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Introducing terahertz technology into plant biology: A novel method to monitor changes in leaf water status

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Summary

We present a novel, non-destructive method for determination of changes in leaf water content in the field based on terahertz (THz) technology. In this method, terahertz waves, which are strongly absorbed by water, are generated and detected using a photomixer that converts the optical beat signal of two interfering diode lasers into THz radiation. This allows a coherent detection as basis for the determination of changes in leaf water contents.

The reliability of this innovative method was verified by monitoring changes in the leaf water content of young coffee plants in parallel using classical, destructive thermogravimetric measurements as well as by THz spectroscopy. The broad applicability of this novel device was shown by long- and short-term measurements. The changes in leaf water content during drought stress induced dehydration as well as during the course of rapid re-hydration after re-watering vividly highlight the tremendous potential of this novel technique and its high reliability. The findings presented here provide the basis for THz-based *in vivo* determination of changes in the leaf water content under field conditions.

Introduction

Determination of the leaf water content is of high importance for numerous aspects in plant science including basic research and various fields of applied plant biology. Due to the effects of global climatic changes leading to increasing aridification, there is a crucial need for effective tools to select drought stress resistant plants. Accordingly, measurement of plant water stress by remote sensing is actually of high scientific interest. In this context, estimation of the leaf water content is inevitable. Unfortunately, most techniques, such as the common thermogravimetric quantification of water content, are destructive. Additionally, there are various non-destructive methods for the detection of water that are based on the absorption or reflection of electromagnetic or radioactive radiation; however, these techniques all have significant drawbacks. For example, during microwave based determination of the leaf water content, the absorbance of the radiation (wavelengths between 3 mm and 1 m, corresponding to frequencies between 100 GHz and 0.3 GHz) is strongly influenced by the inorganic salt content of the leaves, which markedly reduces the reliability of this method (ULABY and JEDLIKA, 1984). Furthermore, the resolution of the imaging measurements is limited due to the relatively large wavelength that this method employs. Due to the problems related to radioactivity, the application of β -radiation based sensors is not recommended. Indeed, nuclear magnetic resonance (NMR) techniques provide a reliable tool for the determination of leaf water contents, but this approach is not suitable for use in a regular biochemical lab or in field trials due to the size of the NMR-machines. Recently, an optimized method for the estimation of leaf water content based on the reflectance of the visible spectrum was published (ZYGIELBAUM

et al., 2009). Unfortunately, when applying this approach, leaf water contents could only be determined in comparison to other leaves and could not be estimated as real values.

The most common methods used for non-destructive quantification of the leaf water content are based on infrared spectroscopy (IR; wavelengths between 0.75 μm and 100 μm , corresponding to frequencies between 400 THz and 3 THz) and employ the reflectance of infrared radiation (TUCKER, 1980; HUNT et al., 1987; HUNT and

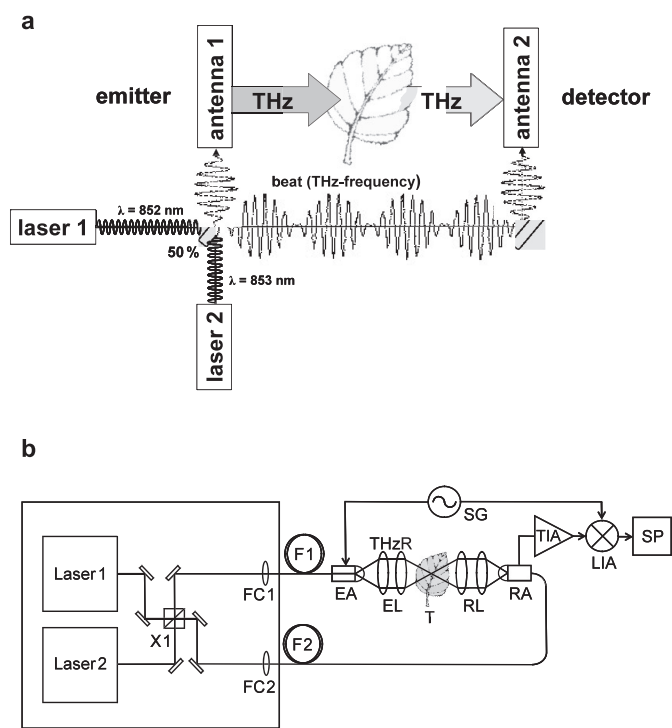


Fig. 1: Principle of the THz-based approach for determination of changes in the leaf water content and schematic drawing of the device

a) General scheme: Two continuous-wave frequency-tunable lasers emitting very similar wavelengths produce an optical beat in the THz range. This beat induces electromagnetic THz radiation in a photomixing emitter (antenna 1). When these THz rays impinge on the detector (antenna 2), an electric current is elicited that varies based on the intensity of the THz radiation. From this data the THz transmission of the sample, e.g. a leaf between the two antennas, can be calculated. As the detector antenna is also irradiated with the original laser generated beat, any phase shifting can be determined (coherent detection).

b) Schematic drawing of the measurement set-up of the device. X1: beamsplitter; FC1, FC2: fiber couplers; F1, F2: fibers; EA: emitting antenna; RA: receiving antenna; EL, RL: lenses for THz radiation; SG, TIA, LIA, SP: components for lock-in amplification and signal recording; T: target, e.g. leaf.

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ROCK, 1989; EITEL et al., 2006; SEELIG et al., 2008). Due to water absorption features in the near- (NIR) and far-infrared (FIR) region, IR-reflectance spectra have great potential to specify the water content. For example, PEÑUELAS et al. (1997) estimated the plant water content by determining the ratio of the reflectance at 970 nm to that at 900 nm ($R_{970}:R_{900}$), while the shortwave infrared water stress index (SIWSI) was used by FENSHOLT and SANDHOLT (2003), the three-band ratio indices were applied by PU et al. (2003), and the normalized difference water index (NDWI) was used by GAO (1996) and by SERRANO et al. (2000). To date, many studies have been conducted to define the optimal formulas and wavelengths (FENSHOLT and SANDHOLT, 2003; PU et al., 2003; SERRANO et al., 2000; SIMS and GAMON, 2003) however, it is difficult to accomplish this due to the complex reflection geometry of the leaves and the vulnerability of the technique to disturbances. Accordingly, there has been relatively little validation of the field data to date (CLAUDIO et al., 2006). In addition to these remote sensing approaches, analogous conditions also apply to the estimation of changes in the water content of certain vital leaves. Although the parameter required in these cases can be specified by solid calibrations, the technique is not sensitive enough to record small differences in the water content of an individual leaf. Moreover, because the complex reflection geometry of leaves changes when the turgor of the cells decreases as a result of water deficit, NIR-based techniques are not suitable for recording small changes in the water content of leaves (HUNT et al., 1987). Consequently, an alternative is required when such changes should be monitored.

HADJILOUCAS et al. (1999) introduced the measurement of leaf water content using terahertz waves. THz radiation is characterized by wavelengths between 0.1 mm and 3 mm, corresponding to frequencies between 3 THz and 0.1 THz (100 GHz). Water strongly absorbs terahertz radiation, while non-polar organic material does not. Therefore a transmission measurement setup is very effective for the quantification of the water present in leaves. However, due to its dimensions, the system used by HADJILOUCAS et al. (1999) is not applicable for field experiments. The set-up presented in this paper (Fig. 1) is based on an electromagnetic model of the complex THz-permittivity as a function of the water content of the plant leaf described by JÖRDENS et al. (2009) and allows that all optical components can be arranged in a small box for field measurements.

Material and methods

Plant material

One-year-old coffee plants (*Coffea arabica* L.) with 10 to 12 leaves were used for the experiments. The plants were grown from coffee seeds in green houses under long-day conditions (16 h light / 8 h dark) at about 22 °C in single pots ($\varnothing = 11$ cm) under approximately 70% rel. humidity in about 700 mL of standard garden soil. Watering was conducted twice a week to maintain the soil water content of approximately 60 % (w/w). To induce drought stress, watering was suspended and the humidity was reduced to 55 % (w/w) for the verification experiments (Fig. 2) and to 45 % (w/w) for the monitoring of long- and short-term changes in the water content (Fig. 3 and 4). During the course of the experiment, the soil water content declined down to 10 % (w/w). In general, all THz measurements were performed in the midmorning. The short term measurements (re-hydration) started at 10 am and lasted till 5 pm.

Thermogravimetric measurement

For the determination of the actual dry weight of the leaves, directly after detaching, the coffee leaves were weighed and subsequently placed in a lab oven for 36 h at 110 °C. After cooling down, the dried leaves were weighed again and the water content was calculated.

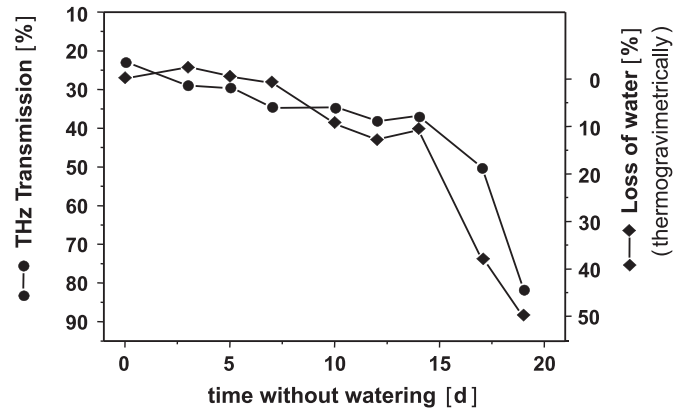


Fig. 2: Verification of THz based determination of the leaf water content

Coffee leaves still attached to plants that had been subjected to massive drought stress were monitored by periodically determining the THz transmission. At any point of the THz measurement the water content of four individual leaves (from at least two different plants) was determined by classical, destructive thermogravimetric measurements. Each point represents the mean value of at least four different individual samples or leaves, respectively. The mean value of the standard deviation was about 3%.

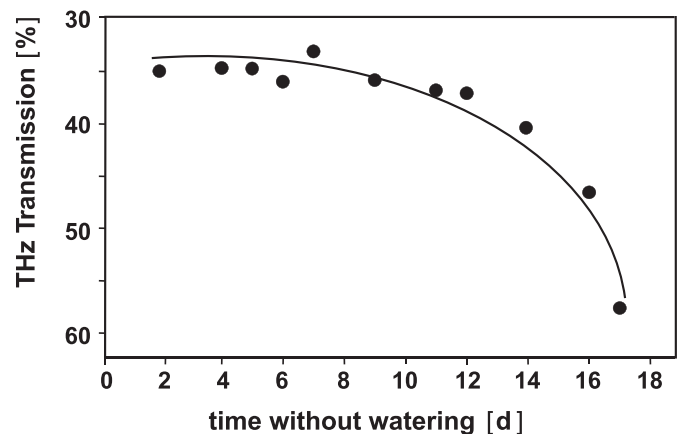


Fig. 3: Long-term monitoring of dehydration induced changes in leaf water content

Drought stress related changes in the water content of coffee leaves were induced by suspending watering. Every day the same leaf on the experimental plant was measured by analyzing the THz transmission of the intercalary leaf area. To mimic realistic conditions in which plants shall be analyzed, the leaf was removed from the THz system after each measurement. Consequently, the appointed area used for the THz measurement may have differed slightly. Each point represents the mean value of at least three independent estimations from the same leaf. The mean value of the standard deviation was about 3%.

Measurement of THz transmission

In this study, terahertz radiation indicates electromagnetic waves with a frequency in the range of 100 GHz to 10 THz (corresponding to a wavelength of 3 mm to 30 μ m). In this sparsely explored gap between microwaves and infrared light, only few types of radiation sources are available, which employ both, microwave and optical techniques. For the investigations presented here, the waves were emitted from a small dipole antenna (length 200 μ m). By the use of three lenses, the diverging waves were bundled into a beam and

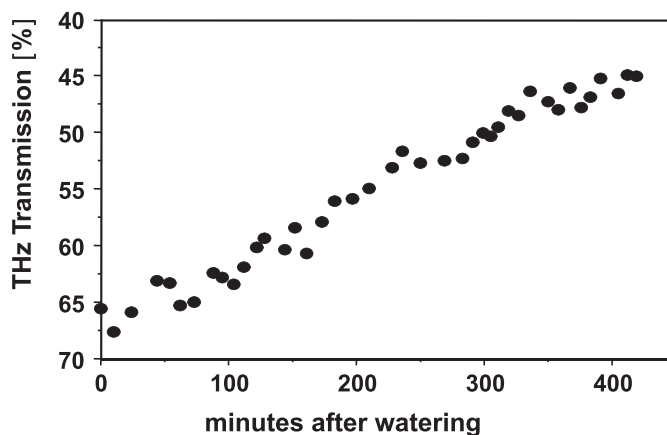


Fig. 4: Determination of short-term changes in leaf water content during re-hydration

The rapid water uptake of drought stressed coffee plants was monitored by quantifying the water content of one leaf. After re-watering, the THz absorbance of one individual coffee leaf was determined every ten minutes without removing the plant from the system. Every point represents a single measurement.

focussed on a small spot with a diameter of approximately 2 mm (see Fig. 1b). In turn, a complementary set of lenses collects the rays that emanate from the focus and redirects them to a receiver antenna of the same type. A photomixing effect in the emitting antenna converts the optical beat note of two interfering diode lasers into THz radiation (Fig. 1). At the detector antenna, the resulting THz radiation generates an electric current that depends on both the amplitude and the phase of the incident THz wave. Because the detector antenna is also irradiated with the original laser generated beat, the phase shifting effects can also be specified. This coherent detection (VERGESE et al., 1998; WILK et al., 2008) allows the simultaneous determination of both, the absorption of THz radiation (mainly by the water present in the sample), and the changes in the refractive index of the material through which the radiation passes. In contrast to the phase-sensitive set-up employed by HADJIOUCAS et al. (1999), in our device there is no need to build in an interferometer for microwave or submillimeter wave radiation. All optical components – except for the THz focussing lenses – are standard components for near-infrared light. The major advantage of our set-up is its compactness. All components can be encased in a small box allowing its transfer into the field. Moreover, the new principle enables to apply a much larger THz wavelength range.

Prerequisite for generation of the required effects were two continuous-wave frequency-tunable lasers that have only a small difference in their wavelengths. The laser beams were superimposed in a beamsplitter and then coupled into polarization-maintaining single-mode fibers. Each of the beams obtained after collimation at the fiber outputs then contains 50% of the light of each laser. This is about 3 to 5 mW per laser per fiber. As the lasers have different optical frequencies, the interference of the two beam components results in a beat note at the difference frequency, which excites the emitter antenna. By changing either the temperature or the supply current of the laser diodes, the beat frequency could be tuned to arbitrary values between 0 and 2 THz. The measurements presented here were carried out at 200 to 220GHz. It turned out that the measurement results were independent from the frequency throughout this range. The emitted THz electromagnetic power was estimated in the range of several nW. The current detected at the receiver antenna was in the range of some nA, which made it necessary to use a lock-in amplifier for signal recovery. However, the relatively small cost of the laser system and the fact that the THz frequency can be chosen almost

arbitrarily in the range 0.1 to 2 THz outweigh the disadvantage of the low THz power. On the other side, the low THz power ensures that the leaves could be measured over a long time period without getting any damages (i.e. necroses, injuries, burnings etc.).

The optomechanical laser set-up (including laser-to-fiber coupling) was mounted on a 15mm thick aluminium board and housed against dust and humidity. So the system could be transported from one institute to another, though slight realignment of the fiber entrances was sometimes necessary. Therefore, future versions will incorporate an already existing automatic beam alignment system (e.g. FiberLock of TEM Messtechnik GmbH). The laser set-up can then be hermetically sealed, as it will no longer require any user service.

Results and discussion

Verification

To validate the suitability of use of the novel terahertz based approach to quantify the leaf water content, approximately 50 individual young coffee plants were subjected to massive drought stress by lowering the soil water content from 50 % to 10 % (w/w). The resulting decrease in the leaf water potential was monitored by periodically determining the THz absorbance and the transmission of leaves still attached to the plants. Subsequently, the water content of four individual leaves (from at least two different plants) was determined by classical, destructive thermogravimetric measurements. Comparison of both parameters revealed the same progression, and thus demonstrates the applicability of the novel THz based quantification to estimate the plant water content (Fig. 2). Although each point of the graphic represents the mean value of at least four different individual leaves, the data match, demonstrating the high reliability of the novel technique presented here.

It should be noted that the actual terahertz technique applied only allows determination of the absolute amount of water present in the measuring focus. For a valid quantification of the relative water content, i.e. the percentage of water, the leaf thickness must also be known. Because the phase shift between the original beat and the THz wave that passes through the leaf strongly depends on the propagation length, an exact estimation of the leaf thickness can be achieved. In a forthcoming evolution of the terahertz system applied here, this technique will be included while focusing on automatic evaluation and calculation computer programs. These future studies should permit co-estimation of both the absolute water content and the leaf thickness, thereby providing reliable data regarding the actual water concentration of a leaf.

Applications

Even without the advanced technique of co-estimation of the water content and leaf thickness, the novel terahertz approach presented here represents a high-end tool for various applications of non-contact online monitoring of changes in the leaf water content such as estimation and recording of its short- or long-term changes. To demonstrate the broad applicability of the novel technique, both long-term and short-term measurements of single plants were performed.

Long term measurement of a single plant: dehydration

The drought stress induced decrease in the leaf water content represents a process that typically lasts for a longer period of time. Accordingly, we used the THz system to determine the water content of one leaf of a coffee plant, while watering was suspended for a time period of about three weeks. Each day, the water content of the same leaf of this experimental plant was measured by analyzing the THz transmission of the intercalary leaf area. All measurements were performed in the same room in which the plant had been

grown. To mimic realistic conditions, the plant was removed from the THz system after each measurement. Consequently, the appointed area used for the THz measurement may have differed slightly. Despite such putative location-related variations, the data recorded (Fig. 3) demonstrate the high performance of the new technique, which indeed enables reliable long term measurements of changes in the leaf water content.

Short term measurement of a single plant: rehydration

In contrast to the slow changes in the leaf water content described above, the increase in the water content after re-watering of a drought stressed plant occurs much more rapidly. Accordingly, we quantified this process by measuring the THz transmission of one leaf on a coffee plant every ten minutes without removing the plant from the system. It is interesting to note that the uptake of water could be monitored efficiently (Fig. 4). In contrast to the long term measurements, the area used for THz measurement in the short-term experiment was fixed throughout the entire experiment. Consequently, putative location-related variations as described above are excluded, resulting in a smaller deviation. The data describing the short-term changes in water content underline the high potential of this novel technique and provide the basis for the further development of a portable THz-device to determine *in vivo* the leaf water content under field conditions.

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