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STUDY PROTOCOL

REVISED Protocols for *in situ* continuous monitoring of water relations/potential in soil and leaf

[version 3; peer review: 2 approved, 2 approved with reservations]

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Abstract

Within the soil-plant-atmosphere continuum, water movement is driven by the water potential gradients between these three domains. To have a comprehensive understanding of such water relations, an examination of how plants respond to variations in soil water availability is required. The methodologies employed for measuring water potential in leaf (Ψ_{leaf}) and soil (Ψ_{soil}) have undergone a significant evolution; transitioning from qualitative assessments to the use of high-precision digital sensors over the past few decades. The present protocol aims to provide a comprehensive, step-by-step guide from the germination phase of maize and tomato plants to the installation of two sensors that continuously monitor water potential in the leaf (PSY1 psychrometer) and in the soil (Teros 21 matric potential sensor). Additionally, we present the code for processing the raw data files in RStudio.

Plan language summary

In this study, we provide a step-by-step guide for in situ continuous monitoring of water potential in soil and leaves with Teros 21 and PSY1-leaf psychrometers, respectively. Additionally, we present the code necessary for the analysis of the collected data using the RStudio platform.

Keywords

Soil water potential; leaf water potential; Teros 21; psychrometer; PSY1; maize; tomato; data analysis

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REVISED **Amendments from Version 2**

The revised version changes include addition of statements like As a support, Venturas et al. (2017) summarized that frictional pressure drops 1~2 MPa in trees under wet soil and dry air conditions, while this pressure drop is smaller in herbs under a shorter transport distance and Whether this technique measures Ψ_{tot} or Ψ_p remains a matter of debate (Boyer, 1969; Rodriguez-Dominguez et al., 2022). The method assumes that equilibrium is reached between the applied balancing pressure and the negative water potential in the leaf xylem, while the osmotic potential of the xylem/apoplastic sap is negligible (Boyer, 1969). However, this assumption may not always hold, as differences among measurement techniques and the presence of non-negligible xylem osmotic potential under certain physiological or environmental conditions can influence the interpretation of pressure chamber measurements (Rodriguez-Dominguez et al., 2022; Enns et al., 2000; Westhoff et al., 2008).

Figure 7 and Figure 8 caption revised and conclusion line redefined.

Any further responses from the reviewers can be found at the end of the article

Introduction

In the soil-plant-atmosphere continuum (SPAC), water movement is driven by the water potential gradients between the soil (Ψ_{soil}), the plants (e.g. Ψ_{leaf}) and the atmosphere (Ψ_{atm}). The fundamental mechanism underlying this phenomenon in vascular plants, as depicted by the cohesion-tension theory (Bohm, 1893; Dixon & Joly, 1895), is the transpiration pull. This force in turn generates a negative pressure potential (Ψ_p) in xylem vessels. When water flows across non-selective tissues, such as cell walls and plasmodesmata, it follows the "downhill" gradients of summed Ψ_p and gravitational potential (Ψ_g) (i.e. from high to low potential). When water flows across a semi-permeable membrane, such as cell plasma membranes, a third additive component comes into play: the osmotic potential (Ψ_o). This component, which is a negative pressure, favors water flow towards the side with the highest solute concentration. At the root cylinder scale, radial water flow rate per unit root surface area (J [LT^{-1}]) is generally described by the Fiscus (1975) equation, describing the root as a single "big membrane". This equation assumes radial water flow rate to be the difference between the soil-root interface and xylem vessels "*total*" water potential (Ψ_{tot} [$ML^{-1}\ T^{-2}$], expressed in units of pressure), multiplied by the root hydraulic conductivity (L_{pr} [$L^2\ M^{-1}\ T$]), (Fiscus, 1975):

$$J = L_{pr} \times \left(\Psi_{tot,soil} - \Psi_{tot,leaf}\right)$$

where the degree of selectivity of the tissues on the radial pathway comes into play as a dimensionless reflection coefficient (σ_r, null when nonselective, one when fully selective) in the calculation of Ψ_{tot}:

$$\Psi_{tot} = \Psi_p + \Psi_g + \sigma_r \Psi_o$$

Note that for simplicity, the notation Ψ_p is used for both pressure and matric potentials in the soil. Negative $\Psi_{p,soil}$ expresses the pressure needed to counteract forces retaining water in the unsaturated soil matrix. Additionally, it will be assumed herein that frictional pressure drop along xylem vessels are negligible for the small plants of this study, and xylem water potential in the root and leaf are equivalent. As a support, Venturas et al. (2017) summarized that frictional pressure drops 1~2 MPa in trees under wet soil and dry air conditions, while this pressure drop is smaller in herbs under a shorter transport distance.

Ψ_{leaf} (herein may refer to water pressure potential or total water potential according to the method of quantification) is a central physiological variable that reflects the water status of plants, and influences critical processes such as photosynthesis, transpiration, and growth (De Swaef *et al.*, 2022). Ψ_{leaf} acts as a primary indicator of plant hydration, offering insights into plant responses to environmental stresses, including drought (very negative $\Psi_{p,soil}$), salinity, and extreme temperatures *etc.* Understanding the concept of both Ψ_{leaf} and Ψ_{soil} is essential for research efforts in plant water relations, drought resistance, and agricultural irrigation management (Shackel, 2011). Over the past century, the methods for measuring Ψ_{leaf} and Ψ_{soil} have evolved significantly, driven by advancements in technology, materials science, and our understanding of soil-plant-water relations (Ding *et al.*, 2020). This evolution has transformed the field of plant physiology and soil physics, enabling more accurate, efficient, and non-invasive monitoring of plant and soil water status.

In the early stages of research, Ψ_{leaf} was primarily measured or estimated by visual assessment (such as leaf wilting) and gravimetric techniques (measurement of water loss) (Veihmeyer & Hendrickson, 1927). In the 1950s and 1960s, direct measurements of Ψ_{leaf} were achieved by using thermocouple psychrometers (TCP), which typically measure the sum of Ψ_p and Ψ_o (Boyer & Knipling, 1965; Richards & Ogata, 1958). Concurrently, the Scholander pressure chamber proposed a direct but destructive measurement of (Ψ_{leaf}) (Scholander *et al.*, 1965). Whether this technique measures Ψ_{tot} or Ψ_p

remains a matter of debate (Boyer, 1969; Rodriguez-Dominguez et al., 2022). The method assumes that equilibrium is reached between the applied balancing pressure and the negative water potential in the leaf xylem, while the osmotic potential of the xylem/apoplastic sap is negligible (Boyer, 1969). However, this assumption may not always hold, as differences among measurement techniques and the presence of non-negligible xylem osmotic potential under certain physiological or environmental conditions can influence the interpretation of pressure chamber measurements (Rodriguez-Dominguez et al., 2022; Enns et al., 2000; Westhoff et al., 2008). Later, plant physiologists devoted much effort to develop new techniques for the measure of Ψ_{leaf} with Dew Point Hygrometry (Banks & Hirons, 2019; Brown & Tanner, 1981). Modern approaches now include infrared thermography (Cohen *et al.*, 2005), microtensiometers (Pagay *et al.*, 2014), optical and spectral methods (Cotrozzi *et al.*, 2017; Wang *et al.*, 2020), and nanosensors (Jain *et al.*, 2021), allowing for continuous, *in situ*, and highly sensitive monitoring of plant water status. In the context of *in-situ* continuous monitoring of $\Psi_{stem/leaf}$, TCP (Bellasio *et al.*, 2024; Delval *et al.*, 2024; Haverroth *et al.*, 2025; Jerszurki *et al.*, 2017) and microtensiometers (Bellasio *et al.*, 2024; Christenson *et al.*, 2024; Delval *et al.*, 2024; Haverroth *et al.*, 2025; Villalobos *et al.*, 2024) have been predominantly utilized in recent years. The microtensiometer is restricted in its application to woody stems; whereas, the TCP can be utilized on both stems and leaves.

Ψ_{soil} measurement techniques have also evolved from qualitative assessments to high-precision digital sensors (Bittelli, 2010; Singh *et al.*, 2024; Yao *et al.*, 2025; Yu *et al.*, 2021). Most of the techniques rely on the measurement of the hydraulic equilibrium between soil water and external substances, i.e., solid, liquid and gas (Campbell & Campbell, 2005; Luo *et al.*, 2022). The techniques are clarified into three main categories, which include solid-based (e.g., filter paper) (Al-Khafaf & Hanks, 1974; Gardner, 1937), liquid-based (e.g., tensiometer) (Cassel & Klute, 1986), and vapor-based (e.g., hygrometer) (Campbell & Campbell, 1974). In field, the most commonly used instruments include piezometers, tensiometers, heat dissipation sensors, dielectric sensors, and TCP. In the laboratory, methods include soil water retention, suction plates, pressure plates apparatus, dew point method, and freezing apparatus (Bittelli, 2010).

This protocol paper will provide a step-by-step guide for using PSY1 and Teros 21 sensors for *in situ*, continuous measurements of Ψ_{leaf} (including pressure potential and osmotic potential) and Ψ_{soil} (soil matric potential), respectively, on maize and tomato plants. The PSY1 psychrometer (ICT International, Armidale, Australia) was selected for this study due to its capacity to measure leaf/stem water potential in situ and with a high degree of continuity (at 10-minute intervals). The installation process for crop plants is also straightforward. Teros 21 (Meter group, Washington, United States) is a digital sensor that uses dielectric permittivity changes to estimate matric potential in soil. It works from wet to extremely dry soils, with a detection range of 0 to −100000 kPa and a high precision of 0.1 kPa. Based on these features, we selected Teros 21 for soil water potential measurement in this study. As demonstrated in Table 1, a physical interpretation and comparability of Teros 21 and PSY1 measurements was established. Although PSY1 measurements are influenced by soil water availability, as reflected by the soil matric potential measured with TEROS 21, the absolute values of leaf water potential and soil matric potential should not be directly equated because they differ in temporal dynamics and magnitude along the soil–plant hydraulic continuum. Instead, the two measurements provide complementary information and should be interpreted together to characterize plant and soil water status.

Table 1. Physical interpretation and comparability of Teros 21 and PSY1 measurements (For more details of Teros 21 and PSY1, please read the user manual from meter group (https://metergroup.com/products/teros-21/teros-21-support/) and ICT international (https://ictinternational.com/wp-content/uploads/2023/08/PSY1-Manual-DOC-00087v2.pdf), respectively).

Sensor	Quantity reported	Physical meaning	Remarks
Teros 21	Soil matric potential	Teros 21 measures the dielectric permittivity of a solid matrix (porous ceramic discs) to determine its water potential. There is hydraulic equilibrium between the ceramic discs and surrounding soil. So, Teros 21 requires good hydraulic contact with the surrounding soil for accurate measurements.	In salty and dry soils, soil osmotic potential becomes non-negligible, and we need to take the sum of osmotic and matric potential to estimate soil total water potential.
PSY1	Leaf/Xylem total water potential	PSY1 measures the vapor pressure in the chamber attached to the water conducting tissue of the plant. It relies on the fact that the liquid phase water in the xylem sets up a vapor pressure equilibrium with the chamber.	It reads both pressure potential and osmotic potential in the measured tissues under thermal and vapor equilibrium between chamber and leaf/xylem, stable temperature, proper installation, no condensation.

Additionally, we present user-friendly codes for the analysis and cleaning (e.g. remove outliers and irrelevant values) of the collected data in RStudio.

Experimental protocols

Seeds germination and plant growth

Seed resources: *Zea mays L.* (in this example, B104 inbred maize line from previous study (Ding *et al.*, 2020), referred to as maize later) or *S. lycopersicum var.* Moneymaker (referred to as tomato later, purchased from the market, "Oh! Green", Belgium, originated from Vilmorin (product number 784)). The accession number for B104 maize is "PI 594047" from the USDA National Plant Germplasm System. The plant passport for tomato seeds is "A *Solanum lycopersicum L.* B FR-RH00867".

Timing: maize, four weeks; tomato, six weeks.

1. Sterilization and germination of seeds

a. Prepare 50 mL of 1/5-strength bleach solution by mixing 10 mL of commercially available bleach (LODA, Bleach 15°, 1815B) with 40 mL demineralized water in a 50 mL falcon tube.

b. Add maize or tomato seeds to the bleach solution for 5 min. Discard the solution and wash five times with demineralized water.

Note: To avoid losing the seeds, especially for tomato seeds, a nylon mesh may be used to cover the falcon tube when discarding the bleach solution or water.

c. In a Petri dish, prepare a "Sandwich" consisting of two 'layers' of absorbent paper saturated with water, one on the top and bottom respectively (Figure 1C–G). Place the seeds with tweezers on the bottom layer and cover them with the other layer of paper, to form the "Sandwich". Close and wrap the Petri dish with aluminum foil and let it stand vertically at 25~30°C in a climate chamber, growth chamber or oven. Typically, the seeds will germinate after 48 h, at 25~30°C for both maize and tomato. If maize seeds were to be kept in these conditions for more than 48 h, the roots would risk being too long making them difficult to transfer to the soil media in the next step. Instead of using the "Sandwich" technique, the seeds may be directly sown in the soil after the sterilization for germination in greenhouse or growth chamber according to the protocol for transplanting (Figure 2A–C).

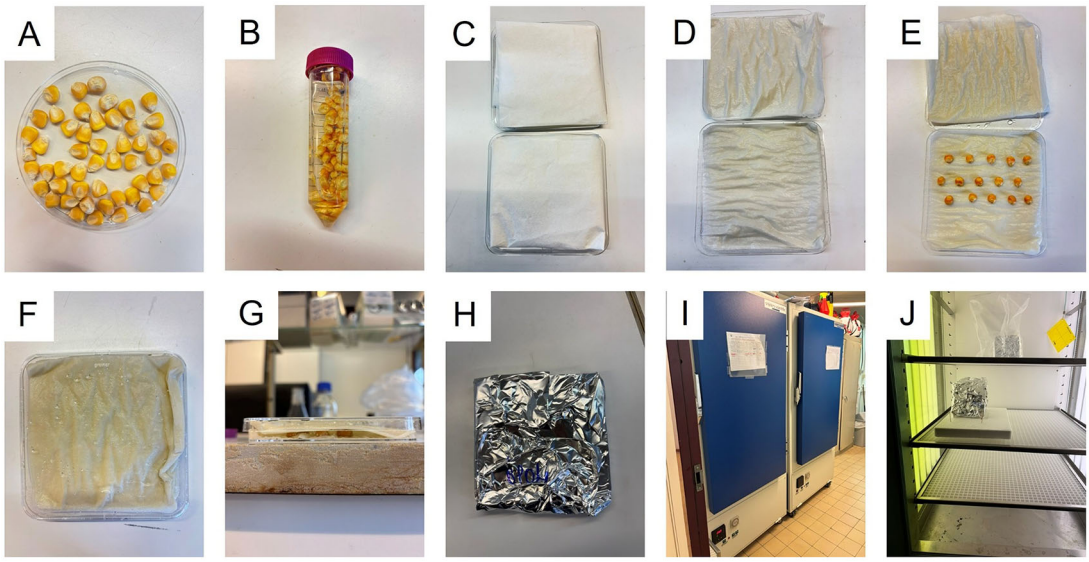


Figure 1. Sterilization and germination of maize seeds. A, Prepare maize seeds. **B**, Sterilize seeds. **C**, Prepare the "Sandwich" germination system. **D**, Saturate the paper with demineralized water. **E**, Place seeds on the paper. **F**, Assemble the "Sandwich". **G**, Side view of the "Sandwich". **H**, Wrap the "Sandwich" with aluminum foil for dark conditions. **I** and **J**, Germinate the seeds at 25~30°C in a growth chamber.

2. Transplanting, repotting and growing the plants in greenhouse

The plants were grown in the greenhouse of SEFY at UCLouvain (https://www.uclouvain.be/en/technology-platforms/plant-cultivation-facilities-sefy). During the experiment periods, the humidity was 50%~70%; the temperature was 21–26°C; and the artificial light intensity was 160~170 μmol.m^{-2}.s^{-1} at table level. For more information about the greenhouse, please visit the SEFY website.

Maize

a. Prepare pots (e.g. 6 × 6 × 6 *cm* [*L*× *W*× *H*] with drainage holes at the bottom) and fill them with soil. This experiment used seedling & cuttings potting soil (https://dcm-info.be/fr/pro, DCM, product 1004525), ensuring good water distribution, drainage and allowing the soil to retain its aerated structure (Figure 2A).

b. If the "Sandwich" technique was used, dig a hole in the soil according to the length of the maize roots and transfer the germinated seeds into the soil (one seed in each pot) (Figure 2B). Cover the seeds with ~1 cm soil and try to delicately cover the coleoptile (i.e. emerging organ that will give rise to the shoot).

In the case of direct sowing, seeds are placed at a depth of approximately 1 cm in the soil.

c. Place the pots in a tray and add tap water/rainwater to the tray to allow for water uptake by capillary action (Figure 2C–D). In this study, rainwater was utilized, as it is employed in the greenhouse facility. Water the plants regularly to keep the soil moist by the same method.

d. After two weeks, repot the plants into a larger pot (diameter: 15 cm, height: 15 cm) with a new type of potting soil (for example: https://dcm-info.be/fr/pro, product 1002381) (Figure 2–F). This type of soil should have a high-water holding capacity. Keep watering regularly, around two to three times a week. At each time, we stopped watering until water drained from the bottom of pots for control treatment. For stress treatment, plants were stopped watering and rewatered according to the experiments. *Note: At this step, Teros 21 sensors were installed in the pots to monitor soil matric potential (Please refer to the Ψ_{soil} measurement protocol).*

e. After another two weeks, the plants are ready for the installation of PSY-1 leaf psychrometers, detailed in a subsequent section.

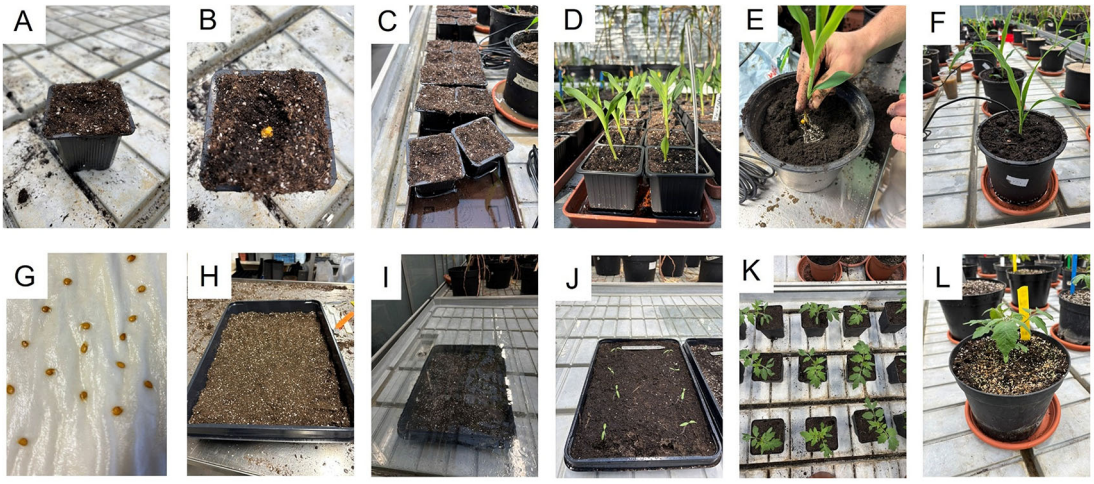


Figure 2. Transplanting and repotting processes. A-F, maize. **A**, prepare small pots filled with soil. **B**, transfer the germinated seeds into the pot. **C-D**, water the plants and grow them in greenhouse. **E**, transfer the maize seedlings to larger pots (diameter: 15 cm, height: 15 cm) after two weeks. **F**, grow plants in greenhouse. **G-L**, tomato. **G**, tomato seeds after two days germination at 27°C. **H**, prepare a big tray and fill it will soil. **I**, cover the tray with glass to keep moisture of the soil. **J**, remove the glass after 3–4 days. **K**, re-pot the seedling into a larger pot (diameter: 6 cm*6 cm*6 cm [L*W*H]) after 2 weeks. **L**, re-pot the seedling into a larger pot (diameter: 15 cm, height: 15 cm) after 2 weeks.

Tomato

a. Prepare a plastic tray (e.g. 38 × 25 × 6 *cm* [*L*× *W*× *H*]) with drainage holes at the bottom, and fill it with a seedling & cuttings potting soil (Figure 2H).

b. Transfer the germinated seeds (Figure 2G) to the top of the soil (plant spacing ~3–4 cm, row spacing ~3–4 cm) and cover the seeds with ~0.5 cm of soil.

c. Water the soil until water drainage is seen from the bottom of the tray and cover the tray with glass to reduce the evaporation (remove it after one week) (Figure 2I–J).

d. After two weeks, repot each plant into a larger pot (e.g. 6 × 6 × 6 *cm* (*L*× *W*× *H*) with seedling & cuttings potting soil (Figure 2K). Keep watering regularly, around two to three times a week.

e. After two weeks, repot the plants into a larger pot (diameter: 15 cm, height: 15 cm) with potting soil (https://dcm-info.be/fr/pro, DCM, product 1002381) (Figure 2L). Keep watering regularly, around two to three times a week. *Note: At this step, Teros 21 sensors were installed in the pots to monitor soil water conditions (Please refer to the Ψ_{soil} measurement protocol).* Note: Repotting should be performed as appropriate for the experimental objectives and plant growth stage. Experiments investigating root architecture or other root traits may require different establishment protocols to minimize disturbance.

f. After another two weeks, the plants are ready for the installation of PSY-1 leaf psychrometers, detailed in the next section.

Soil and leaf water potential measurements

1. Soil water potential (Ψ_{soil})

Timing: 30~60 minutes for the installation. The duration of the measurements will depend on the experiments.

Teros 21 is factory calibrated at 0 kPa, 0~−100 kPa (four points) and −100000 kPa. When soil matric potential < −100 kPa, Teros 21 relies on the linear relationship between the logarithm of water content and the logarithm of water potential. No calibration will be needed prior to use. However, additional considering will be required to use Teros 21 when, 1) measuring in frozen soils; 2) measuring in high salinity; 3) temperature sensitivity at matric potential less than about −500 kPa.

a. Prepare the materials (Figure 3A): Data logger (CR800, Campbell Scientific, Logan, Utah, United states), Teros 21 (Meter group, Washington, United States), RS232_USB cable, 12 V battery (Y7–12, Yuasa), isolation box.

b. Transfer the plant to the pot (Figure 3B), as explained in the protocol for transplanting and repotting (maize, step "d"; tomato, step "e").

c. To favor the contact between the sensor and the soil, cover Teros 21 with moist soil by gently pressing soil around the sensor (Figure 3C-D).

d. Make a hole in the soil, put the sensor in the pot (away from the pot wall and install horizontally) and fill the pot with soil (Figure 3E-F). Keep the plants in the greenhouse for further growth before the installation of PSY1 (see the protocols for the installation of PSY1).

e. Connect the Teros 21 sensors with a CR800 in an isolation box (Figure 3G-L). In this study, we used "C1" and "C3" ports from SDM channel (Figure 4). In each port, it is possible to connect a maximum of 10 Teros 21 sensors with different addresses. Splitter were used for multiple connections of Teros 21 to one port (Figure 3I and K).

   Note: To check and change the address number for each sensor, connect an individual Teros 21 sensor to CR800 (Figure 5), then follow the protocol from Campbell Scientific (https://s.campbellsci.com/documents/us/technical-papers/sdi12-troubleshooting.pdf).

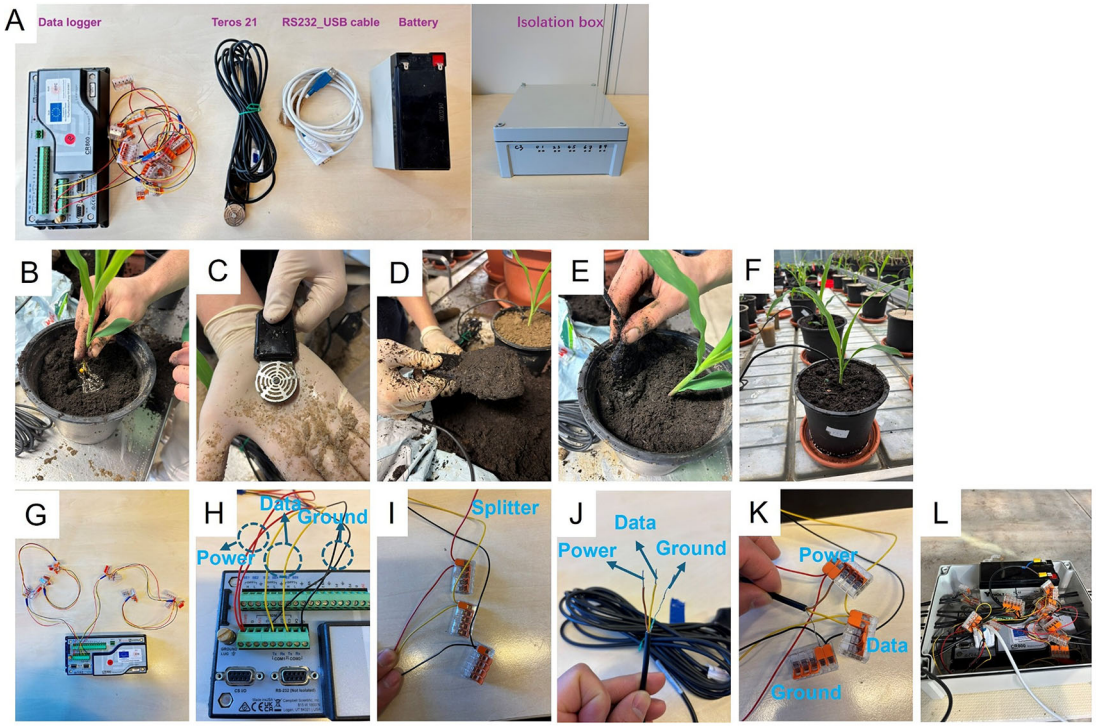


Figure 3. Installation of Teros21 in the pots. A, Materials for the installation of Teros 21. **B**-**F**, the installation of Teros 21 during repotting at section 2 (maize, steps d; tomato, steps e.) **G**-**L**, the connection between Teros 21 and data logger CR800.

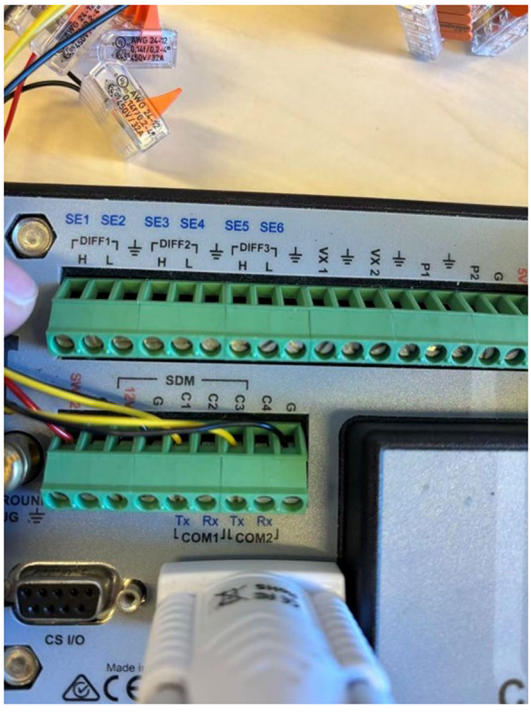


Figure 4. The data transporting ports between Teros21 and CR800.

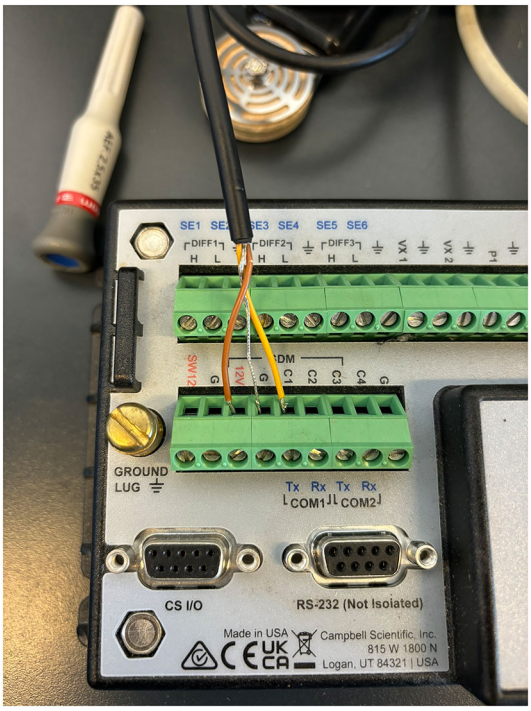


Figure 5. The connection of Teros21 and CR800 for checking and changing the address of the sensor.

f. On a computer, write a program for the data logger with a programming tool (in this study, "CRBasic Editor" from Campbell Scientific was used, https://help.campbellsci.com/crbasic/cr1000x/). CR800 needs a program to send an operation to the Teros 21, read the SDI-12 bus communication protocol received by the Teros 21, and store the data. In this program, key information that will be sent and received by the SDI-12 signal, needs to be specified, i.e. (1) the address of each Teros 21; (2) the data transporting port ("C1" or "C3"); (3) the creation of dataset files to store the recorded soil matric potential and temperature, as well as the voltage of the battery for power supply; (4) the time interval for the data recording.

   An example of the program was deposited on Zenodo (https://doi.org/10.5281/zenodo.17158115), with the document name of "Program-CR800"). Before starting, install the software of "Device Configuration Utility" and "PC400" from Campbell Scientific (https://www.campbellsci.com/devconfig; https://www.campbellsci.com/pc400). "CRBasic Editor" is integrated inside PC400. For more details about the programming, please refer to https://www.campbellsci.eu/videos/datalogger-programming?slid=101#slide=99.

g. Flash the program to the CR800. Firstly, connect CR800 with power supply (either a 12 V battery or a 12 V adapter socket directly plugged from the wall (220–240 V)). Then, connect CR800 with computer by RS232_USB cable. In this study, "Device Configuration Utility" was used to interact with CR800, which includes sending the program, identifying Teros 21 address, and downloading the data.

   Note: For more details of how to use "Device Configuration Utility", please refer to the protocols from Campbell Scientific (https://www.campbellsci.com/devconfig).

h. During the next day or after few hours of the installation, download the data from CR800 with "Device Configuration Utility" software to validate if all the sensors are working properly.

   Note: For more details of how to use Teros 21, please also read the user manual from meter group (https://metergroup.com/products/teros-21/teros-21-support/).

2. Leaf water potential (Ψ_{leaf})

Duration: ~5 minutes per installation. The duration of the measures will depend on the experiments. However,one installation could generate reliable data for one to two weeks for maize and tomato. After this period, the installed area of

the leaf will be in senescence and non-functional for water transport, so a new installation will be required to change the position on the leaves. An additional explicative video for the psychrometer installation on leaves is available on Zenodo (Degand *et al.*, 2025).

Note: PSY1 must be calibrated prior to first use or before installing in a new condition to guarantee accurate results. It is recommended to calibrate the instrument at or near the temperature at which it will normally be used. The calibration includes making measurements with calibration solutions of 0.1 M, 0.2 M, 0.3 M, 0.4 M,0.5 M, 1 M, 1.2 M NaCl on paper disk loaded to lid, to plot the data, generating a slope and an intercept that can be stored to calculate corrected water potentials in the ICT international software. After calibration, a good practice is to test that calibration with a 1 M NaCl solution that should give a value around −4.64 MPa after equilibration time (30 min to 2 hours). For other details of calibration, please check the manual from ICT international (https://ictinternational.com/wp-content/uploads/2023/08/PSY1-Manual-DOC-00087v2.pdf).

a. Prepare the materials (Figure 6A): PSY1-leaf psychrometer (head and data logger, ICT international, Armidale, Australia), leaf clamps (PSY1-Leaf Psychrometer Clamps, ICT international, Armidale, Australia), retort stand, grease (Bardahl 1688 Silicone Grease, Bardahl, France), sandpaper (P400, grit size ~35 µm), demineralized water, precision wipes (Kimberly-Clark).

b. Choose newly expanded leaf for maize and fully expanded leaf for tomato (blue arrows in Figure 6B).

c. Set up the retort stand (with the leaf chamber attached) beside the selected leaf. The leaf should be able to rest freely inside the leaf chamber without being bent.

d. Put the selected leaf inside the leaf clamp and use a marker pen to mark the position on each side of the leaf (Figure 6C).

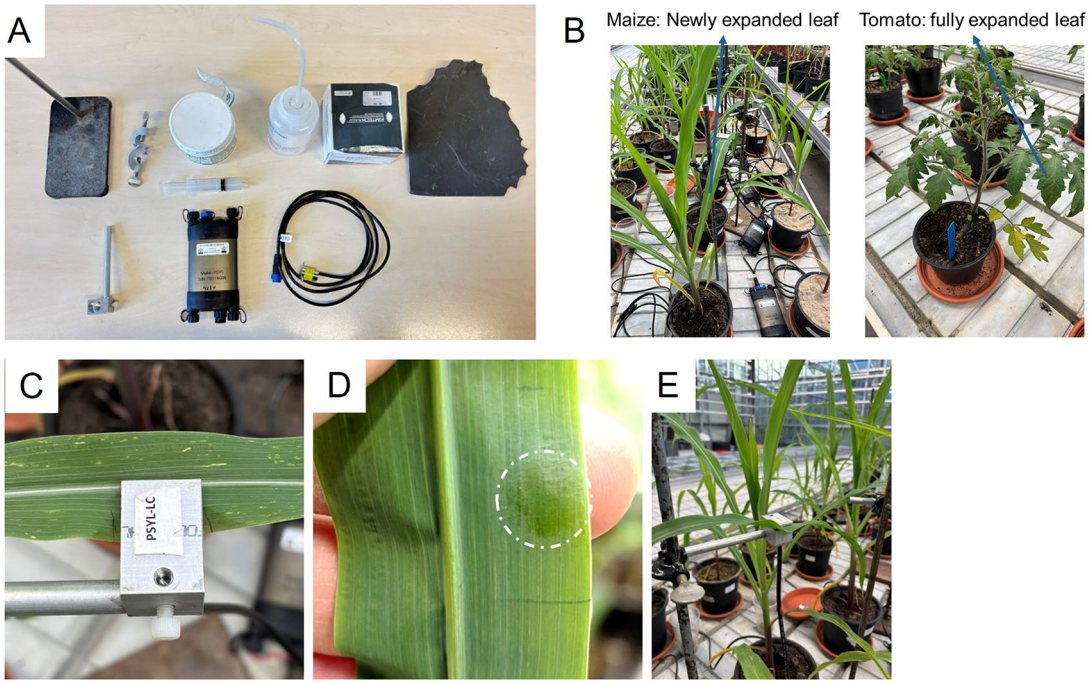


Figure 6. Installation of PSY1-Leaf on maize leaves. A, Materials needed for the installation. **B**, leaves selected for the measurement in maize and tomato. **C**, select the region for the installation. **D**, leaf after the abrasion with sandpaper. **E**, fix the head in the leaf clamp.

e. Remove the leaf from the chamber, take a piece of sandpaper (~1 cm^2), lightly wet the sandpaper with a few drops of demineralized water for lubrication, and use the sandpaper to abrade the marked region on the abaxial side of the leaf (Figure 6D). This abrasion will remove the cuticle on the surface of the leaf, in turn reducing cuticle resistance and improving water vapor diffusion in leaves. By removing cuticles, it will also reduce the equilibration time of leaf and psychrometer chamber. Ideally, the abrasion should just remove the cuticles without damaging the epidermal cells. It is recommended by the training from ICT international company to use a grit size of P400 for thick cuticles, such as citrus, a grit size of P600 for grasses, and a grit size of P800 for thin cuticles, such as beans.

f. Clean the abrasion region with demineralized water and dry it with tissue paper. Put the leaf back into the leaf clamp.

g. Take one PSY1-leaf psychrometer and put silicone grease around the edge of the cylindrical face. Spread the grease to have a ~0.5 mm layer of grease. This can be done either with a finger, or by placing the psychrometer lid back onto the chamber and turning slightly (remove lid after this action).

h. Put the psychrometer into the leaf clamp and fix it with the screw (Figure 6E).

   Note: In this study, the psychrometer was used in the greenhouse, with no thermal insulation around the leaf clamp. In a separate experiment, it was found that the measurements of PSY1-leaf showed similar water potential values between insulated and non-insulated treatments in the greenhouse. However, limiting temperature gradients and maintaining constant instrument temperature are two highly desirable factors in the reliability of the measurements. In the field, insulating the chamber creates a thermal buffer zone to avoid rapid temperature change during the measurement. This insulation is not intended to prevent the diurnal temperature change but rather serves as a way to avoid condensation in the chamber, which would compromise the measurement. (For more details of the insulation, please refer to the manual from ICT international, https://ictinternational.com/wp-content/uploads/2023/08/PSY1-Manual-DOC-00087v2.pdf).

i. Connect the data logger of the psychrometer to a computer to configure the settings by following the instructions from ICT international. In this study, data was recorded every 15 minutes, with a cooling time of 10 seconds, waiting time of 6 seconds, warming duration of 0 seconds, waiting time before measurement of 15 seconds, and chamber heating of 0 seconds. However, these settings can be changed according to the experiment by following the instructions from ICT international (see "0" error section).

   Note: Installation of the ICT international software is required to connect psychrometers (Combined Instrument Software, ICT international, https://ictinternational.com/product/psy1-psychrometer-for-plant-water-potential/). Please follow the protocols given by ICT international to install the software and connect the PSY1-leaf sensors to a computer.

j. After allowing the leaf to equilibrate (typically between 30 minutes and 2 hours), the next step is to download and review the Ψ_{leaf} data. It is also good practice to check the data again about 24 hours after installation to confirm that a normal day–night pattern is observed. To retrieve the Ψ_{leaf} data, you can either connect the PSY1 leaf data logger directly to a computer using the dedicated software or remove the memory card from the logger and read it on a computer. If the installation was not done properly, the PSY1 may need to be reinstalled. Signs of improper installation often appear clearly in the data. For example, a typical dataset should show a diurnal pattern, with Ψ_{leaf} decreasing during the day as transpiration increases and recovering during the night. If this pattern is absent, or if the data consistently shows values close to "0", it likely indicates an issue with the installation.

The "0" recording can be induced due to the abrasion technique, or due to a bending of the thermal couple, which might lead to a loss of contact with the leaf. In this case, check the thermocouple under a microscope and adjust the position if necessary, by following the protocols from ICT international (https://www.youtube.com/watch?v=kNsZnSYDBPE&list=PL4F3FBBF0881B16D7&index=16).

Lastly, a common issue when working with psychrometers is condensation. If the sample tissue is cooler than the chamber, water may condense directly on the sample, where it can be absorbed or redistributed. On the other hand, if the chamber is cooler than the sample, condensation may form on the chamber walls, introducing uncertainty and potential errors in the measurements. This problem is often noticeable in the data when there are strong temperature gradients (dT).

In most cases, condensation issues can be resolved by cleaning the system and reinstalling the components properly. However, if the problem persists, it may be necessary to adjust certain parameters, such as the cooling time or chamber heating settings. For more details on these adjustments, refer to Chapter 6 ("Settings and Measurement Options") of the PSY1 Manual DOC-00087v2. If none of these solutions resolve the issue—particularly when measurements remain close to 0 MPa—it is important to recognize a fundamental limitation of the psychrometric method. Near the wet end of the spectrum (around 0 MPa), the energy required to convert water from liquid to vapor is extremely low, which reduces measurement sensitivity and accuracy. This situation is uncommon in potted plants but can occur more frequently in hydroponic systems.

Download and check the data regularly (once a day). This regular check will help in tracking the data, and in identifying any issues (as mentioned above) as early as possible.

As a complement to Ψ_{leaf}, plant transpiration can be monitored using weighing scales. In this study, whole-plant transpiration was monitored continuously by recording pot weight over the same duration of the psychrometers. The pots were placed on a scale (A&D, EK-15KL, Japan; the resolution is 0.1 g), and the weight was recorded every 15 minutes using the RsWeight program from the Windows Communication Tools (WinCT) package. This software, developed by A&D, collected data via a StarTech USB 16- or 8-port adapter connected to the scale through an RS232 cable. To minimize soil evaporation, the pots were covered with aluminum foil.

Additionally, destructive measurements of Ψ_{leaf} can be taken using a Scholander pressure bomb to compare with the data measured by TCP. However, it is still unclear whether the Scholander pressure bomb measures both Ψ_p and Ψ_o or just the Ψ_p of leaves, while TCP measures both Ψ_p and Ψ_o.

Data analysis

Timing: ~10 minutes.

We made an R quarto document as well as custom functions to load raw measurements files, clean the data files, and make graphs. This R quarto document is available on Zenodo (D'Agostino, 2026). The folder contains a user-friendly notebook to load files in R, custom functions to extract and compile data, and a simple dataset containing data about 4 plants. Figure 7 shows compiled and cleaned data from the example dataset.

1) Code setup

Download the repository (D'Agostino, 2026). Extract the content to a local disk. Launch Rstudio. Open the file *"Data_Analysis_Main.qmd*" located in the repository folder. Ensure all required libraries are installed.

2) Data collection

Collect data from both Teros 21 and PSY1-leaf by connecting the data loggers to a computer with the relevant software mentioned previously. PSY1-leaf will generate a.csv format file and store all the data inside, while CR800 generates a DAT format file. In both cases, copy each individual file to *data/raw/LWP/*folder. Make sure that each file is named as the ID of the sensor.

3) Encode your metadata

Create a metadata file that contains identifiers for plants and sensors, similar to *Plants_ID.csv* in *data/clean/*folder. Make sure that each sensor ID corresponds to the right Plant_ID.

4) Run the code to extract and clean Ψ_{leaf} data

The code automatically extracts data from each individual Ψ_{leaf} file and integrates it into one unique table that contains Ψ_{leaf} data per date and per Plant_ID. The table is saved in the *data/raw/*folder. Statistical manipulations or data corrections can then be applied as deemed appropriate. A cleaning algorithm has been included in the code that filters data based on: the number of "0" and *"NAN"* values; incorrect day-night cycle amplitude; the global slope, and the global level (more details are provided in the notebook).

A web version of the notebook is available here: https://theplantwaterpump.github.io/TS_Protocol/

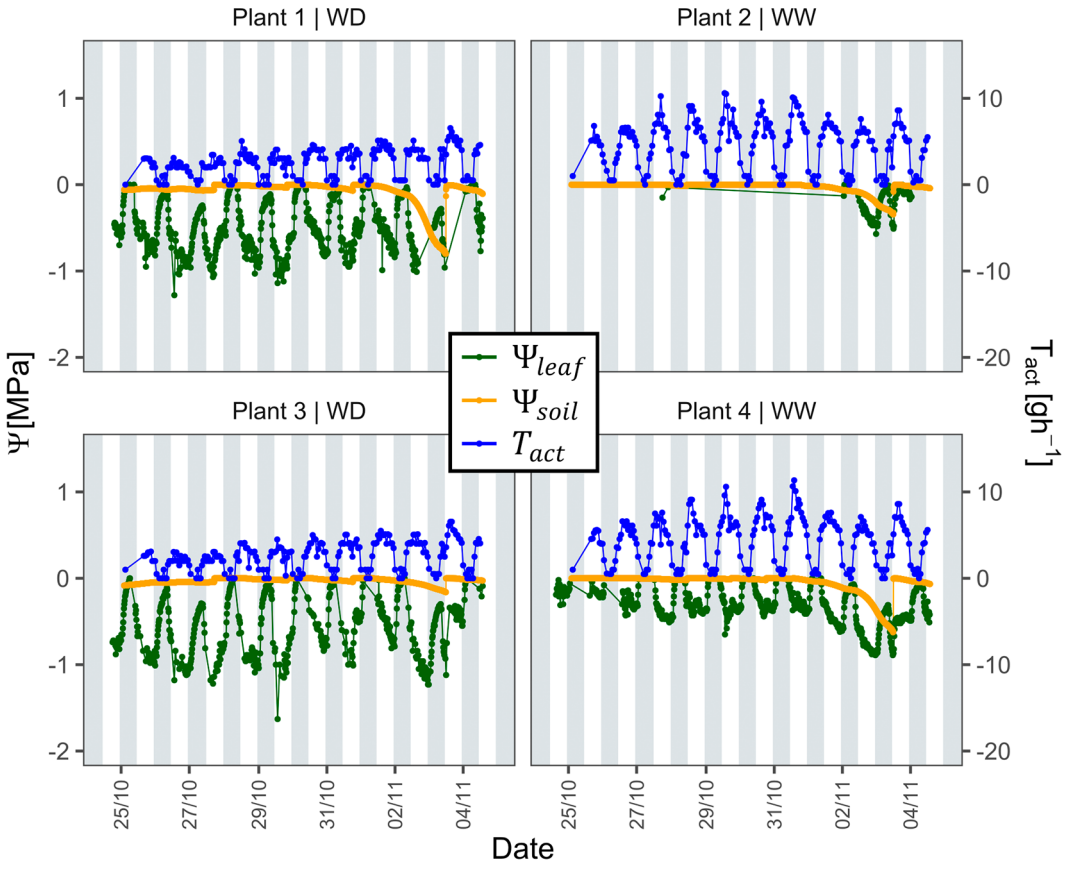


Figure 7. Example of compiled and cleaned data from the R notebook. Plants were grown in greenhouse as explained in the section of "Seeds germination and plant growth". Plant 2 and 4 were subjected to well-water (WW) conditions, while plant 1 and 3 were subjected to water-deficit (WD) conditions. Left y-axis indicated Ψ_{leaf} and Ψ_{soil}. Right y-axis indicated actual transpiration rate (Tact) recorded by weighing scale.

5) Run the code to extract soil water potential and transpiration data

A similar process to the Ψ_{leaf} is done for Ψ_{soil} data and weight data within the code. Follow instructions set in the notebook for further detail for these two data types. Additionally, the code includes the computation of transpiration rate, and allows all variables to be plotted together (Figure 7).

Because this protocol paper is intended to demonstrate PSY1 operation and interpretation, the example dataset is illustrative and not designed for rigorous statistical treatment; users should include sufficient biological replication, typically at least 3–5 replicates where possible depending on the experimental objective and biological variability, together with calibration checks and uncertainty analysis.

Troubleshooting

Heterogeneity of water distribution in the soil

As the soil dries out, there will be heterogeneity of water distribution in the soil. To have more representative Ψ_{soil} measurements in the pot, we recommend to:

(1) Install Teros 21 at different depths and/or the same depth with different horizonal positions.

(2) Avoid watering in one location and instead try to spray the water uniformly over the whole surface of the top layer of the soil. If a large amount of water is required to be given to the plant, try to split the amount into several portions and give it to the plant during the day.

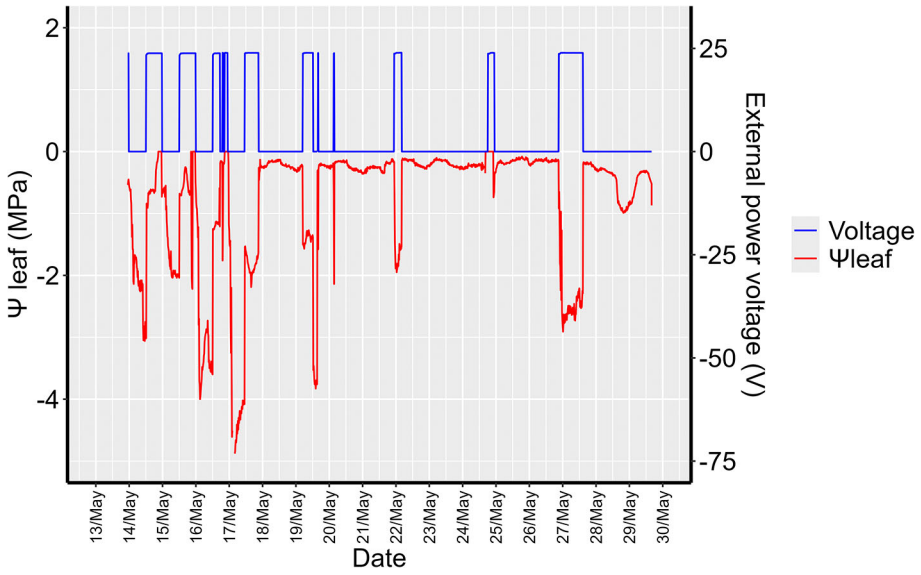


Figure 8. Example of the charging effects on the data recordings. Ψ_{leaf} is indicated by red line and voltage is indicated by blue line. The charging voltage was 24 V and "0" voltage indicates there was no charging. When voltage changed between "0 V" and "24 V", Ψ_{leaf} started jump.

Charging issue for the psychrometer data recording

During this study, difficulties with the psychrometer data were encountered due to the impact of the data logger's charging process. Fluctuations in the PSY1 data were exhibited when the charging process was initiated or terminated (as shown in Figure 8). This occurred particularly when the PSY1 data loggers were connected to a power socket simultaneously used by other devices, such as weighing scales. To avoid this issue, an external 12 V battery was used to charge the data loggers.

Data cleaning with an algorithm

The initial dataset from the psychrometer measurements may contain several outliers, missing values (skipped due to low battery), incorrect entries, and aberrant patterns. To resolve this, a data cleaning algorithm was implemented. This algorithm was designed to remove any values that were missing, zero, or otherwise aberrant. Data were considered aberrant when values deviated markedly from the typical day- or night-time distribution and/or were accompanied by abnormal Wet Bulb Depression or dT signals indicative of unreliable psychrometric measurements. It was also programmed to identify and remove inverted day-night cycle patterns, as well as values that were statistically insignificant. The inverted day/night cycle could be due to the charging issue explained above. Figure 9 shows

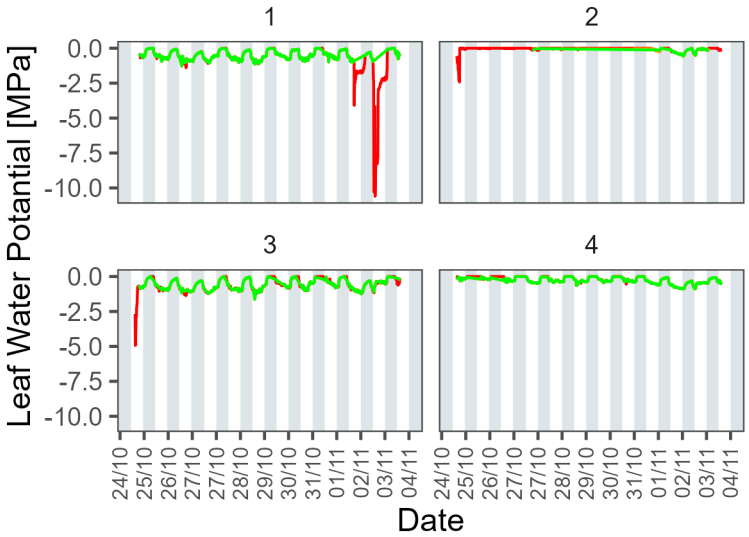


Figure 9. Example of data cleaning using the algorithm. Green is kept data and red is discarded data.

applications of data cleaning on the example dataset. For more details, please check codes that have been deposited on Zenodo (https://doi.org/10.5281/zenodo.20080750, D'Agostino, 2026).

Conclusion

In summary, the present protocol is not confined to the descriptive monitoring of Ψ_{soil} and Ψ_{leaf}; it also furnishes an experimental framework to relate continuous water-potential measurements to plant hydraulic properties. As previously mentioned, radial water flow can be expressed as a function of the driving force in Ψ_{tot} and the corresponding hydraulic conductivity. Accordingly, when Teros 21 measurements of soil matric potential are interpreted in conjunction with PSY1-derived Ψ_{leaf} and an independent estimate of water flux, such as whole-plant transpiration obtained gravimetrically, these time series can be used to assess changes in apparent root or whole-plant hydraulic conductance during the processes of drying and rewetting under near-steady-state conditions and with appropriate assumptions. Such an interpretation necessitates explicit assumptions, including hydraulic equilibration at the measurement interface, limited temperature artefacts, and a physically consistent comparison between the water-potential components under consideration. It has been demonstrated that, within the established limits, the combined measurements are not only useful for tracking plant water status over time, but also for identifying phases of hydraulic limitation, soil-root disconnection, and recovery. Consequently, this linkage of the protocol to mechanistic analyses of water transport in the SPAC is more direct.

Ethics and consent

Ethical approval and consent were not required.

Data availability

The datasets and codes to analyze the data have been deposited on Zenodo (https://doi.org/10.5281/zenodo.20080750, D'Agostino (2026)).

Data are available under the terms of the Creative Commons Zero v1.0 Universal.

An additional explicative video for the psychrometer installation on leaves is available on Zenodo (https://doi.org/10.5281/zenodo.17510720, Degand *et al.* (2025)).

The author(s) declare that this video is released under the Creative Commons CC0 1.0 Universal Public Domain Dedication. This means the video is free of all copyright restrictions and may be copied, modified, distributed, and used without permission, including for commercial purposes.

Data are available under the terms of the Creative Commons Attribution 4.0 International.

Acknowledgement

We thank Marc Migon and Thomas Dagbert for their technical support for the plants grown in the greenhouse.

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Publisher Full Text

Open Peer Review

Current Peer Review Status: ✔ ✔ ? ?

Version 2

Reviewer Report 01 July 2026

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?

Tina Koehler
Technical University of Munich, Freising, Germany

General comments:
The manuscript entitled “Protocols for in situ continuous monitoring of water relations/potential in soil and leaf” by Ding and colleagues is timely (i.e., it relates well to recently published work on the topic, e.g., in reference), and important in terms of offering a standardized protocol for applying in-situ measurements of soil- and leaf water potential, thereby enhancing the comparability across experiments conducted by different groups. The scripts they share on publicly available repositories are especially appreciated. I believe the present manuscript can contribute meaningfully to the community conducting those measurements, and, more importantly, help bridge the gap to communities that are not yet employing these measurements. However, I have some suggestions for refinements, which are specified below.

- Avoid jargon like “Light was …” when you mean “*light intensity was* …”, or “To retrieve the data, *you can* either…”
- Unify use of abbreviations (e.g., sometimes you use LWP, sometimes “Leaf water potential”, and sometimes Ψ_{leaf}, in the text as well as in the figure axis titles and legends)
- Unify spelling (e.g., sometimes you use Teros21 and sometimes TEROS21)
- Use consistent variables, titles, and colors across plots (e.g., no proper left y-axis title nor units in Fig. 8, and no meaningful subplot titles in Fig. 7)

P3:

- “It will be assumed herein that frictions along xylem vessels are negligible for the small plants of this study, and xylem water potential in the root and leaf are equivalent” - regarding this statement, it would be good to add a “Note that..” and mention some of the studies addressing, e.g., xylem-internal friction.
- When referring to methods for quantifying leaf water potential, it would be good to refer to the article in the reference section.
- Consider referring to key papers contributing to the debate around whether the Scholander pressure chamber measures total leaf water potential or merely the pressure potential.

P4:

- Helpful introduction to which sensors and methods are commonly used to quantify leaf and

soil water potential. I would add a sentence on why exactly TEROS21 and PSY1 psychrometers were chosen amongst all of the introduced ones for *this* study.
- TEROS21 does not work well in the wet range. METER even specifies –8 to –100.000 kPa.
- "Despite the fact that PSY1 measurements are affected by the soil matric water potential reported by TEROS 21, a direct comparison between the two is not recommended" - What do you mean by *a direct comparison*? Of course, there is a temporal and magnitude offset between leaf water potential and soil water potential, and diurnal patterns of leaf water potential do not necessarily translate into (measurable) diurnal fluctuations in soil water potential (especially in wet soil). However, interpreting leaf water potential without knowing the water status of the soil and vice versa will lead to invalid comparisons. It would help if this statement could be made clearer.

P5:
- In 1c., you refer to "the Sandwich technique in d.", and I am not sure which d. you mean.
- "Artificial light [intensity] was 160-170 µmol m^{-2} s^{-1}" - that seems quite low. I would report the actual light intensity the plants experienced.

P6:
- Maize b.: How deep to plant the seeds if they are not pre-germinated?
- Maize c.: Why irrigation with rainwater, specifically? Do you imply de-mineralized water?
- Maize d.: Why plant maize in a new type of potting soil upon transplantation?
- Maize d.: Instead of saying that the soil should be watered "regularly", which is very vague, it would be more helpful (and in line with the paper) to indicate a specific soil water potential level/ range to aim for and possibly suggest a protocol for determining how much water to add (weight or volume).

P7:
- Tomato e.: I would avoid going into the details of recommending how often to repot. For example, researchers interested in root architecture might not want to repeatedly disturb root development. I think this aspect of the plant establishment and growth will be rather context specific.
- Soil water potential a.: The METER ZL6 logger could also be used instead of programming a Campbell logger. Is there any specific reason why you decided on the other way?
- Soil water potential d.: The installation of the TEROS21 could be specified in greater detail, as this is crucial for the quality of the data. For example, the sensors should be as far away from the pot walls as possible and, ideally, installed horizontally rather than vertically.

P8:
- Soil water potential e.: Are "Terminal blocks" the same as "splitters" (in Fig. 3I)? If yes, use same terminology.
- Soil water potential f.: Thank you for depositing the file for programming the Campbell logger on Zenodo, I am sure this will be very helpful for the community.

P9:
- Soil water potential h.: Fix grammar: "to validate [if] all the sensors [are] working properly"
- Leaf water potential: Does water transport functionality decline gradually as the leaf on which the psychrometer is installed senesces? If yes, from when on do the data become unreliable?
- Leaf water potential b.: When you say "newly expanded leaf for maize and fully expanded leaf for tomato", do you mean "youngest fully developed leaf for maize"? It should already be fully expanded, also for maize, right?

P11:
- The comment on the importance of buffering psychrometers against temperature changes is really important here. Could you add a plot of temperature (and possibly relative humidity) fluctuations over the experimental period in the greenhouse here? Typically, it can get quite hot inside greenhouses. Hence, I am surprised that you do not see a signal of this in your psychrometer data.
- Specify the resolution of the balance. This matters for resolving diurnal plant water-use patterns in small, potted plants.

P14:
- Not sure if changes in whole-plant hydraulic conductance *during* the process of drying and rewetting can be inferred from time series of transpiration rate, soil- and leaf water potential, as Darcy's law, that is typically being used for that, applies under steady-state conditions only...

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Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: soil physics, soil-plant hydraulics, plant/ crop physiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 07 Sep 2026

Lei Ding

Reviewer 4 General comments:
The manuscript entitled "Protocols for in situ continuous monitoring of water relations/potential in soil and leaf" by Ding and colleagues is timely (i.e., it relates well to

recently published work on the topic, e.g., in reference), and important in terms of offering a standardized protocol for applying in-situ measurements of soil- and leaf water potential, thereby enhancing the comparability across experiments conducted by different groups. The scripts they share on publicly available repositories are especially appreciated. I believe the present manuscript can contribute meaningfully to the community conducting those measurements, and, more importantly, help bridge the gap to communities that are not yet employing these measurements. However, I have some suggestions for refinements, which are specified below.

- Avoid jargon like "Light was …" when you mean "*light intensity was* …", or "To retrieve the data, *you can* either…"

Answer: Thanks for this suggestion! We revised the text.

- Unify use of abbreviations (e.g., sometimes you use LWP, sometimes "Leaf water potential", and sometimes Ψ_{leaf}, in the text as well as in the figure axis titles and legends)

Answer: We used Ψ_{leaf} and Ψ_{soil} throughout the text and figures in the revised manuscript.

- Unify spelling (e.g., sometimes you use Teros21 and sometimes TEROS21)

Answer: Teros 21 was used in the revised manuscript.

- Use consistent variables, titles, and colors across plots (e.g., no proper left y-axis title nor units in Fig. 8, and no meaningful subplot titles in Fig. 7)

Answer: We have revised Figure 7 and 8, please check! P3:

- "It will be assumed herein that frictions along xylem vessels are negligible for the small plants of this study, and xylem water potential in the root and leaf are equivalent" - regarding this statement, it would be good to add a "Note that.." and mention some of the studies addressing, e.g., xylem-internal friction.

Answer: We thank the reviewer for this good suggestion. We cited Venturas et al. (2017). Reference Venturas MD, Sperry JS, Hacke UG: Plant xylem hydraulics: What we understand, current research, and future challenges. Journal of Integrative Plant Biology. 2017;59 (6): 356–389 10.1111/jipb.12534

- Consider referring to key papers contributing to the debate around whether the Scholander pressure chamber measures total leaf water potential or merely the pressure potential.

Answer: Thank you for this suggestion. We have included several key references discussing the ongoing debate regarding whether the Scholander pressure chamber measures total leaf water potential (Ψ_tot) or primarily xylem pressure potential (Ψ_p). The corresponding text has been revised as follows: Concurrently, the Scholander pressure chamber proposed a direct but destructive measurement of (Ψ_{leaf}) (Scholander *et al.*, 1965). Whether this technique measures Ψ_{tot} or Ψ_{p} remains a matter of debate (Boyer, 1969; Rodríguez-Domínguez et al., 2022). The method assumes that equilibrium is reached between the applied balancing pressure and the negative water potential in the leaf xylem, while the osmotic potential of the xylem/apoplastic sap is negligible (Boyer, 1969). However, this assumption may not always hold, as differences among measurement techniques and the presence of non-negligible xylem osmotic potential under certain physiological or environmental conditions can influence the interpretation of pressure chamber measurements (Rodríguez-Domínguez et al., 2022; Enns et al., 2000; Westhoff et al., 2008). P4:

- Helpful introduction to which sensors and methods are commonly used to quantify

leaf and soil water potential. I would add a sentence on why exactly TEROS21 and PSY1 psychrometers were chosen amongst all of the introduced ones for *this* study.

Answer: We explained why we selected Teros 21 and PSY1 in this study in the revised manuscript, please check.

- TEROS21 does not work well in the wet range. METER even specifies –8 to –100.000 kPa.

Answer: According to the user manual, Teros 21 detection range is 0 to –100.000 kPa.

- "Despite the fact that PSY1 measurements are affected by the soil matric water potential reported by TEROS 21, a direct comparison between the two is not recommended" - What do you mean by *a direct comparison*? Of course, there is a temporal and magnitude offset between leaf water potential and soil water potential, and diurnal patterns of leaf water potential do not necessarily translate into (measurable) diurnal fluctuations in soil water potential (especially in wet soil). However, interpreting leaf water potential without knowing the water status of the soil and vice versa will lead to invalid comparisons. It would help if this statement could be made clearer.

Answer: Thank you for this helpful comment. We agree that leaf water potential and soil water potential provide complementary information and should be interpreted together to understand the soil–plant water continuum. Our original wording was unclear. By "direct comparison", we intended to indicate that the absolute values of leaf water potential and soil matric potential should not be directly equated because they differ in both temporal dynamics and magnitude due to hydraulic resistance, plant water storage, and environmental conditions. We have revised the text accordingly to clarify this point. P5:

- In 1c., you refer to "the Sandwich technique in d.", and I am not sure which d. you mean.

Answer: Sorry for this confusing, we removed the text of "in d."

- "Artificial light [intensity] was 160-170 µmol m^{-2} s^{-1}" - that seems quite low. I would report the actual light intensity the plants experienced.

Answer: In the original text, we mentioned "the artificial light intensity was 160~170 µmol.m^{-2}.s^{-1} at table level". However, the plants were grown in a glasshouse and there was sunlight as well. We did not record the actual light intensity during the period of this protocol study. P6:

- Maize b.: How deep to plant the seeds if they are not pre-germinated?

Answer: We have now added the following clarification to the revised manuscript: "In the case of direct sowing, seeds are placed at a depth of approximately 1 cm in the soil."

- Maize c.: Why irrigation with rainwater, specifically? Do you imply de-mineralized water?

Answer: Sorry for this unclear! In our greenhouse facility, rainwater is collected and routinely used for plant irrigation. In this study, the maize plants were therefore irrigated with collected rainwater rather than demineralized water. We do not intend to imply that rainwater is equivalent to demineralized water, as it may contain small amounts of dissolved minerals and nutrients. We have revised the manuscript to clarify this point.

- Maize d.: Why plant maize in a new type of potting soil upon transplantation?

Answer: In a, we mentioned that "This experiment used seedling & cuttings potting soil (https://dcm-info.be/fr/pro, DCM, product 1004525), ensuring good water distribution, drainage and allowing the soil to retain its aerated structure". In d, we mentioned that

"...with a new type of potting soil (for example: https://dcm-info.be/fr/pro, product 1002381) (Figure 2–F). This type of soil should have a high-water holding capacity. Keep watering regularly, around two to three times a week."

- Maize d.: Instead of saying that the soil should be watered "regularly", which is very vague, it would be more helpful (and in line with the paper) to indicate a specific soil water potential level/ range to aim for and possibly suggest a protocol for determining how much water to add (weight or volume).

Answer: We thank the reviewer for this valuable suggestion. We agree that specifying a target soil water status is more informative than using the vague term "regularly." However, we prefer not to prescribe a fixed irrigation volume because plant water requirements vary with environmental conditions, including radiation, temperature, and humidity, as well as with plant size and developmental stage. In our experiments, irrigation was adjusted according to soil water potential measured with the TEROS 21 sensor, together with transpiration-driven water loss. For well-watered conditions, pots were irrigated until drainage occurred from the bottom of the pot. For drought treatments, irrigation was withheld until the desired soil water potential was reached when TEROS 21 was installed and was then resumed according to the experimental design. We have revised the manuscript to clarify this procedure. P7:

- Tomato e.: I would avoid going into the details of recommending how often to repot. For example, researchers interested in root architecture might not want to repeatedly disturb root development. I think this aspect of the plant establishment and growth will be rather context specific.

Answer: Thank you for this helpful suggestion. We agree that the timing and frequency of repotting are highly dependent on the experimental objectives. Our recommendation was intended for studies focusing on continuous in situ monitoring of soil and leaf water potential rather than experiments investigating root architecture or other root-related traits, where minimizing root disturbance is important. We have therefore revised the manuscript to remove the prescriptive recommendation on repotting frequency and instead state that repotting should be adjusted according to the specific experimental objectives.

- Soil water potential a.: The METER ZL6 logger could also be used instead of programming a Campbell logger. Is there any specific reason why you decided on the other way?

Answer: Yes, there are several options for communicating with and recording data from the TEROS 21 sensor, including the METER ZL6 data logger. We used a Campbell Scientific data logger in this study because it was already available in our experimental setup and could be readily integrated with our other measurements. There is no specific methodological requirement to use a Campbell logger, and the ZL6 or other compatible logging systems can also be used.

- Soil water potential d.: The installation of the TEROS21 could be specified in greater detail, as this is crucial for the quality of the data. For example, the sensors should be as far away from the pot walls as possible and, ideally, installed horizontally rather than vertically.

Answer: Thank you for this helpful suggestion! We revised the text in the revised manuscript. P8:

- Soil water potential e.: Are "Terminal blocks" the same as "splitters" (in Fig. 3I)? If yes, use same terminology.

Answer: Yes, they are the same. We changed “Terminal blocks” to “splitters”.

- Soil water potential f.: Thank you for depositing the file for programming the Campbell logger on Zenodo, I am sure this will be very helpful for the community.

Answer: Thanks! P9:

- Soil water potential h.: Fix grammar: “to validate [if] all the sensors [are] working properly”

Answer: Done.

- Leaf water potential: Does water transport functionality decline gradually as the leaf on which the psychrometer is installed senesces? If yes, from when on do the data become unreliable?

Answer: Water transport functionality does not decline gradually. We mentioned that “However, one installation could generate reliable data for one to two weeks for maize and tomato. After this period, the installed area of the leaf will be in senescence and non-functional for water transport, so a new installation will be required to change the position on the leaves.” We will need to check the data regularly to validate if the data becomes unreliable.

- Leaf water potential b.: When you say “newly expanded leaf for maize and fully expanded leaf for tomato”, do you mean “youngest fully developed leaf for maize”? It should already be fully expanded, also for maize, right?

Answer: Yes, we mean the youngest fully expanded leaf in maize. Leaves that are still elongating or expanding are not suitable for measurement because ongoing leaf growth can disturb sensor contact and may also cause mechanical damage to the leaf during the measurement period. P11:

- The comment on the importance of buffering psychrometers against temperature changes is really important here. Could you add a plot of temperature (and possibly relative humidity) fluctuations over the experimental period in the greenhouse here? Typically, it can get quite hot inside greenhouses. Hence, I am surprised that you do not see a signal of this in your psychrometer data.

Answer: We thank the reviewer for this helpful suggestion. We have now added the PSY1 chamber temperature recorded over the experimental period together with the corresponding leaf water potential measurements (Figure 1). As expected under greenhouse conditions, chamber temperature showed a clear diurnal pattern. It is important to distinguish these variations in absolute chamber temperature from temperature gradients within the psychrometer system. The PSY1 can operate across changes in ambient temperature provided that the leaf, chamber, and sensor remain sufficiently close to thermal equilibrium. The leaf water potential measurements also showed diurnal dynamics. These variations may partly reflect the physiological response of the plants to changing daytime environmental conditions, including temperature and associated changes in transpiration demand, rather than a direct temperature artefact of the psychrometer. As mentioned in the manuscript, abnormal temperature gradients (dT), rather than chamber temperature alone, were used as an indicator of potentially unreliable PSY1 measurements.

https://openreseurope-files.f1000.com/linked/280181.Lei_Ding.png

Figure 1 Continuous recording of leaf water potential (Ψleaf; left y-axis, lower/thicker curve in each panel) and PSY1 chamber temperature (right y-axis, upper/thinner curve) for four

plants during the experimental period.
- Specify the resolution of the balance. This matters for resolving diurnal plant water-use patterns in small, potted plants.

Answer: In this study, the resolution of the balance is 0.1 g. We mentioned this in the revised manuscript. P14:
- Not sure if changes in whole-plant hydraulic conductance *during* the process of drying and rewetting can be inferred from time series of transpiration rate, soil- and leaf water potential, as Darcy's law, that is typically being used for that, applies under steady-state conditions only…

Answer: We agree with the reviewer that changes in whole-plant hydraulic conductance during the process of drying and rewetting may not be inferred from time series of transpiration rate, soil- and leaf water potential, as Darcy's law, that is typically being used for that, applies under steady-state conditions. In the revised manuscript, we mentioned that the assessment should be working under near-steady-state conditions and with appropriate assumptions.

Competing Interests: No competing interests were disclosed.

Reviewer Report 22 June 2026

https://doi.org/10.21956/openreseurope.25594.r76008

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?

Simon Kraatz
USDA ARS Hydrology and Remote Sensing Laboratory, Beltsville, Maryland, USA

This work is to provide measurement protocols for measuring water potential in tomato and maize starting at germination. The protocols are tailored to the PSY1 psychrometer and the TEROS21 matric potential sensor, but the concepts and basic steps provided likely also are more generally applicable.

The authors also provide an installation video of the leaf psychrometer, and an R code for data processing.

Given the level of detail (plus the installation video and code) this protocol should now be reproducible by others.

I agree with the previous two reviewers on the issue about errors inherent to psychrometry, but they have since been added to the protocol.

However, a key item still missing, the expected level of uncertainty for applying this protocol under standard conditions. There is instrument error but also between ideal conditions and in the

theory, and how temperature gradient / non-equilibrium conditions etc. propagate.

There should be more detail on the 'otherwise aberrant' part in the data cleaning, and rationale should be given for removing inverted day night cycle patterns (why does this occur?).

The manuscript text did define the abbreviations for the legend items shown in Figure 7 for SWP but not for Tr or LWP. Figure 7 caption could have more details.

It is important to caution about the use of "Measure" in the caption, because the potentials are not direct measurements, they are calculated from e.g. vapor pressure measurements using the presented theory. All three quantities should have some error bar or uncertainty estimate due to instrument error & theory error propagation.

Figure 8 should have a legend for the blue and red line, and the caption could have more information on what issue is presented in the Figure.

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Partly

Are sufficient details of the methods provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Sensing of plant and soil properties

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 07 Sep 2026

Lei Ding

Reviewer 3 This work is to provide measurement protocols for measuring water potential in tomato and maize starting at germination. The protocols are tailored to the PSY1 psychrometer and the TEROS21 matric potential sensor, but the concepts and basic steps provided likely also are more generally applicable. The authors also provide an installation video of the leaf psychrometer, and an R code for data processing. Given the level of detail (plus the installation video and code) this protocol should now be reproducible by others. I agree with the previous two reviewers on the issue about errors inherent to psychrometry,

but they have since been added to the protocol. However, a key item still missing, the expected level of uncertainty for applying this protocol under standard conditions. There is instrument error but also between ideal conditions and in the theory, and how temperature gradient / non-equilibrium conditions etc. propagate.
Answer: We thank the reviewer for this important comment. We agree that understanding the expected level of uncertainty is important for the appropriate application and interpretation of this protocol. Based on our experience, high-quality and reproducible datasets can be obtained under standard experimental conditions, including both greenhouse and growth-chamber experiments, when the sensors are properly calibrated and installed and when temperature stability and sufficient equilibration are maintained. In the manuscript, we have clarified the main sources of uncertainty associated with the measurements, including instrumental and calibration uncertainty, temperature gradients, incomplete equilibration, condensation, sensor–leaf contact, and spatial heterogeneity in soil water potential. However, we prefer not to assign expected level to the overall uncertainty of the protocol. The effective uncertainty depends strongly on experimental conditions, sensor installation, temperature stability, equilibration, sample characteristics, and data-quality criteria. In addition, deviations from ideal psychrometer conditions, such as temperature gradients or non-equilibrium, may introduce systematic bias rather than simply increasing random measurement error. Therefore, a single uncertainty value would not adequately represent the range of conditions under which this protocol may be applied.

There should be more detail on the 'otherwise aberrant' part in the data cleaning, and rationale should be given for removing inverted day night cycle patterns (why does this occur?).
Answer: We thank the reviewer for this comment. We agree that the term “otherwise aberrant” was too vague and have clarified the criteria used during data cleaning in the revised manuscript. In our dataset, measurements were considered aberrant when they deviated markedly from the typical day- or night-time distribution, or when abnormal values were accompanied by diagnostic indicators from the PSY1 output, particularly unusual Wet Bulb Depression values or abnormal temperature-gradient signals (dT). These diagnostic variables were therefore used together with the water potential time series to distinguish physiologically plausible variation from likely measurement artefacts. We have also clarified the rationale for removing inverted day/night cycle patterns. Under our experimental conditions, these inverted patterns were associated with the charging/power issue described in the manuscript, which disturbed the normal measurement sequence and produced non-physiological temporal patterns in the PSY1 output. Because these patterns were linked to a technical artefact rather than to expected plant water-potential dynamics, the affected data were excluded from further analysis. This explanation has now been added to the revised manuscript (lines 564-567 and 569-570).

The manuscript text did define the abbreviations for the legend items shown in Figure 7 for SWP but not for Tr or LWP. Figure 7 caption could have more details.
Answer: We have revised the figure 7 legend, please check.
It is important to caution about the use of "Measure" in the caption, because the potentials are not direct measurements, they are calculated from e.g. vapor pressure measurements using the presented theory. All three quantities should have some error bar or uncertainty estimate due to instrument error & theory error propagation.

Answer: We thank the reviewer for this important comment. We have removed “measure” in Figure 7. We agree with the reviewer that all three quantities should have some error bar or uncertainty estimate due to instrument error & theory error propagation. As we explained to reviewer 1 and 2, Figure 7 is intended primarily as an illustrative example of PSY1 and Teros 21 use rather than as a dataset for formal statistical comparison. Because the number of biological replicates in this example was limited, we chose not to display standard deviations in Figure 7, as they would not provide a robust estimate of variability. Although a full error analysis is beyond the scope of this protocol paper, we agree that these aspects should be explicitly discussed so that users can better interpret PSY1 measurements. To clarify the importance of replication, we included the following sentence in the revised manuscript: “Because this protocol paper is intended to demonstrate PSY1 operation and interpretation, the example dataset is illustrative and not designed for rigorous statistical treatment; users should include sufficient biological replication, typically at least 3–5 replicates where possible depending on the experimental objective and biological variability, together with calibration checks and uncertainty analysis.” (line 529-534) In addition, we are currently preparing a research manuscript in which Teros 21 and PSY1 are used extensively for water potential measurements with a replication scheme suitable for formal statistical analysis.
Figure 8 should have a legend for the blue and red line, and the caption could have more information on what issue is presented in the Figure. Answer: We added the legend and detailed the caption in the revised Figure 8, please check!

Competing Interests: No competing interests were disclosed.

Reviewer Report 22 June 2026

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Piyush Jain
Cornell University, Ithaca, New York, USA

I appreciate that the authors have given due consideration to the comments and have revised the manuscript.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant Physiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 17 June 2026

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yijian zeng
University of Twente, Enschede, The Netherlands

I thank the authors' efforts in revising this protocol.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: soil-plant hydraulics; and its coordination with photosynthesis; soil-plant digital twin;

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 16 March 2026

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yijian zeng
University of Twente, Enschede, The Netherlands

The manuscript presents a useful and timely protocol for continuous in situ monitoring of plant and soil water status using PSY1 leaf psychrometers and TEROS 21 sensors, together with a data-processing workflow and open materials on Zenodo. The practical value is clear, especially for groups that are new to continuous water-potential measurements. The step-by-step instructions, photographs, and linked code/video resources are strengths. The paper is also commendable for making the workflow accessible beyond a narrow instrumentation community.

My main concern is that the manuscript needs a stronger treatment of measurement meaning, assumptions, and uncertainty. At present, the paper gives readers a workable installation protocol, but not yet enough guidance to ensure that the resulting measurements are interpreted correctly and reproducibly across systems.

1. the experimental section states that TEROS 21 measures soil matric potential, while PSY1 is presented as measuring leaf water potential, including pressure and osmotic components, and the pressure chamber is discussed separately as potentially measuring either pressure potential alone or total potential.
For physical consistency, the manuscript should explicitly state:

- what the TEROS 21 reports;
- what the PSY1 reports under equilibrium assumptions;
- under what assumptions these quantities can be compared directly;
- when osmotic effects, temperature gradients, or non-equilibrium invalidate a simple interpretation of soil-to-leaf gradients.

As written, the paper risks encouraging readers to combine Ψsoil and Ψleaf as if they were directly interchangeable without stating the required assumptions.

2. The treatment of calibration, uncertainty, and replicate requirements is also too limited at present; directing readers to the instrument manual is not sufficient for a protocol paper.

3. I agree with the previous reviewer that the manuscript should add a short concluding section linking the measurements back to hydraulic conductivity/conductance theory

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Yes

Are sufficient details of the methods provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: soil-plant hydraulics; and its coordination with photosynthesis; soil-plant digital twin;

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 20 May 2026

Lei Ding

Reviewer 2 The manuscript presents a useful and timely protocol for continuous in situ monitoring of plant and soil water status using PSY1 leaf psychrometers and TEROS 21

sensors, together with a data-processing workflow and open materials on Zenodo. The practical value is clear, especially for groups that are new to continuous water-potential measurements. The step-by-step instructions, photographs, and linked code/video resources are strengths. The paper is also commendable for making the workflow accessible beyond a narrow instrumentation community.
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- when osmotic effects, temperature gradients, or non-equilibrium invalidate a simple interpretation of soil-to-leaf gradients.

As written, the paper risks encouraging readers to combine Ψsoil and Ψleaf as if they were directly interchangeable without stating the required assumptions.
Answer: We thank the reviewer for this valuable suggestion. To compare TEROS 21 and PSY1 measurements, we included comparison of both in the revised manuscript. Please check line 94-100 and Table 1 in the revised manuscript. 2. The treatment of calibration, uncertainty, and replicate requirements is also too limited at present; directing readers to the instrument manual is not sufficient for a protocol paper. Answer: We thank the reviewer for this important comment. We agree that, for a protocol paper, calibration procedures, measurement uncertainty, and replication strategy should be described explicitly rather than referring readers only to the instrument manual. As noted in our responses to Reviewer 1, we have revised the manuscript accordingly by adding a paragraph on calibration (Teros 21: line 216-222; PSY1: line 303-318) and by expanding the discussion of uncertainty associated with PSY1 measurements. To clarify the role of replication, we also added the following sentence to the revised manuscript: “Because this protocol paper is intended to demonstrate PSY1 operation and interpretation, the example dataset is illustrative and not designed for rigorous statistical treatment; users should include sufficient biological replication, typically at least 3–5 replicates where possible depending on the experimental objective and biological variability, together with calibration checks and uncertainty analysis.” (line 507-512) In addition, we are currently preparing a separate research manuscript in which Teros 21 and PSY1 is used extensively for water potential measurements with a replication scheme suitable for formal statistical analysis. We hope these revisions make the protocol more practically useful for readers seeking guidance on the proper implementation and interpretation of Teros 21 and PSY1 measurements. We have updated our code to address these errors and uncertainties to analyze the PSY1 datasets.

3. I agree with the previous reviewer that the manuscript should add a short concluding section linking the measurements back to hydraulic conductivity/conductance theory
Answer: A concluding section was included in the revised manuscript (line 550-569).

Competing Interests: No competing interests were disclosed.

Reviewer Report 17 February 2026

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?

Piyush Jain
Cornell University, Ithaca, New York, USA

The authors present a protocol for in situ continuous monitoring of leaf and soil water potential. This contribution will help reduce the time needed to prepare and standardize water potential measurements. It is helpful for those unfamiliar with these measurements. I suggest the following additions to help readers apply this protocol more broadly:

1. Add a concluding paragraph that connects the data presented here with hydraulic conductivity measurements. The paper currently reads as two separate sections. The final measurements are not connected back to theory presented in the introduction to support the calculations.
2. State assumptions explicitly around errors inherent to psychrometry. These include temperature gradients, non-equilibrium measurements, condensation, and other issues typically encountered during these measurements.
3. Include more direction on calibrations and error analysis. Reporting standard deviations in water potential, calculating uncertainty with current methods, and determining the number of replicates needed are all critical to mitigating experimental and measurement uncertainties.

Overall, such protocols are helpful, but, they should always be accompanied with thorough presentation of pros and cons of the approach along with the uncertainties and errors associated with the measurements.

Is the rationale for, and objectives of, the study clearly described?
Partly

Is the study design appropriate for the research question?
Yes

Are sufficient details of the methods provided to allow replication by others?
Partly

Are the datasets clearly presented in a useable and accessible format?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant Physiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 20 May 2026

Lei Ding

Reviewer 1 The authors present a protocol for in situ continuous monitoring of leaf and soil water potential. This contribution will help reduce the time needed to prepare and standardize water potential measurements. It is helpful for those unfamiliar with these measurements. Answer: We thank the reviewer for this positive feedback. Please find our detailed point-by-point responses below. I suggest the following additions to help readers apply this protocol more broadly: 1. Add a concluding paragraph that connects the data presented here with hydraulic conductivity measurements. The paper currently reads as two separate sections. The final measurements are not connected back to theory presented in the introduction to support the calculations. Answer: Thanks for this nice suggestion. We agree that the final measurements should be more clearly connected to the theoretical framework introduced earlier in the manuscript. Accordingly, we have added a concluding paragraph in the revised manuscript to explicitly link the presented data with hydraulic conductivity measurements and to clarify how these observations support the calculations outlined in the Introduction. Please check line 550-569 in the revised manuscript. In addition, we are currently preparing a separate manuscript that will present a full dataset generated using this protocol together with inverse modeling, allowing a more detailed quantitative interpretation of hydraulic properties. 2. State assumptions explicitly around errors inherent to psychrometry. These include temperature gradients, non-equilibrium measurements, condensation, and other issues typically encountered during these measurements. Answer: We thank the reviewer for this important suggestion. We have revised the manuscript accordingly to make the assumptions and potential sources of error inherent to psychrometric measurements more explicit. First, we now explicitly acknowledge the limitation of the PSY1 near the wet end of the water potential range (around 0 MPa), where measurement sensitivity decreases due to the underlying psychrometric principle (line 417-435). We also clarified the importance of allowing sufficient equilibration time for the leaf within the chamber, noting that this duration may vary depending on leaf conditions (line 384-397). In addition, we expanded the discussion on condensation, a common source of measurement error. We now explain how condensation can be identified in the dataset (e.g., through abnormal temperature gradients, dT), and outline practical solutions such as cleaning and reinstalling the system, as well as adjusting parameters like cooling time and chamber heating when necessary (line 417-435). Finally, we emphasized the importance of minimizing thermal gradients during measurements and included a brief discussion on proper insulation to ensure stable and

reliable data acquisition (line 357-364). We have updated our code to address these errors and uncertainties to analyze the PSY1 datasets. Please check the updated code, https://doi.org/10.5281/zenodo.20080750 and revised Figures 7 and 9. 3. Include more direction on calibrations and error analysis. Reporting standard deviations in water potential, calculating uncertainty with current methods, and determining the number of replicates needed are all critical to mitigating experimental and measurement uncertainties. Answer: We thank the reviewer for this important suggestion. We agree that calibration procedures, error analysis, and replication strategy are critical for mitigating experimental and measurement uncertainties when using Teros 21 and PSY1. In the revised manuscript, we have included information about calibrations (Teros 21: line 216-222; PSY1: line 303-318), uncertainty and error analysis, especially for PSY1. In this protocol paper, Figure 7 is intended primarily as an illustrative example of PSY1 use rather than as a dataset for formal statistical comparison. Because the number of biological replicates in this example was limited, we chose not to display standard deviations in Figure 7, as they would not provide a robust estimate of variability. Although a full error analysis is beyond the scope of this protocol paper, we agree that these aspects should be explicitly discussed so that users can better interpret PSY1 measurements. To clarify the importance of replication, we included the following sentence in the revised manuscript: “Because this protocol paper is intended to demonstrate PSY1 operation and interpretation, the example dataset is illustrative and not designed for rigorous statistical treatment; users should include sufficient biological replication, typically at least 3–5 replicates where possible depending on the experimental objective and biological variability, together with calibration checks and uncertainty analysis.” (line 507-512) In addition, we are currently preparing a research manuscript in which Teros 21 and PSY1 are used extensively for water potential measurements with a replication scheme suitable for formal statistical analysis.

Competing Interests: No competing interests were disclosed.