Plant Soil (2013) 369:257–268
DOI 10.1007/s11104-012-1568-x

REGULAR ARTICLE

Non-invasive pressure probes magnetically clamped to leaves to monitor the water status of wheat

Helen Bramley · Wilhelm Ehrenberger · Ulrich Zimmermann · Jairo A. Palta · Simon Rüger · Kadambot H. M. Siddique

Received: 26 August 2012 / Accepted: 11 December 2012 / Published online: 29 December 2012

Abstract
Background and aims Being able to monitor the hydration status of a plant would be useful to breeding programs and to providing insight into adaptation to water-limited environments, but most current methods are destructive or laborious. We evaluated novel non-invasive pressure probes (commercial name: ZIM-probe) for their potential in monitoring the water status of wheat (*Triticum aestivum* L.) leaves.
Methods The probes consist of miniature pressure sensors that clamp to the leaves via magnets and detect relative changes in hydration status. Probes were clamped to leaves of six individual plants of the cultivar Wyalkatchem at the stem elongation stage and compared against traditional plant water relations measurements.
Results Output from the probes, called patch-pressure (P_p), correlated well with leaf water potential and transpiration of individual plants. Variation between plants in the original clamp pressure exerted by the magnets and leaf individual properties led to variations in the amplitude of the diurnal P_p profiles, but not in the kinetics of the curves where P_p responded simultaneously in all plants to changes in the ambient environment (light and temperature).
Conclusions Drying and rewatering cycles and analysis of the curve kinetics identified several methods that can be used to test comparisons of water status monitoring of wheat genotypes under water deficit.

Keywords Turgor · Water potential · Temperature · Drought

Responsible Editor: John McPherson Cheeseman.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-012-1568-x) contains supplementary material, which is available to authorized users.

H. Bramley (✉) · K. H. M. Siddique
The UWA Institute of Agriculture, M082,
The University of Western Australia,
35 Stirling Highway, Crawley, WA 6009, Australia
e-mail: helen.bramley@uwa.edu.au

W. Ehrenberger · U. Zimmermann · S. Rüger
ZIM Plant Technology GmbH,
Neuendorfstr. 19, 16761 Hennigsdorf, Germany

W. Ehrenberger
Lehrstuhl für Biotechnologie, Biozentrum,
Universität Würzburg,
Am Hubland, 97074 Würzburg, Germany

J. A. Palta
CSIRO Plant Industry,
Private Bag No. 5, Wembley, WA 6913, Australia

J. A. Palta
School of Plant Biology,
The University of Western Australia,
35 Stirling Highway, Crawley, WA 6009, Australia

Introduction

All land plants must take up water from the soil and transport it to leaves to replace the water lost to the atmosphere through transpiration. Mechanisms that

improve efficiency of water transport and minimise water loss are therefore, advantageous in ephemeral or arid environments where water is the most limiting resource (Smith et al. 1989). Efficiency in water transport and transpiration efficiency (amount of carbon fixed per unit of water transpired) also influence crop productivity and drought resistance, and where crops are irrigated, the amount of water applied to achieve optimal growth (Flexas et al. 2010; Galmés et al. 2010; Morison et al. 2008). With the demand for increased crop production to feed the world's escalating population and the adverse changes in climate predicted for many cropping regions (Easterling et al. 2007), there is a need to identify hydraulic traits that breeders can select to develop cultivars better adapted to their target growing regions. However, plant hydraulic properties form a complex interactive network so that decoupling of a single trait is unlikely to make a dramatic impact on crop productivity or drought resistance (Sinclair 2012).

The hydration status of a plant incorporates all of its hydraulic traits. The hydration status here is defined as how well the plant maintains the water balance of its leaves, whether it is through stomatal regulation, water uptake and transport capacity of roots, desiccation resistance, volumetric elasticity (water holding capacity), osmotic adjustment or any combination of these and other hydraulic traits. Therefore, being able to monitor the hydration status of a plant would be particularly useful to breeding programs and also provide insight into adaptation to particular environments or responses to abiotic stress.

Many methods have been used previously to measure plant water use or water balance. One of the most standard techniques is the determination of leaf water potential using a pressure chamber (Scholander et al. 1965). However, this method is destructive, using detached leaves, and temporal and spatial variations limit sampling (O'Toole et al. 1984). Stomatal conductance and transpiration are commonly measured using porometry and gas exchange equipment and although these measurements can be carried out on intact leaves, they are disruptive and suffer from the same temporal and spatial resolution problems as leaf water potential measurements (O'Toole et al. 1984). Thermal imaging using infra-red technology to measure leaf and canopy temperatures, as a surrogate for stomatal conductance, has been used for several decades (Ehrler et al. 1978), but recent advances in remote sensing and methods to normalise spatial and temporal variation has led to rapid adoption of thermal imaging in modern phenotyping programs (Jones et al. 2009; Munns et al. 2010; Reynolds 2002). While thermal imaging has obvious advantages in scaling from leaves to whole fields, proponents of the technology have suggested that accompanying measurements of leaf water status, especially turgor, would provide the extra information needed to understand the effect of stomatal behaviour on plant adaptation and growth rate (Munns et al. 2010).

New magnetic patch-clamp pressure probes (ZIM-probes) potentially provide an alternative or complementary method of directly monitoring leaf hydration. ZIM-probes are non-invasive and continuously monitor the hydration status of leaves in real-time (Zimmermann et al. 2008, 2010). Because the devices communicate wirelessly to a remote controller connected by GSM to an Internet server, the data can be viewed online over the Internet at any time. The ZIM-probe technology uses miniature pressure sensors that are clamped to leaves via magnets, improving on the spring-loaded connection mechanism in the original prototype (Zimmermann et al. 2008). The magnets apply a constant clamp pressure to the leaf, so that the pressure sensors are able to detect relative changes in leaf turgidity. Turgor is related to the hydration status as cell and bulk leaf turgor pressure decline when leaves dehydrate during transpiration and in response to drought (Kramer and Boyer 1995). The ZIM-probe technology has been used for monitoring water status of trees and irrigation scheduling of horticultural crops (Ehrenberger et al. 2012; Rüger et al. 2010; Zimmermann et al. 2010), but can also potentially be adapted to monitoring the water use of agricultural crops. In this paper we evaluate ZIM-probes for monitoring the hydration status of wheat. Adaptation of the technology for crops such as wheat poses a greater challenge than that of trees because wheat leaves are more delicate and less rigid than tree leaves. Wheat leaves are also narrow; typically 5–20 mm depending on age and genotype, and have prominent mid-ribs that limit the region where the 10 mm diameter ZIM-probes can be clamped. We clamped the probes to mature leaves of a popular Australian wheat (*Triticum aestivum* L.) cultivar when the plants were at the stem elongation stage (Z30 in Zadok's growth stage for cereals) (Zadoks et al. 1974) and compared plant water use/balance measured with

traditional methods against the output from ZIM-probes under controlled environmental conditions.

Materials and methods

Plant material

Seeds of the bread wheat (*Triticum aestivum* L.) cultivar Wyalkatchem were kindly provided by Dr. Ben Biddulph (Department of Agriculture and Food, Western Australia). Seeds were surface sterilised with commercial bleach (active constituent 2.1 % sodium hypochlorite) and then pre-germinated on moist filter paper in Petri-dishes. Seedlings were transferred to pots (75 mm diameter, 350 mm depth) filled with commercial potting mix (Doublegee cocopeat mix with macrominerals and trace elements, Richgro Garden Products, Canningvale, Western Australia) when the roots were 2–3 cm long. Plants were watered every third day with water and once every 2 weeks with water soluble fertiliser (Thrive Soluble All Purpose Plant Food, N:P:K 27:5.5:9). Plants were grown in a growth cabinet with 12 h light/dark and 60–75 % relative humidity. The temperature and light intensity in the cabinet were programmed to gradually increase and decrease during the day to mimic diurnal changes (Supplementary Fig. 1).

Experimental protocol

ZIM-probes (ZIM Plant Technology GmbH, Hennigsdorf, Germany) were clamped to the penultimate leaf of the main stem of six individual plants when the plants were at the stem elongation stage of development. All probes were located 6–7 cm from the leaf tip, where the leaf blade was smooth and flat. ZIM-probes were clamped with the sensor facing the abaxial leaf surface. Although the probes only weigh 5.5 g, wheat leaves could not support a probe by themselves unlike previous studies on trees (Ehrenberger et al. 2012; Rüger et al. 2010) or vines (Westhoff et al. 2009; Zimmermann et al. 2008). Therefore, each probe was supported by an iron rod impaled in the soil, which allowed the clamped leaf to maintain its original orientation (Fig. 1a).

The output from the probes was monitored for 2 days to ensure that there was no damage to the leaves during clamping. All plants were then watered thoroughly and the traditional plant water relations measurements conducted over a diurnal cycle the following day (day 5).

After the diurnal cycle measurements, the ZIM-probes continued monitoring for a further 9 days. Three plants were watered after 3 days (day 8), whereas water was withheld from the other three plants until 6 days (day 11), when all plants were rewatered. The probes were removed from the plants after 14 days monitoring and the leaves inspected for damage.

Plant water relations

Because of the destructive nature of some of the plant water relations measurements, these measurements were conducted on plants grown and treated identically as those clamped with the ZIM-probes.

Whole plant transpiration (E_{plant}) was measured gravimetrically. The evening before measurements and after all plants had been thoroughly watered and allowed to drain, the plant pots were covered with plastic bags and sealed to the base of the plant. Four individual plants were weighed at each time point and then again 1 h later. E_{plant} was standardised by total leaf area, which was measured with a LI-COR LI-3000A Portable Leaf Area Meter (LI-COR Environmental, Nebraska, USA). E_{plant} was also measured on plants clamped with ZIM-probes, to ensure that there was no difference between clamped and unclamped plants.

After measuring E_{plant}, gas exchange (E_{leaf}, CO_2 assimilation and stomatal conductance) was measured on the same plant with a portable differential gas analyser (CIRAS-2 Portable Photosynthesis System, PP Systems, Amesbury, US) on the penultimate leaves of the main stem. Measurements were only conducted when the lights were on in the growth cabinet.

The water potential (Ψ_{leaf}) of the same leaf used for gas exchange was measured using a Scholander-type pressure chamber fitted with a specimen holder for grass blades (Soil Moisture Equipment Corp., Santa Barbara, USA). The leaf was enclosed in a plastic bag before excision from the plant and during the measurement.

Leaf patch clamp pressure probes (ZIM-probes)

The measuring principle of the magnetic leaf patch clamp pressure probe (commercial name: ZIM-probe)

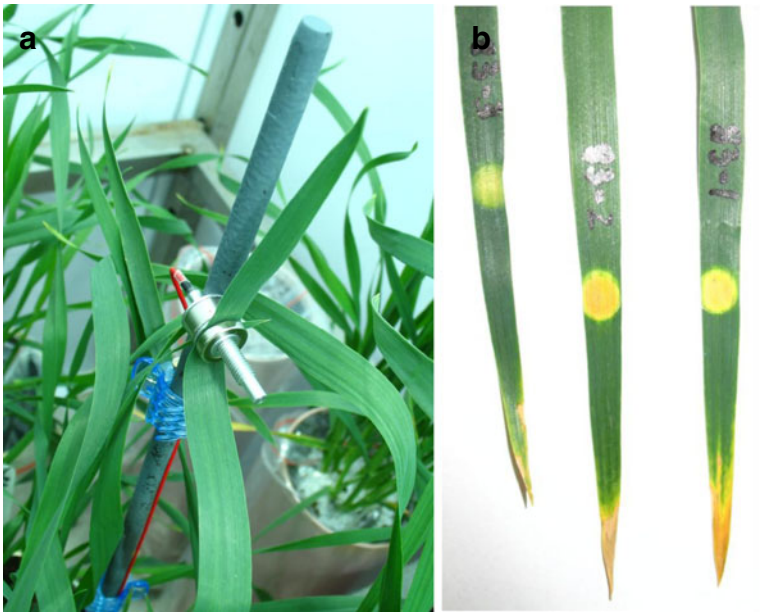

Fig. 1 A ZIM-probe clamped to a wheat leaf (**a**) and examples of leaf patches beneath the probes at the end of the experiment (2 weeks) (**b**)

is described in detail elsewhere (Zimmermann et al. 2008, 2010). Briefly, an intact leaf is positioned between the two pads of the probe (diameter 10 mm), each of which is connected with magnets. The probe measures the pressure transfer exerted by the two magnets through the leaf patch. The leaf patch is assumed to be in hydraulic contact with the surrounding unclamped leaf tissue. The output pressure signal (i.e. the so-called patch pressure P_p) is sensed by a pressure sensor that is integrated in one of the pads. The clamp pressure (P_{clamp}) that is exerted by the two magnets onto the leaf patch can be adjusted to the rigidity of the leaf by varying the distance between the two magnets and is constant during the measurements. Essentially, leaf turgor opposes the clamp pressure and the pressure sensor detects changes in turgor by monitoring the change in pressure opposing the magnetic force (i.e. turgor). Therefore, P_p is inversely correlated with leaf turgor pressure (Zimmermann et al. 2008, 2010), such that when the leaf dehydrates during stomatal opening and in response to water deficit P_p increases and conversely, decreases again when the leaf rehydrates.

The P_p signals were transmitted via wireless transmitters to a controller (ZIM-transmitter and ZIM radio-controller, ZIM Plant Technology GmbH, Hennigsdorf, Germany), which sent the data by a GPRS modem (fitted with a commercial prepaid SIM card) to an Internet server, from which the data were downloaded. Ambient temperature and relative humidity of the growth cabinet were simultaneously recorded by microclimate probes (ZIM Plant Technology GmbH, Hennigsdorf, Germany) that were sent to the controller via the same wireless transmitters as the P_p signals.

Tests under the same conditions as the experiments, but without leaves connected between the magnets showed that ZIM-probes were relatively independent of temperature (Supplementary Fig. 2).

Statistical analyses

Statistical analyses were performed using GraphPad Prism version 5.02. Diurnal values of Ψ_{leaf}, E_{plant} and gas exchange were compared with One-Way ANOVA and Tukey multiple comparison post-test. The relationship between P_p and traditional plant water relations measurements were compared with correlation analyses.

Results

ZIM-probes were clamped to wheat leaves where the blade was smooth and flat, 6–7 cm from the tip (Fig. 1a). The prominent mid-rib prevented clamping further from the tip.

Typical diurnal P_p recordings on well-watered wheat plants are shown in Fig. 2, together with the corresponding changes in ambient temperature (T) and relative humidity (RH) of the growth cabinet. P_p

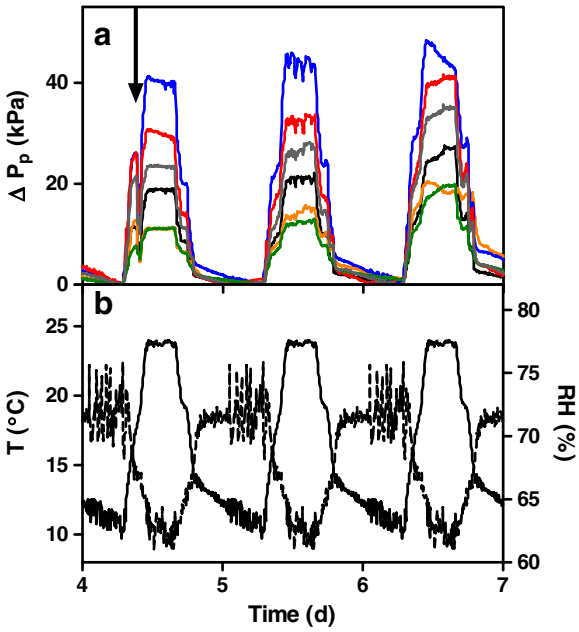

Fig. 2 Typical patch pressure (P_p) recordings from penultimate leaves of the main stems of well-watered wheat plants (**a**) and the corresponding ambient temperature (T; *solid line*) and relative humidity (RH; *dashed line*) of the growth cabinet (**b**). Each line in (**a**) represents the recording from a different plant/probe. The *arrow* indicates when the plants were watered. P_p was normalised to the value at 07:00 h on day 4 to give ΔP_p

increased when the plants were illuminated during the day and decreased again during the afternoon. Apart from variation in amplitude, the kinetics of the P_p curves from different plants were the same; the rise and fall in P_p occurred at the same time, and reached the plateau at the same time (Fig. 2). Fluctuations in P_p followed similar patterns to changes in T, but did not coincide exactly. For example, the midday plateau in P_p was reached 10 min earlier than the corresponding T plateau value. In addition, small fluctuations in P_p occurred during the midday plateau, when T was constant (24 °C) and during the afternoon hours, the continuous drop of P_p was interrupted twice by a small increase (at 17:00 and at 18:30) whereas the drop of T was only interrupted once for 55 min at 17:00 when T was constant at 15 °C. Sudden decrease in P_p when plants were watered was not seen in T recordings and noisy fluctuations in T recorded during the night were also not reflected by the P_p recordings.

Leaf water potential (Ψ_{leaf}) decreased during the day, with the minimum Ψ_{leaf} values occurring over the same time period as maximum P_p (Fig. 3a). Correlation between Ψ_{leaf} and P_p was highly significant ($P<0.0001$, $r=-0.973$) (Fig. 3b), although slopes and intercepts for individual leaves varied ($P<0.0001$). Ψ_{leaf} was also highly correlated with ambient temperature ($P<0.0001$, $r=-0.87$) (Fig. 4). E_{plant} and E_{leaf} (data not shown) and P_p also correlated well ($P=0.0002$, $r=0.9591$) (Fig. 3c and d), whereas G_s and P_p were less related ($P=0.0115$, $r=0.9113$) (Fig. 3f). Although G_s appeared to show a diurnal trend, values during illumination hours were not significantly different ($P=0.1596$) (Fig. 3e). G_s in the dark was too low for the CIRAS machine to detect.

Half of the plants connected with ZIM-probes were watered on days 4, 8 and 11, whereas the other half were only watered on days 4 and 11. Typical P_p recordings of these drying and re-watering cycles are shown in Fig. 5a and b, respectively. P_p peak values at noon and, to a lesser extent, minimum P_p values at night continuously increased when plants were not watered (Fig. 5a). Re-watering caused an instantaneous fluctuation in P_p (Fig. 5a inset) and lower P_p peak values on subsequent days. P_p peak and night values were constant with only 2 days between watering events (Fig. 5a). Longer periods without water (7 days) caused continuous increase of P_p peak and night values until the sixth day (Fig. 5b). The drop in P_p during the afternoon also continuously occurred later until on the sixth day without water, half-inversed curves of P_p occurred during the end of the light where P_p increased again instead of declining to the night value (Fig. 5b, day 10). P_p dropped again during the night but to higher values than on preceding days. P_p curves returned to their original shape with watering the next morning (day 11), i.e. peaking at noon and continuous increase in peak P_p on the following days when the plants were not watered (Fig. 5b).

The rate of leaf rehydration may take longer when the soil is dry. To analyse whether this could be detected by the probes, single exponential decay curves were fit to each afternoon's data between peak P_p and minimum P_p night values. Best fits for comparison of the curves parameters incorporated 67 % of the data points (between 16:00 and 02:00 h). The rate constant (K) of P_p relaxations during afternoon hours decreased with ongoing water deficit between watering events, while the half-time ($T_{1/2}$) of P_p relaxations increased (Fig. 6). Watering three plants on day 11 reduced $T_{1/2}$ from 188 to 90 min and increased K from 4 to 8 min^{-1}. $T_{1/2}$ of the remaining unwatered plants could not be determined

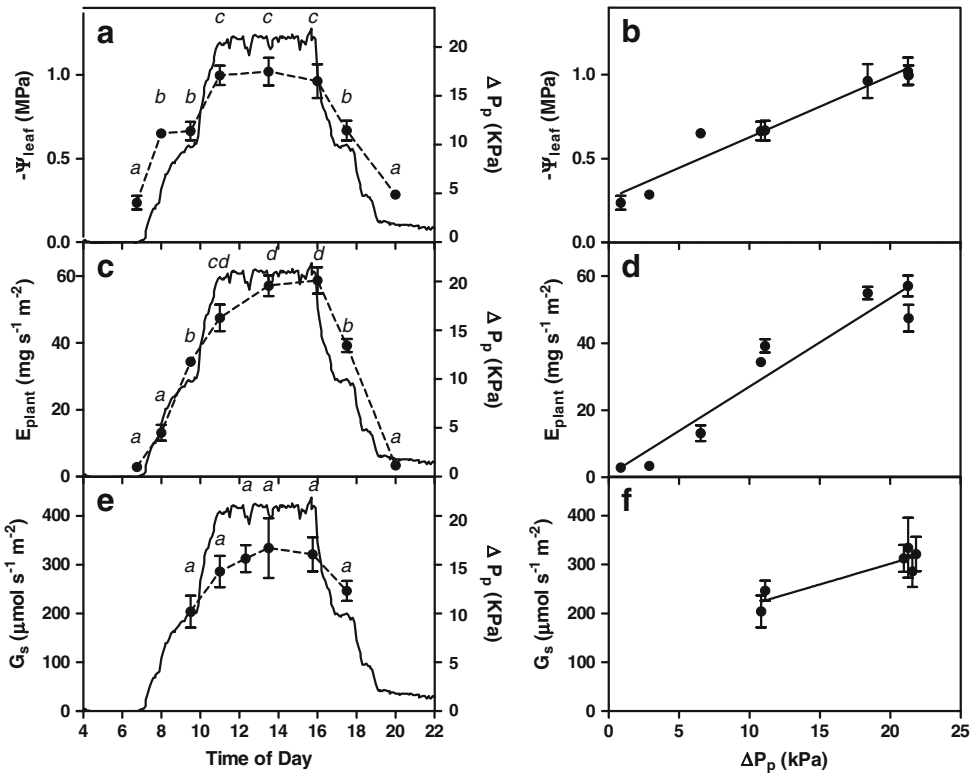

Fig. 3 Concomitant diurnal changes in patch pressure (P_p; *solid line*) (**a**, **c** and **e**) and correlation with traditional leaf water relations measurements (**b**, **d** and **f**; *filled circles*, *dashed line*). ΔP_p shown is for a single ZIM-probe normalised to the value at 07:00 h. Leaf water potential (Ψ_{leaf}) was measured with a pressure chamber (**a** and **b**), transpiration was measured gravimetrically and normalised by leaf area (E_{plant}) (**c** and **d**) and stomatal conductance (G_s) was measured with a portable differential gas analyser (**e** and **f**). Values for Ψ_{leaf}, E_{plant} and G_s are means ± SEM, n=4

due to the development of the half-inversed curves during the end of the light period (Fig. 5).

Similar to the afternoon recovery phase analysis, comparison of plots of the P_p values against T for morning and afternoon hours also relates to the water status of the wheat plants (Fig. 7). When plants were well-watered, P_p and T exhibited an inverse hysteresis (Fig. 7b). P_p and T were linearly related in the morning but the afternoon data was curvilinear as the decline in P_p occurred much faster than the decline in T. Conversely, when water uptake was limited, P_p and T were linearly related and exhibited no hysteresis between morning and afternoon (Fig. 7a). The intensity of the inverse hysteresis loops was related to the plant water supply, decreasing as the area between the morning and afternoon P_p = f (T) curves decreased. In order to quantify the level of hysteresis, the area between the two P_p = f (T) curves was calculated and normalised to the rectangular area given by the co-ordinates of the lowest and highest P_p values that encompass the hysteresis loop (inset of Fig. 7b). In between watering events the area of the inverse hysteresis loop decreased and increased again upon watering (Table 1).

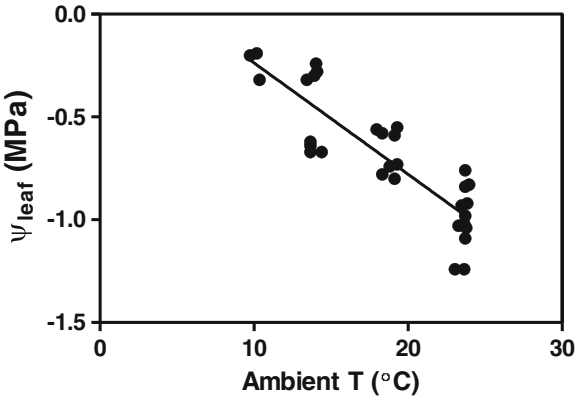

Fig. 4 Relationship between leaf water potential (Ψ_{leaf}) and ambient temperature (T) of the growth cabinet for the diurnal experiment shown in Fig. 3a. Each point represents an individual leaf

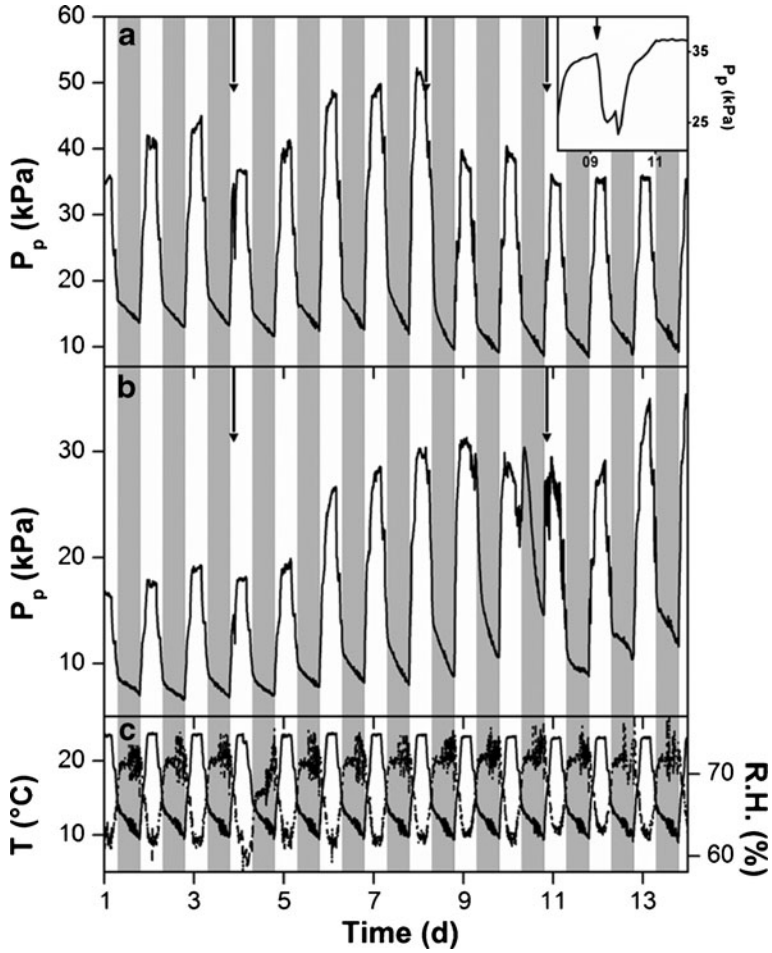

Fig. 5 Diurnal changes in patch pressure (P_p) of plants subjected to different watering regimes (**a** and **b**) and concomitant changes in ambient temperature (T; *solid line*) and relative humidity (RH; *dotted line*) (**c**). Plants were watered two (**b**) or three (**a**) times (indicated by *arrows*). Noon and night P_p values gradually increased between watering events. Inset in (**a**) is an enlargement of the immediate fluctuation in P_p due to watering on day 4

At the end of the experiment (2 weeks), the probes were removed from the leaves. The leaf patches beneath the probes were yellow and only slight impressions from the probe pads could be seen on the leaf surface with closer inspection under the microscope (Fig. 1b).

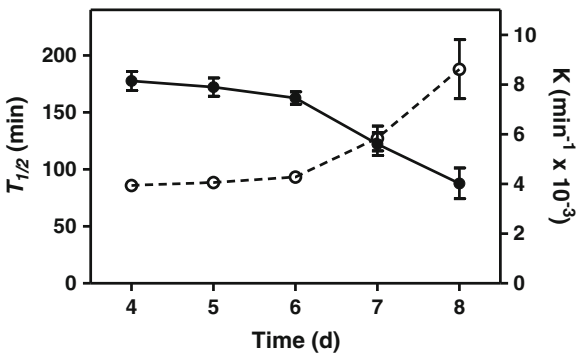

Fig. 6 Half-times ($T_{1/2}$; *open circles*, *dashed line*) and rate constants (K; *filled circles*, *solid line*) of the patch clamp recovery phases during the afternoons after watering on day 4. $T_{1/2}$ and K were determined by fitting exponential decay curves to the afternoon/night data between 16:00 and 02:00. Values are means ± SEM, n=6

To examine the variability in P_p between individual probes and the dependence of temperature in more detail a separate experiment was conducted by clamping probes to three individual plants within the same 7.5 L pots. Each pot represented a different clamping pressure by accurately adjusting the distance between the magnets (Fig. 8). Diurnal fluctuations in P_p were much larger when the magnets were wound fully to the base of the probe pad (i.e. distance between magnets=0 mm) than when the magnets were wound out from the probe pad 1.6 or 2.6 mm (Fig. 8a–c). However, the variability between the individual probes was also largest when P_{clamp} was maximum (mean P_p SEM in 8a 5.02, 1.41 in 8b and 0.88 in 8c).

Figures 8 and 9 also show the response of P_p when plants were placed in the dark (covered with

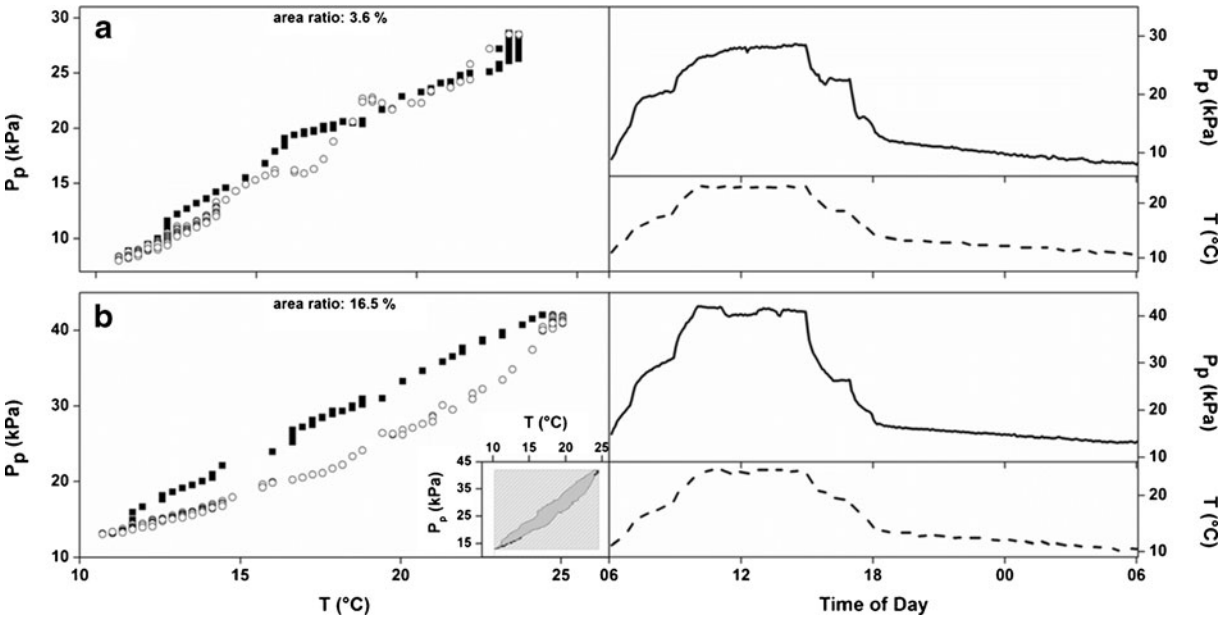

Fig. 7 Typical examples of the dependency of the leaf patch pressure (P_p) values against T measured for the morning (*filled squares*) and afternoon hours (*open circles*). **a** P_p = f (T) curve together with the diurnal changes of P_p and T measured on a water-limited wheat plant (no inverse hysteresis). **b** P_p = f (T) curve together with the diurnal changes of P_p and T measured on a well-watered wheat (occurrence of inverse hysteresis). The area of the inverse hysteresis was normalised by referring to the co-ordinates of the lowest and highest P_p readings of the probes and determining the rectangular area which encompassed the elliptical inverse hysteresis (see inset in **b**)

aluminium foil) and subsequently re-illuminated while ambient temperature and relative humidity varied diurnally. P_p rapidly decreased when the plants were placed in the dark while temperature was constant and conversely, rapidly increased when the plants were reilluminated (Fig. 9). The maximum diurnal amplitude of ΔP_p was smaller in the dark than when the plants were illuminated (Fig. 9). In addition, P_p increased more rapidly than ambient temperature at the start of the day when the first lights came on in the growth cabinet (PAR 155 $\mu mol s^{-1} m^{-2}$), but when the plants were covered the rate of change in P_p at the start of the day was slower than ambient temperature (Fig. 9).

Discussion

This is the first report of the application of the leaf patch-clamp pressure probes (ZIM-probes) to agricultural crop plants. We chose to evaluate the application on wheat plants because wheat is the most important agricultural crop in Australia (Australian Bureau of Statistics 2011). Cereal crops also dominate the world's agricultural production (FAOSTAT, http://faostat.fao.org/site/339/default.aspx, accessed 13/6/12). Water stress is often the most limiting factor for cereal yield production, particularly in southern Australia (Loss and Siddique 1994). On-line monitoring of plant water status will not only aid farmers of horticultural crops in determining when to irrigate, but could also potentially

Table 1 Area ratios of the hysteresis between patch pressure (P_p) vs. ambient temperature (T), corresponding to the recordings shown in Fig. 5. The plant in Fig. 5a was watered on days 4, 8 and 11, whereas the plant in Fig. 5b was only watered on days 4 and 11

Day	2	3	4	5	6	7	8	9	10	11	12	13
Fig. 5a: area ratio (%)	24	15	34	16	17	8	13	23	20	21	16	18
Fig. 5b: area ratio (%)	20	14	24	11	14	9	1	1	12	46	10	5

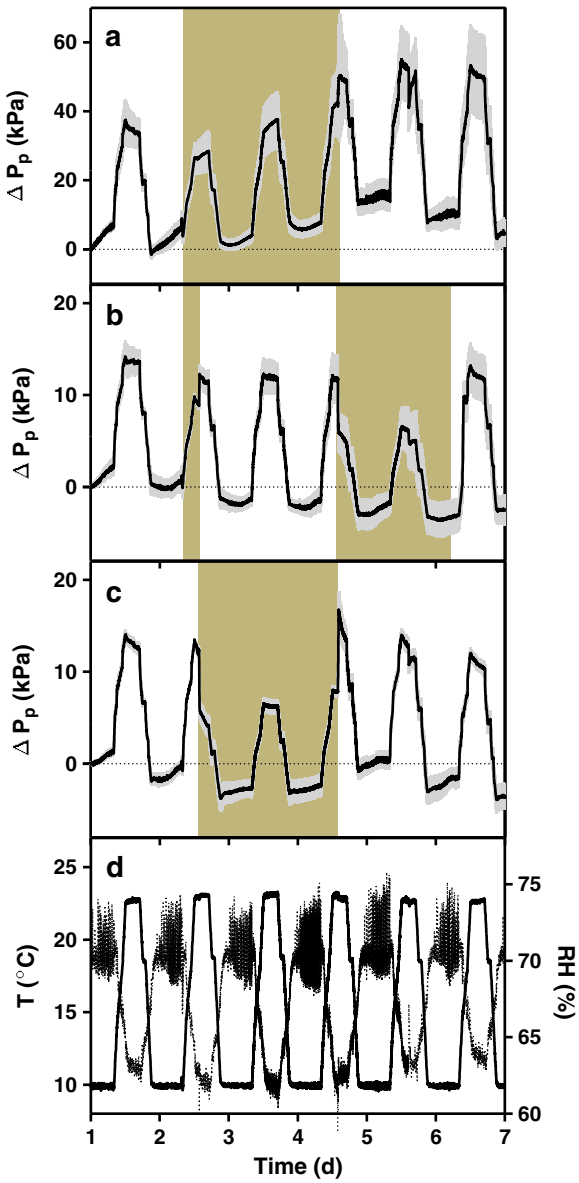

Fig. 8 Relative change in leaf patch pressure (ΔP_p) for different clamp pressures. Different clamp pressures were applied by winding the opposing magnets fully to the stamp base (**a**), or winding out the magnets 1.6 mm (**b**) and 2.6 mm from the stamp base (**c**). Each figure shows the mean ± SEM for three plants. Note the different ΔP_p-axes. ΔP_p was calculated by normalising to the first value at time=1.00. The *shaded blocks* indicate when the plants were in the dark (covered with aluminium foil). Corresponding ambient temperature (T) and relative humidity (RH) are shown in D

complement existing technologies in cereal phenotyping programs for drought resistance. We tested application of the technology on wheat to determine first, whether it is possible to clamp the devices on the small leaves of wheat and second, how well the output correlates with plant water relations using traditional methods. Our study demonstrates that ZIM-probes can be adapted to wheat and that the probes monitor leaf hydration status non-destructively.

ZIM-probes were relatively easy to clamp to wheat leaves despite their narrow shape and more delicate structure compared with tree leaves, as long as the probes were supported. The loss of chlorophyll beneath the ZIM-probes patches after 2 weeks of clamping is to be expected, but the hydraulic connection with the rest of the leaf was maintained. Longer-term monitoring is potentially possible, but reclamping may be needed under severe stress and as leaves age and senesce.

The output from the probes correlated well with traditional measurements of leaf water relations on individual plants, particularly leaf water potential and transpiration. P_p did not correlate so well with stomatal conductance, but stomatal conductance did not vary much during daylight hours. The leaf water potential threshold for stomatal closure in wheat is low and even under water deficit wheat plants tend to keep their stomata open (Henson et al. 1989). Consequently, increasing transpiration with increasing ambient temperature led to a decrease in leaf water potential and concomitant increase in P_p. ZIM-probes also detected differences between watered and unwatered plants and P_p profiles during drying and rewatering cycles demonstrated dehydration and rehydration kinetics, respectively. These combined elements indicate that the probes monitor the relative hydration status of wheat leaves.

Variation in the diurnal amplitude of P_p on different plants was due to differences in initial clamp pressure and variation in the compressibility and thickness of individual leaves, although all clamped leaves were of the same age and the probes were clamped a similar distance from each leaf tip. Variation in the diurnal P_p amplitude consequently led to variation from leaf to leaf in the slopes and intercepts of the P_p and Ψ_{leaf} correlations. P_p can obviously be normalised so that all values fall within the same max-min range. Initial clamp pressure can also be coarsely adjusted by increasing the distance between the magnets. A screw thread has been incorporated onto the counter-pad, so that the magnet can be wound closer to or further away from the sensor magnet. Variability in P_p amplitudes between individual probes on wheat leaves was reduced when the magnets were 1.6 to 2.6 mm apart. This optimum distance is likely to vary between leaf types. Nevertheless, clamp pressure will not be identical without prior knowledge

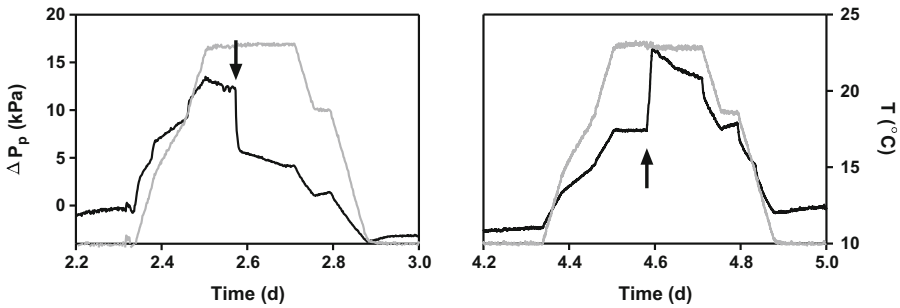

Fig. 9 Typical example of the effects of illumination and ambient temperature (T; *grey line*) on mean leaf patch pressure (ΔP_p; *black line*). *Arrows* indicate when the plants were covered with aluminium foil and then uncovered. The example shown is a zoomed in view of Fig. 8c

of the leaf properties and a measurement of the force applied by the magnets. The probes therefore, show the relative changes in leaf hydration. If more detailed information is needed, such as predicting absolute turgor pressure values, calibration would be needed. Several reports on tree species showed a correlation between P_c measured with a cell pressure probe and P_p that could be predicted by the original theoretical basis (Ehrenberger et al. 2012; Rüger et al. 2010). The usefulness of absolute values will depend on the application. For genotypic comparisons, relative changes in leaf hydration will mostly be sufficient.

Despite variation in P_p minimum and maximum amplitudes, the P_p profiles reflected changes in the environment. All leaves responded at the same time to changes in ambient temperature and light intensity. P_p of all plants started to increase at the same time each morning when the lights came on and temperature began to increase, with illumination causing more rapid changes. All P_p profiles reached the peak at the same time. Any temporal differences that were observed in P_p were due to the level of the water stress. The P_p profiles were similar to the changes in ambient temperature, particularly under well-watered conditions, but there were disparities between P_p and T throughout the experiment. Moreover, illumination caused dramatic changes in the P_p profiles that is to be expected with irradiance-induced changes in stomatal opening and hence, leaf hydration. The underlying diurnal fluctuations of P_p are not an artefact of the probe as demonstrated when there are no leaves clamped between the magnets, but are due to changes in leaf properties in response to ambient conditions. The volumetric elasticity of leaves is temperature dependent (Woodward and Friend 1988), but it is also dependent on the hydration of cell walls and cell turgor pressure (Murphy and Ortega 1996; Rascio et al. 1988; Steudle and Zimmermann 1977). It is impossible to separate these interacting components, but knowledge of the absolute contribution of each component is not needed for comparing the overall relative hydration status of different plants and temporal responses to environmental conditions.

The drying and rewatering cycles identified similar P_p responses in wheat that has been seen for other plant species (Ehrenberger et al. 2012; Zimmermann et al. 2008). Peak and minimum P_p gradually increased between watering events as it is more difficult for the plant to fully rehydrate with increasing soil water deficit. Osmotic adjustment in the leaves of some wheat genotypes can occur in response to water deficit (Johnson et al. 1984), which will aid in maintaining turgor and hence, leaf hydration. However, with increasing time between watering events rehydration was slower as indicated by the slower recovery rates in the afternoon (increasing half-times). With-holding water for 6 days inhibited the afternoon recovery in P_p, but generated an inverse curve during part of the night. Inverse curves during the night have been attributed to complete turgor loss (Ehrenberger et al. 2012). Assuming that this was the case for wheat, the process was reversible upon rewatering. In addition, the afternoon recovery half-times for P_p also decreased again upon rewatering. Another method of analysing the P_p profiles that demonstrated dehydration/rehydration kinetics was the relationship between P_p and T during the drying and rewatering cycles. When plants were well hydrated, P_p = f(T) exhibited hysteresis as P_p recovered quicker than T during the afternoon. The hysteresis declined between watering events and increased again upon watering, i.e. the area between the morning and afternoon plots decreased and then increased again.

In conclusion, the ZIM-probe technology is a potentially useful tool for assessing relative temporal variations in hydration status of wheat. The methods of analysing the P_p profiles in this study will be useful for genotypic comparisons of hydration status under

water deficit. Afternoon recovery times may differ between genotypes, as well as the hysteresis relationship between P_p and T. The timing of the onset of inverse curves due to turgor loss may differ with drought resistance and speed of the decline in soil water content.

Acknowledgments Funding for HB and research costs were provided by The UWA Institute of Agriculture and research facilities by CSIRO Plant Industry as part of the UWA/CSIRO PI partnership. This project is supported by the Group of Eight Australia - DAAD German Research Cooperation Scheme to KS and ZIM Plant Technology (Project-ID: 54387088) and a grant of the Europäischer Fonds für regionale Entwicklung (EFRE 80145650; State Brandenburg) to ZIM Plant Technology GmbH. Sam Henty provided technical assistance. We are grateful to Professor Steve Tyerman for comments on the manuscript.

References

Australian Bureau of Statistics (2011) Value of principal agricultural commodities produced, Australia, 2010–11. Australian Government, Canberra

Easterling WE, Aggarwal PK, Batima P, Brander KM, Erda L, Howden SM, Kirilenko A, Morton J, Soussana J-F, Schmidhuber J and Tubiello FN (2007) Food, fibre and forest products. In: Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE (Eds) Climate change 2007: Impacts, adaptation and vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge, UK, pp 273–313

Ehrenberger W, Rüger S, Rodríguez-Domínguez CM, Díaz-Espejo A, Fernández JE, Moreno J, Zimmermann D, Sukhorukov VL, Zimmermann U (2012) Leaf patch clamp pressure probe measurements on olive leaves in a nearly turgorless state. Plant Biol 14:666–674

Ehrler WL, Idso SB, Jackson RD, Reginato RJ (1978) Wheat canopy temperature: relation to plant water potential. Agron J 70:251–256

Flexas J, Galmés J, Gallé A, Gulías J, Pou A, Ribas-Carbo M, Tomàs M, Medrano H (2010) Improving water use efficiency in grapevines: potential physiological targets for biotechnological improvement. Aust J Grape Wine Res 16:106–121

Galmés J, Conesa MA, Ochogavía JM, Perdomo JA, Francis DM, Ribas-Carbó M, Savé R, Flexas J, Medrano H, Cifre J (2010) Physiological and morphological adaptations in relation to water use efficiency in Mediterranean accessions of *Solanum lycopersicum*. Plant Cell Environ 34:245–260

Henson IE, Jensen CR, Turner NC (1989) Leaf gas exchange and water relations of lupins and wheat. I. Shoot responses to soil water deficits. Aust J Plant Physiol 16:401–413

Johnson RC, Nguyen HT, Croy LI (1984) Osmotic adjustment and solute accumulation in two wheat genotypes differing in drought resistance. Crop Sci 24:957–962

Jones HG, Serraj R, Loveys BR, Xiong L, Wheaton A, Price AH (2009) Thermal infrared imaging of crop canopies for the remote diagnosis and quantification of plant responses to water stress in the field. Funct Plant Biol 36:978–989

Kramer PJ, Boyer JS (1995) Water relations of plants and soils. Academic, San Diego

Loss SP, Siddique KHM (1994) Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. Adv Agron 52:229–276

Morison JIL, Baker NR, Mullineaux PM, Davies WJ (2008) Improving water use in crop production. Philos Trans R Soc B Biol Sci 363:639–658

Munns R, James RA, Sirault XRR, Furbank RT, Jones HG (2010) New phenotyping methods for screening wheat and barley for beneficial responses to water deficit. J Exp Bot 61:3499–3507

Murphy R, Ortega J (1996) A study of the stationary volumetric elastic modulus during dehydration and rehydration of stems of pea seedlings. Plant Physiol 110:1309–1316

O'Toole JC, Turner NC, Namuco OP, Dingkuhn M, Gomez KA (1984) Comparison of some crop water stress measurement methods. Crop Sci 24:1121–1128

Rascio A, Cedola MC, Sorrentino G, Pastore D, Wittmer G (1988) Pressure-volume curves and drought resistance in two wheat genotypes. Physiol Plant 73:122–127

Reynolds MP (2002) Physiological approaches to wheat breeding. In: Curtis BC, Rajaram S, Gomez Macpherson H (eds) Bread wheat: Improvement and production. Food and Agricultural Organisation of the United Nations, Caracalla. www.fao.org/docrep/006/y4011e/y4011e0a.htm

Rüger S, Ehrenberger W, Arend M, Geßner P, Zimmermann G, Zimmermann D, Bentrup FW, Nadler A, Raveh E, Sukhorukov VL, Zimmermann U (2010) Comparative monitoring of temporal and spatial changes in tree water status using the non-invasive leaf patch clamp pressure probe and the pressure bomb. Agr Water Manag 98:283–290

Scholander PF, Bradstreet ED, Hemmingsen EA, Hammel HT (1965) Sap pressure in vascular plants. Science 148:339–346

Sinclair TR (2012) Is transpiration efficiency a viable plant trait in breeding for crop improvement? Funct Plant Biol 39:359–365

Smith JAC, Popp M, Lüttge U, Cram WJ, Diaz M, Griffiths H, Lee HSJ, Medina E, Schäfer C, Stimmel KH, Thonke B (1989) Ecophysiology of xerophytic and halophytic vegetation of a coastal alluvial plain in northern Venezuela. VI. Water relations and gas exchange of mangroves. New Phytol 111:293–307

Steudle E, Zimmermann U (1977) Effect of turgor pressure and cell size on the wall elasticity of plant cells. Plant Physiol 59:285–289

Westhoff M, Reuss R, Zimmermann D, Netzer Y, Gessner A, Geßner P, Zimmermann G, Wegner LH, Bamberg E, Schwartz A, Zimmermann U (2009) A non-invasive probe for online-monitoring of turgor pressure changes under field conditions. Plant Biol 11:701–712

Woodward FI, Friend AD (1988) Controlled environment studies on the temperature responses of leaf extension in species of *Poa* with diverse altitudinal ranges. J Exp Bot 39:411–420

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

Zimmermann D, Reuss R, Westhoff M, Gessner P, Bauer W, Bamberg E, Bentrup FW, Zimmermann U (2008) A novel, non-invasive, online-monitoring, versatile and easy plant-based probe for measuring leaf water status. J Exp Bot 59:3157–3167

Zimmermann U, Rüger S, Shapira O, Westhoff M, Wegner LH, Reuss R, Gessner P, Zimmermann G, Israeli Y, Zhou A, Schwartz A, Bamberg E, Zimmermann D (2010) Effects of environmental parameters and irrigation on the turgor pressure of banana plants measured using the non-invasive, online monitoring leaf patch clamp pressure probe. Plant Biol 12:424–436