, these findings confirm that non-resonant air-coupled ultrasound provides a comprehensive and quantitative framework for non-destructive assessment of leaf water status and turgor dynamics.

Conclusions

This study demonstrates that non-resonant air-coupled ultrasound provides a precise, contactless, and non-destructive framework for quantifying hydration dynamics in potato leaves. The normalized peak-to-peak (pk–pk) amplitude exhibited a strong characteristic dependence on relative water content (RWC), with a TLP candidate ($TLP_c = 0.973$) that closely matched the physiological turgor loss point obtained from pressure–volume (P–V) analysis ($RWC_{TLP} = 0.978$). This close agreement confirms that ultrasonic propagation reflects the same turgor-driven mechanical and hydraulic transitions identified by conventional water-relations analysis. Because pk–pk amplitude can be extracted directly from the time-domain waveform with minimal processing, it emerges as the most practical and robust indicator of leaf water status for real-time field implementation. Complementary parameters including time of flight, spectral amplitude, and transmission coefficient followed consistent dehydration trends and showed frequency-dependent sensitivity that peaked near 650 kHz. These findings confirm that high-frequency ultrasonic waves are especially responsive to the early mechanical relaxation that occurs in the mesophyll as leaves lose turgor. As dehydration progresses, reduced cell-wall tension, increased curvature, and the development of microscopic wrinkling make the mesophyll more deformable. These structural changes lower the effective elastic modulus, decrease acoustic velocity, and increase internal friction, which collectively raise ultrasonic attenuation. These processes fully explain the ultrasonic behavior observed in this study. The high physiological TLP of potato leaves indicates that turgor is lost very early during dehydration, consistent with their limited osmotic adjustment capacity and strong drought sensitivity. Early detection of this transition through ultrasonic sensing offers a promising tool for irrigation management in water-sensitive crops. The main limitation of this study is that all measurements were performed on detached leaves under controlled ambient conditions. In situ validation on intact plants across diurnal cycles and variable microclimates is required to evaluate environmental influences. Future work will also include quantitative estimation of the frequency-dependent attenuation coefficient to distinguish intrinsic viscoelastic attenuation and absorption processes. Ultrasonic monitoring may also support early detection of pest-induced physiological stress, because pests often disrupt turgor and cellular water balance before visible symptoms appear. Overall, pk–pk amplitude is a simple, sensitive, and computationally efficient parameter for real-time monitoring of hydration status and stress physiology in precision agriculture.

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Lesk, C., Rowhani, P. & Ramankutty, N. Influence of extreme weather disasters on global crop production. *Nature* **529**, 84–87, DOI: https://doi.org/10.1038/nature16467 (2016).
2. Quemada, C. *et al.* Remote sensing for plant water content monitoring: A review. *Remote. Sens.* **13**, 2088, DOI: https://doi.org/10.3390/rs13112088 (2021).

3. Zhang, K., Li, X., Zheng, D., Zhang, L. & Zhu, G. Estimation of global irrigation water use by the integration of multiple satellite observations. *Water Resour. Res.* **58**, e2021WR030031, DOI: https://doi.org/10.1029/2021WR030031 (2022).
4. Siddique, Z., Jan, S., Imadi, S. R., Gul, A. & Ahmad, P. Drought stress and photosynthesis in plants. *In: Plant Metal Interaction: Emerging Remediation Techniques*, pp. 1–11. https://doi.org/10.1002/9781119054450.ch1 (2016).
5. Tyree, M. T. & Hammel, H. T. The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *J. Exp. Bot.* **23**, 267–282, DOI: 10.1093/jxb/23.1.267 (1972).
6. Álvarez Cansino, L. *et al.* Turgor loss point predicts survival responses to experimental and natural drought in tropical tree seedlings. *Ecology* **103**, e3700, DOI: 10.1002/ecy.3700 (2022).
7. Castillo-Argaez, R. *et al.* Spectral ecophysiology: hyperspectral pressure–volume curves to estimate leaf turgor loss. *New Phytol.* **242**, 935–946, DOI: 10.1111/nph.19669 (2024).
8. Jacques, M. M., Gumiere, S. J., Gallichand, J., Celicourt, P. & Gumiere, T. Impacts of water stress severity and duration on potato photosynthetic activity and yields. *Front. Agron.* **2**, 590312, DOI: https://doi.org/10.3389/fagro.2020.590312 (2020).
9. Survase, S. A. & Singhal, R. S. Chapter 16 - novel applications and non-food uses of potato: Potatoes in biomedical/pharmaceutical and fermentation applications. *In: Advances in Potato Chemistry and Technology*, edited by Jaspreet Singh and Lovedeep Kaur, Academic Press, pp. 447–463. https://doi.org/10.1016/B978-0-12-374349-7.00016-7 (2009).
10. Zaki, H. E. M. & Radwan, K. S. A. Response of potato (*Solanum tuberosum* l.) cultivars to drought stress under in vitro and field conditions. *Chem. Biol. Technol. Agric.* **9**, 1, DOI: https://doi.org/10.1186/s40538-021-00266-z (2022).
11. Akkamis, M. & Caliskan, S. Responses of yield, quality and water use efficiency of potato grown under different drip irrigation and nitrogen levels. *Sci. Reports* **13**, 9911, DOI: https://doi.org/10.1038/s41598-023-36934-3 (2023).
12. Fariñas, M. D., Jimenez-Carretero, D., Sancho-Knapik, D. *et al.* Instantaneous and non-destructive relative water content estimation from deep learning applied to resonant ultrasonic spectra of plant leaves. *Plant Methods* **15**, 128, DOI: https://doi.org/10.1186/s13007-019-0511-z (2019).
13. Rodriguez-Dominguez, C. M. *et al.* Leaf water potential measurements using the pressure chamber: Synthetic testing of assumptions towards best practices for precision and accuracy. *Plant, Cell & Environ.* **45**, 2037–2061, DOI: https://doi.org/10.1111/pce.14330 (2022).
14. Boyer, J. S. Plant water potential: Methods and meanings. *J. Exp. Bot.* **70**, 4891–4906, DOI: 10.1093/jxb/erz467 (2019).
15. Sun, H. *et al.* Assessment of plant water status in winter wheat (*Triticum aestivum* l.) based on canopy spectral indices. *PLOS ONE* **14**, e0216890, DOI: https://doi.org/10.1371/journal.pone.0216890 (2019).
16. Mertens, S., Verbraeken, L., Sprenger, H. *et al.* Monitoring of drought stress and transpiration rate using proximal thermal and hyperspectral imaging in an indoor automated plant phenotyping platform. *Plant Methods* **19**, 132, DOI: https://doi.org/10.1186/s13007-023-01102-1 (2023).
17. Carrasco-Benavides, M., Ortega-Farías, S., Ahumada-Orellana, L. E. *et al.* Assessing olive tree water status using canopy spectral reflectance indices. *Irrigation Sci.* DOI: https://doi.org/10.1007/s00271-024-00967-x (2024).
18. Camoglu, G., Demirel, K. & Genc, L. Use of infrared thermography and hyperspectral data to detect effects of water stress on pepper. *Quant. InfraRed Thermogr. J.* **15**, 81–94, DOI: https://doi.org/10.1080/17686733.2017.1331008 (2017).
19. Qu, F., Nie, P., Lin, L., Cai, C. & He, Y. Review of theoretical methods and research aspects for detecting leaf water content using terahertz spectroscopy and imaging. *Int. J. Agric. Biol. Eng.* **11**, 27–34, DOI: 10.25165/j.ijabe.20181105.3952 (2018).
20. Konings, A. G., Rao, K. & Steele-Dunne, S. C. Macro to micro: microwave remote sensing of plant water content for physiology and ecology. *New Phytol.* **223**, 1166–1172, DOI: https://doi.org/10.1111/nph.15808 (2019).
21. Li, B., Wang, R. & Ma, J. Crop water status monitoring by terahertz imaging. *arXiv preprint* https://arxiv.org/abs/2003.10964 (2020).
22. Zahid, A. *et al.* Machine learning driven non-invasive approach of water content estimation in living plant leaves using terahertz waves. *Plant Methods* **15**, 138, DOI: https://doi.org/10.1186/s13007-019-0522-9 (2019).
23. Sebastian, J. A. *et al.* Assessing engineered tissues and biomaterials using ultrasound imaging: In vitro and in vivo applications. *Biomaterials* **296**, 122054, DOI: 10.1016/j.biomaterials.2023.122054 (2023).
24. Alqurashi, I., Alver, N., Bagci, U. & Catbas, F. N. A review of ultrasonic testing and evaluation methods with applications in civil ndt/e. *J. Nondestruct. Eval.* **44**, 53, DOI: https://doi.org/10.1007/s10921-025-01190-0 (2025).

25. Lv, D., Zi, J., Gao, M., Xi, R. & Huang, X. Detection of water changes in plant stems in situ by the primary echo of ultrasound rf with an improved aic algorithm. *Sensors* **23**, 20, DOI: https://doi.org/10.3390/s23010020 (2023).

26. Collazos-Burbano, D. A., Ealo, J. L. & Villagrán-Muniz, M. Laser-induced ultrasonic guided waves in water-stressed leaves. *Comput. Electron. Agric.* **216**, 108524, DOI: https://doi.org/10.1016/j.compag.2023.108524 (2024).

27. Yang, H., Liu, Y. & Zhao, Y. Effect of moisture content on the ultrasonic acoustic properties of wood. *J. For. Res.* **26**, 421–426, DOI: https://doi.org/10.1007/s11676-015-0079-z (2015).

28. Oliveira, J. T. S., Sales, A. & Gouveia, B. P. A. Moisture content effect on ultrasonic velocity in *Goupia glabra*. *Mater. Res.* **8**, 35–38, DOI: https://doi.org/10.1590/S1516-14392005000100004 (2005).

29. Paniagua, A. C., Ramírez, R. M. & Vargas, C. C. Influence of timber moisture content on the ultrasonic wave velocity of tropical species: Teak and cypress. *Forests* **13**, 1296, DOI: https://doi.org/10.3390/f13081296 (2022).

30. Fariñas, M. D., Sancho-Knapik, D., Peguero-Pina, J. J., Gil-Pelegrín, E. & Gómez Álvarez Arenas, T. E. Contact-less, non-resonant and high-frequency ultrasonic technique: Towards a universal tool for plant leaf study. *Comput. Electron. Agric.* **199**, 107160, DOI: https://doi.org/10.1016/j.compag.2022.107160 (2022).

31. Sattar, M. A. & Laila, D. S. A review of ultrasound monitoring applications in agriculture. *Front. Plant Sci.* **16**, 1620868, DOI: https://doi.org/10.3389/fpls.2025.1620868 (2025).

32. Gómez Álvarez Arenas, T. E., Sancho-Knapik, D., Peguero-Pina, J. J. & Gil-Pelegrín, E. Noncontact and noninvasive study of plant leaves using air-coupled ultrasounds. *Appl. Phys. Lett.* **95**, 193702, DOI: 10.1063/1.3263138 (2009).

33. Sancho-Knapik, D., Gómez Álvarez Arenas, T., Peguero-Pina, J. J. & Gil-Pelegrín, E. Air-coupled broadband ultrasonic spectroscopy as a new non-invasive and non-contact method for the determination of leaf water status. *J. Exp. Bot.* **61**, 1385–1391, DOI: 10.1093/jxb/erq001 (2010).

34. Luiz Ferraresso Conti Junior, J. *et al.* Quantifying turgor loss point and leaf water potential across contrasting eucalyptus clones and sites within the techs research platform. *For. Ecol. Manag.* **475**, 118454, DOI: https://doi.org/10.1016/j.foreco.2020.118454 (2020).

35. Corcuera, L., Camarero, J. J. & Gil-Pelegrín, E. Functional groups in *Quercus* species derived from the analysis of pressure–volume curves. *Trees* **16**, 465–472, DOI: 10.1007/s00468-002-0187-1 (2002).

36. Brodribb, T. J. & Holbrook, N. M. Stomatal closure during leaf dehydration: correlation with other leaf physiological traits. *Plant Physiol.* **132**, 2166–2173, DOI: 10.1104/pp.103.023879 (2003).

Fundings and Acknowledgements

This work was supported by Kempestiftelserna (Project ID: JCSMK24-0080), whose generous funding enabled the research, analysis, and preparation of this manuscript. The authors extend their sincere gratitude to the Kempe Foundation for its support. The authors also acknowledge Luleå University of Technology for providing open access funding.

Author contributions statement

M.A.S. conceived the study, analysed and interpreted the data, and wrote the manuscript. T.E.G.A-A. designed and performed the experiments, validated the results, and contributed to manuscript revision. E.G-P. and D.S-K. designed and performed the experiments to measure the physiological turgor loss point. V.N. initiated the idea of the study and contributed to verifying the results. D.S.L. supervised the project, provided guidance on analysis and interpretation, contributed to the manuscript writing and reviewed the manuscript. All authors approved the final manuscript.

Declarations

Competing Interests

The authors declare no competing interests.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Additional information

Correspondence and requests for materials should be addressed to M.A.S.