



SPECIAL ISSUE PAPER

Monitoring plant and soil water status: established and novel methods revisited and their relevance to studies of drought tolerance

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Abstract

In all studies of the effects of water deficits on plant functioning there is a need for an accurate and comprehensive definition of treatments and their effects on plant water status. The various measures of water status used in plant and soil science are reviewed and their appropriateness for different purposes such as for studies of mechanistic effects of water deficits on plants, for breeding of drought-tolerant plants, or for management of irrigation systems are reviewed. An important conclusion is that the frequent emphasis on water potential rather than on cell turgor can be shown to be misleading, as can be measurements in the leaf. The disadvantages of the current trend towards the omission of necessary water-status measurements, especially common in more molecular studies, are outlined, and recommendations made for minimal sets of measurements for specific types of experiments.

Key words: Drought, relative water content, soil water, turgor, water potential, water stress.

Introduction

Precise definition of both the environmental conditions and of the plant responses is a prerequisite for the conduct of repeatable and interpretable experiments. This paper reviews the range of approaches available for monitoring plant and soil water status, includes discussion of both direct and indirect methods, and aims to provide some recommendations as to how to select the most appropriate technique for specific purposes. The discussion builds on

the general concepts presented previously (Jones, 1990), with the aim being to compare and contrast the general types of measurement available rather than attempting to evaluate all the specific instruments on the market for measurement of water status. Further details of the necessary instrumentation and its use may be found in appropriate texts (e.g. Slatyer, 1967; Boyer, 1995; Kirkham, 2004).

The choice of measurement technique in any experiment depends critically on the experimental objectives, and also on any pre-existing hypotheses concerning the mechanisms of any response or adaptation to the water deficit. All too often, modern plant physiologists do not incorporate an understanding of the control systems underlying the expected physiological responses into the design of their experiments and their analysis or into their choice of water-status measure. For example, recent improvements in the understanding of mechanisms involved in plant adaptation to drought have only developed through the increasing recognition of the involvement of root–shoot signalling (see review by Davies and Zhang, 1991) in the control of stomatal aperture and growth in some water-stressed plants. Although these advances might have been expected to lead to a shift in the ways in which water status is quantified in drought studies, there has been surprisingly little recognition of these changes in much modern experimental work relating to plant response or adaptation to drought. Where water status is measured (and that is all too rarely) it is too commonly based on measurements of water potential in the leaves, neither of which (water potential or leaves) is usually optimal. In this paper, some of the evidence which indicates a need for a change in the general approach to measurement of water status in modern drought studies, including a need for a shift away from the reliance on leaf

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water potential, is reassessed. Although these arguments are not new (Jones, 1990) a survey of typical usage (outlined below) highlights the deficiencies in current practice and the need for a more thoughtful approach to the choice of method for monitoring water status in experimental systems.

Why measure water status?

It is generally accepted that the accurate measurement of plant and/or soil water status is critical in any experiment where one is concerned with understanding the effects of differing water supply. Such measurements are essential to define the conditions of the experiment (both in terms of the treatments applied and in terms of the effects on the plants) and as a first step in facilitating repetition of the experiment (which is an essential part of rigorous scientific method). Precise definition of water status in different parts of the soil-plant system is also required for the formulation and testing of any rigorous mechanistic hypotheses, such as those relating to the mechanisms of drought tolerance or adaptive responses in any plant. It is also essential that the measure of water status chosen is relevant to the physiological process of interest. Reliable measures of water status also provide powerful tools for crop management purposes where there is a need for repeatable control as in irrigation scheduling.

In measuring water status it is useful to distinguish cause and effect (=‘stress’ and ‘strain’ using the terminology of Levitt, 1972). For example, soil water deficits and the consequently lowered soil water potentials are usually considered as the underlying stresses in the system; the leaf water status is then a result of the soil water deficit. Indeed the actual leaf water status is modulated by plant responses such as stomatal closure or changing xylem hydraulic conductance, so it neither uniquely nor usefully describes the experimental treatment. For some purposes, however, the leaf water status can usefully be regarded as a stress: these are where one is concerned with the direct effects of leaf water status on metabolic or physiological processes within the leaf. The leaf water status can therefore be regarded both as a strain and a stress, with its role at any time being dependent on the adaptive mechanisms that occur in the plant.

Unfortunately there is not necessarily any unique or ‘best’ measure of water status that is applicable in all situations. The choice of the most appropriate method(s) for measuring or describing water status in any situation depends on the purpose of the experimenter and may be very different for (i) those concerned primarily with practical management such as irrigation scheduling or quantification of the benefits of management treatments such as mulching or pruning, (ii) those aiming to understand the mechanisms of water movement, (iii) those aiming to understand the mechanisms involved in water

stress effects on growth and physiology and the adaptive plant responses involved, and (iv) those aiming to identify differences in drought tolerance, to isolate the controlling genes, or to breed or test drought-tolerant genotypes.

General principles and approaches to measurement of water status

Before what should be measured and reported in studies of plant water relations is considered in detail, it is worth reviewing some of the fundamental principles of water-status measurement. The available measures of soil or plant water status can be broadly divided into those based either on (i) the amount of water or on (ii) its energy status. The principles and practice underlying the quantification and measurement of soil and plant water status have been well described in a number of texts and reviews (Slatyer, 1967; Boyer, 1995; Nobel, 1999; Mullins, 2001; Kirkham, 2004) and these should be consulted for more detail and background theory.

Amount of water

Obvious measures of water status for soils or plant tissues are based on water content. Although content can be expressed as an absolute amount this needs normalizing so it is more useful to express it as a fraction of other material in the system on either a volumetric or a mass (or molar) base (e.g. $\text{m}^3 \text{m}^{-3}$, %, g g^{-1} fresh mass, etc.).

Soil moisture content measurement: A basic measurement which can be used for calibration of the other methods available is to measure soil moisture directly using gravimetric measurements. These require extraction of a known volume of soil, which is weighed and then dried and reweighed. It is normally assumed that organic material is not lost during the drying process. There are a wide range of approaches and instruments for direct and indirect measurement of soil moisture content; these have been extensively reviewed elsewhere (Gardner *et al.*, 2001; Kirkham, 2004) and include neutron probes and a wide range of capacitance or electromagnetic sensors, including time-domain reflectometry, capacitative probes, resistance probes, etc. The majority of these instruments make use of the fact that the dielectric constant of water is very different from that of other components of the soil so the output signal can be directly related to moisture content, while resistance and voltaic probes depend more on the conductive capacity of soil solutions. In addition, there are several indirect approaches for estimation of soil moisture content based on remote sensing using, for example, passive and active microwave or radar techniques (Gardner *et al.*, 2001).

An alternative approach that is widely adopted, especially for agronomic and irrigation purposes, is the indirect estimation of water status on the basis of soil moisture

balance calculations. Changes in soil moisture content ($\Delta\theta$) are estimated from

$$\Delta\theta = \text{precipitation} + \text{irrigation} - \text{runoff} - \text{drainage} - \text{evapotranspiration} \quad (1)$$

where the various components are estimated using standard methods (Allen *et al.*, 1998). As drainage is generally rather difficult to estimate, this approach works best in situations where drainage can be neglected.

A major limitation of water content measurements in soils is that the moisture release curves are quite different for peat soils, fine-grained clays, and coarser soils, so that a given % water content may represent either a sand fully saturated with free water or a rather dry clay (Townend *et al.*, 2001). Slightly more generality may be obtained for soils through a normalization process involving expression of the soil moisture content as a fraction of either the total volume of pore space (giving a relative saturation) or as the related water-holding capacity of the soil (usually defined as the amount of water released in going from field capacity to a tension of 1.5 MPa). Unfortunately, hysteresis and different release curves for different soils mean that these can only be approximate.

Plant water content measurement: Analogous measures of water content are available for plant tissues where the water content may be expressed per unit fresh weight or per unit dry weight (or even in terms of unit leaf area). Unfortunately, as with soils, these raw water contents are not very useful for comparisons between species with different morphologies as the degree of succulence, for instance, influences the value obtained, even for fully turgid leaves. A very powerful method of normalization of such data is the use of relative water content (RWC) which is defined as

$$\text{RWC} = (\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight}) \quad (2)$$

The various precautions that need to be considered in the determination of RWC have been addressed in detail by Barrs (1968), who emphasized especially the procedure required to obtain a reliable estimate of the turgid weight. This measure of water status goes a substantial way towards effectively normalizing leaf water content for differences in leaf succulence.

Energy status

A major problem with measures of water content, even the relative measures described above, is that such measures do not necessarily relate to the ease with which that water can be extracted or to its effect on plant functioning. It was recognized many years ago that measures of water status based on the energy status of water in the system have

advantages over purely volumetric measures. For soils, the concept of capillary potential (equivalent to the modern matric potential) was introduced by Buckingham (1907), while for plants Ursprung and Blum (1916) introduced the term 'suction pressure deficit' as the basis for describing plant water relations. These concepts were re-expressed on a sound thermodynamic basis and brought into line with modern physico-chemical usage by Slatyer and Taylor (1960) who introduced the term 'water potential' (ψ), as a measure of Gibbs free energy. Dainty (1963) and Slatyer (1967) then expressed water potential in pressure units which were familiar to plant physiologists and soil scientists by defining it in terms of the chemical potential of water (μ) and dividing by the partial molal volume of water

$$\psi = (\mu - \mu_0) / V_w = - (RT / V_w) \log_e (e / e_s) \quad (3)$$

where V_w is the partial molal volume of water (usually assumed to equal that in the liquid phase) and μ_0 is the chemical potential of pure water at a reference level, R is the universal gas constant, T is the temperature (K), and e/e_s is the vapour pressure in equilibrium with the water-containing matrix divided by the saturation vapour pressure at that temperature. The total water potential anywhere within the soil-plant system is then the sum of a number of component potentials

$$\Psi = \psi_\pi + \psi_P + \psi_g [+ \psi_\tau] \quad (4)$$

where ψ_π is the osmotic potential due to dissolved solutes, ψ_P is pressure potential (equal to the hydrostatic pressure; often simply given the symbol P), ψ_g is the gravitational potential reflecting elevation differences between the site of interest and the reference level, and ψ_τ is the matric potential. In practice, the conventions used in soil science (Marshall *et al.*, 1996; Mullins, 2001), where ψ_τ is defined as a component of ψ_P , appear more logical than the separation of matric and pressure terms often adopted somewhat misleadingly by plant physiologists. The alternative of combining the osmotic and matric effects into one (osmotic) term is also well based physically (Passioura, 1988). Where the gravitational potential comes into play, as in water transport in tall trees, it is probably most convenient to use differences in what is often called hydraulic potential ($= \psi_g + \psi_P$).

Notwithstanding the controversies relating to the correct formulation for water potential and its units (see, for example, Passioura, 1988; Roderick, 2001, 2005) it is felt that for compatibility with much current usage it is reasonable to stick with the use of water potential as outlined above, even though it has only restricted relevance to plant physiologists, as will be seen below. In retaining water potential terminology it is important to remember some of the assumptions involved. For example, the standard derivation assumes that the system is in equilibrium and

isothermal, which clearly is not true in most plant systems. It is also necessary to understand and recognize the relevant components for water movement in any specific system, with, for example, the osmotic component only being relevant for systems with semi-permeable membranes as modified by the appropriate reflection coefficient when that is not unity (Dainty, 1963).

Soil water potential and its measurement: By contrast to the measurement of the amount of water in the soil, methods which measure the energy status are of greater value for providing a rigorous indication of the water 'availability' to plants, with values that allow comparisons between any growing substrate. Unfortunately even the use of water potential as a measure of availability is somewhat oversimplified as this strictly refers only to the equilibrium situation; the capacity of a soil to give up water also depends on the hydraulic conductivity of the soil (which itself is a function of both soil type and its water potential). Nevertheless, the water potential gradients do determine whether water can be absorbed at all, even if by themselves they do not determine the rate of uptake. Again there is a substantial amount of literature on this subject (Mullins, 2001; Kirkham, 2004) which will not be repeated here. The most common instruments for the measurement of the energy status of soil water are tensiometers (for fairly low suction) and soil psychrometers (Mullins, 2001), or else one can calibrate volumetric sensors (neutron probes and capacitance sensors) using the moisture release isotherm as derived from, for example, pressure plate calibrations (Townend *et al.*, 2001). Many other indirect sensors of soil moisture tension, such as gypsum blocks depend on the water release characteristics of porous materials (Mullins, 2001).

Plant water potential and its measurement: Measurements of plant water potential are primarily by means of either psychrometric methods (involving tissue equilibration with air in an enclosed chamber and the estimation of the vapour pressure using wet and dry thermocouples) or by means of the pressure chamber. For psychrometry, ψ can in principle be derived from the vapour pressure using equation 3, but in practice ψ is more usually obtained using a calibration curve for known concentrations of salt solutions. The cell pressure probe (reviewed by Tomos and Leigh, 1999), which measures turgor pressure in cells, can be used in combination with estimates of osmotic potential to derive water potentials according to equation 4, while the xylem pressure probe (Balling and Zimmerman, 1990) can be used to estimate mild xylem tensions, although its use for more extreme tensions is controversial (Angeles *et al.*, 2004). The cell pressure probe is a particularly valuable tool for the study of important cell hydraulic properties such as cell wall elasticity and cell membrane permeabilities. Details of these and other methods are well described elsewhere (Jones, 1992; Boyer, 1995; Kirkham, 2004) so will not be reviewed in more detail here.

Indirect measures of water status

In addition to the various methods for the direct measurement of either water content or energy status of plant or soil water, there are a number of widely used indirect indicators of water status based on the analysis of plant growth or physiological responses known to be indicative of water deficits (Jones, 2004b). These range from visible expression of increasing plant water deficits such as wilting (Jones, 1972), through morphometric changes such as stem, leaf, or fruit shrinkage (Jones, 1973b; Hugué *et al.*, 1992; Fereres and Goldhamer, 2003; Naor and Cohen, 2003) and the well-known reductions in cell expansion and growth rate that are associated with water deficits, to physiological responses such as stomatal closure and reductions in photosynthesis rate. A particularly widely used method for detecting water stress-induced stomatal closure as a guide to irrigation scheduling is the use of infra-red thermometry (Idso *et al.*, 1981) or thermography (Jones, 2004a, b).

It is frequently possible to derive repeatable calibration curves to allow estimation of water status for a given cultivar, so such methods are often valuable in agronomic practice. The fundamental problem with all such indirect measures of water status, however, is that, although they may be very valuable for detecting the physiological results of water deficits (whether in terms of damage or adaptive responses such as stomatal closure), they are not very useful in mechanistic studies aiming to understand the process of plant adaptation or response to water deficits because they are not rigorously associated with any underlying measures of water status. As a result they can only be used with circumspection when attempting to identify drought-tolerance mechanisms for incorporation in plant breeding programmes. Nevertheless those techniques, such as thermal imaging (Jones, 2004a), which are suitable for screening large numbers of plants, have real value in drought studies as long as their limitations are remembered.

Choice of water-status measure and its relevance to physiological processes

As has been pointed out previously (Jones, 1990, 1992) the choice of water-status measure for plant physiological studies is not necessarily straightforward, with the most appropriate method being dependent on the uses to which the data are to be put. The following sections review and expand some of these earlier arguments relating to the measures of water status to be preferred in different situations.

What physical measure of water status?

In soils, the total potential, which normally equates to soil moisture tension, appears to be a good indicator of soil moisture status for physiological studies because it is the measure that most closely indicates the potential ability of plants to extract water, although, as indicated above,

the actual rate of extraction will also depend on hydraulic conductivity of the soil–plant pathway. The ease of extraction declines as soil moisture content declines and an increasing proportion of the remaining soil moisture becomes tightly bound to soil particles or in capillaries. On the other hand, a researcher or agronomist concerned with irrigation is often only concerned to replace the soil water lost in transpiration as might be calculated using equation 1. Therefore simple volumetric measures of soil moisture content may be adequate, or indeed most appropriate, as long as information is available on the amount of water loss that can be sustained without yield loss.

A general problem with estimation of soil moisture content or potential arises because of the substantial heterogeneity within soils, with single point measurements rarely being representative. This necessitates either substantial replication of soil moisture sensors or, even better, a combination of a wide distribution of soil moisture sensors with an irrigation system capable of targeted variable water application.

In plants, although water potential terminology has been strongly proposed as the most rigorous basis for assessment of plant water status on the grounds that differences in water potential drive water flow in the soil–plant–atmosphere system, one should note that different sub-components are relevant to flow in different situations with, for example, the osmotic component not being relevant in the absence of semi-permeable membranes (e.g. in the case of xylem flow or water movement in soils). Mass flow in such cases is driven by pressure differences or, where height differences are involved, by differences in hydraulic potential ($= \psi_p + \psi_g$).

It has also been clear for some time that biochemical systems in plants do not appear to have sensing mechanisms that can detect/respond to ψ (or to water activity) over the small range of water activity changes (at most a few per cent; compare equation 3) as plants lose turgor and the capacity to grow or survive (Sinclair and Ludlow, 1985). For example, this lack of physiological sensitivity for processes such as photosynthesis has been demonstrated clearly in experiments comparing the effects of the addition of non-permeating and permeating osmotica (e.g. ethylene glycol) to leaf slices (Jones, 1973a). Addition of concentrations of the permeating solute ethylene glycol that lowered the water potential by around 1.5 MPa had no significant effect on the photosynthesis rate of leaf slices, while addition of an equivalent concentration of non-permeating osmotica (mannitol or sodium chloride) substantially inhibited photosynthesis (Fig. 1). As the permeating osmotica equilibrate rapidly across the plasma membrane, cell volume (and hence cell turgor) would be expected to change little as water potential was lowered in this case, leading to the conclusion that it must have been the change in either the turgor pressure or the cell volume, rather than the change in water potential *per se*, that caused

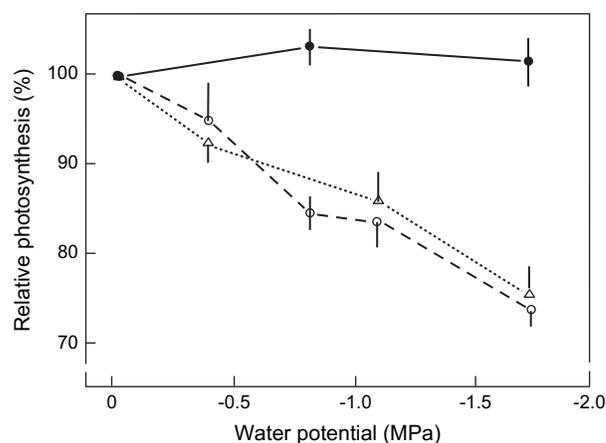


Fig. 1. Relative rate of photosynthesis (measured as $^{14}\text{CO}_2$ fixation) by thin wheat leaf slices in solution with a range of osmotic potentials achieved by varying concentrations of mannitol (open circles), sodium chloride (open triangles), or ethylene glycol (filled circles) (data from Jones, 1973). Bars represent standard errors for 6–10 replicates.

the inhibition of photosynthesis. Though it is usually difficult to devise experiments to distinguish volume from turgor effects, studies with isolated protoplasts (Kaiser, 1982) have suggested the importance of cell volume. It is likely therefore that the many documented cases where drought responses have been associated with changes in water potential result from the common, but often coincidental, close association between turgor and total water potential rather than resulting from any true dependence on water potential.

More recently, substantial evidence has been accumulating that membrane stretch-sensitive channels (Alexandre and Lassalles, 1991; Cosgrove and Hedrich, 1991; Lew, 1996; Ramahaleo *et al.*, 1996) may be primary sensors of mild water deficits, although redox-state sensing may also be important [see, for example, Kacperska (2004) for a review especially covering the downstream signal transduction]. Since the early findings there have been many studies confirming the general importance of processes related to cell turgor as a key signal of water deficit stress, for which RWC can frequently be used as a good proxy measure, although some conversion factor may be needed to make the most of it. A consequence of the physiological importance of turgor pressure is that the cell wall elasticity takes on a crucial role in determining cell responses to declining water availability (Schulte, 1992).

Notwithstanding all this evidence, many workers still consider measurement of water potential the ‘gold standard’ for physiological studies. It seems to me that it is now time for a fundamental reassessment of this view, as there are few cases where total water potential can be shown to be the causal variate underlying physiological responses and adaptations. The emphasis on water potential seems misleading as the real value of water potential is only as a means of deriving the more ‘important’ variables such as turgor

pressure using equation 4. A clear example where it is easy to see that total water potential may be misleading is in the case of osmotic adjustment: this does not directly affect water potential yet may improve the ability of plants to take up water from the soil simply by maintaining positive turgor pressure at lower tissue water potentials than would otherwise be the case. This point is discussed further below.

Also note that different methods of measurement give different answers. The leaf psychrometer attempts to estimate the total leaf water potential ($= \psi_{\pi} + \psi_p$) but the pressure chamber measures the xylem pressure potential ($= \psi - \psi_{\text{xylem}} - \pi$). In most cases the error is small, but the result may be misleading in cases where there are significant apoplastic solutes (James *et al.*, 2006).

Where in the soil–plant system should water status be measured?

The usual (often implied) concept is that water status should be measured ‘at the site of the process of interest’ (Spomer, 1985). For example, those physiologists concerned with the study of leaf processes such as photosynthesis or stomatal opening have frequently concentrated on measurements of leaf water status, while those concerned with growth have concentrated on the water status of the appropriate meristem. The increasing recognition of the importance of within-plant signalling by non-hydraulic means (reviewed by Davies and Zhang, 1991) has cast substantial doubt on the applicability of the above principle. Rather, the existence of root–shoot signalling implies that, in many cases, the water status should be measured at the site of signal generation [e.g. abscisic acid export or re-export in the roots (Hartung *et al.*, 2002), or pH regulation in the xylem (Wilkinson and Davies, 2002)] rather than in the leaf.

Notwithstanding the evidence for the importance of chemical signalling in plants, there is still an important role for conventional hydraulic signalling, which may be more or less important in different plants (Fuchs and Livingston, 1996). For example, cavitation in the stem as water deficits increase can act as a hydraulic signal, substantially lowering ψ_{leaf} (Jones and Sutherland, 1991; Sperry *et al.*, 1998), while some modelling studies suggest that processes such as transpiration in some species are more closely related to leaf water status than to soil water (Lynn and Carlson, 1991). In practice, it is often difficult to determine precisely where in the soil–plant system it is appropriate to measure because of the feedbacks involving control of water status. These feedbacks mean that it is often difficult to disentangle or break the correlations between, for example, ψ_{soil} and ψ_{leaf} that result from conventional hydraulic signalling (Jones, 1997). There are substantial differences between isohydric plants such as cowpea and maize and anisohydric plants such as sunflower (Bates and Hall, 1981; Tardieu and Simonneau, 1998). In the former, stomatal control (as a result of root–shoot signalling) tends to maintain leaf water status, and hence

turgor, stable as soil dries. Therefore, in the isohydric case, leaf turgor (or ψ_{leaf}) cannot be used as a measure of stress, while in more anisohydric plants leaf turgor declines in parallel with soil drying so it is difficult to distinguish the causal stress for any physiological response.

Measurements of ψ_{leaf} can be used to infer ψ at other places in the soil–plant system. For example, predawn ψ_{leaf} is thought to equilibrate closely with soil/root ψ , so it is frequently used as an indicator of soil or root ψ that avoids some of the problems associated with direct soil measurement. Indeed it is widely used as a measure of stress in irrigation experiments (Ameglio *et al.*, 1998; de Souza *et al.*, 2003; Remorini and Massai, 2003). Alternative approaches to the estimation of the potentially more relevant ψ_{root} , based on measurements made at more experimentally convenient times, are either pressure chamber measurements of ψ_{leaf} on root suckers pre-equilibrated in the dark to allow equilibration with root ψ (Higgs and Jones, 1991; Simonneau and Habib, 1991) or the calculation of the root ψ appropriate for a transpiring plant based on ψ_{leaf} and stomatal conductance (Jones, 1983).

When should measurements be made?

When choosing a sampling/measurement strategy for water status it is also necessary to consider the appropriate time of day for measurement. Historically plant physiologists have concentrated on simultaneous measurement of characters such as leaf function (e.g. photosynthesis) and leaf water status. Many physiological and developmental responses and adaptations to drought, however, occur as a result of the temporally and spatially integrative responses controlled through hormonal signalling and involving substances such as cytokinins and abscisic acid. These themselves may have substantial lags in their expression, so instantaneous comparisons with local water status are only likely to be appropriate for those responses directly dependent on local water status (e.g. stomatal apertures are determined directly by differences in turgor pressure of guard and subsidiary cells) (Munns *et al.*, 2000). Even in the case of stomatal aperture, however, the ion fluxes that determine the relevant turgor pressures are dependent on integrative signalling systems.

Schematic low time resolution-smoothed average trends in leaf water potential over a typical field drying cycle are illustrated in Fig. 2a, with the corresponding higher temporal resolution data shown schematically in Fig. 2b. There are several points to note from these graphs: the first is that the absolute value of ψ_{leaf} (or any related variable such as turgor pressure) varies diurnally over perhaps an order of magnitude; the second is that substantial changes occur even over time scales as short as minutes (Jones, 1990); and thirdly these short-term changes can be substantially larger than treatment differences, even when clear differences in growth rate or other physiological response have been achieved.

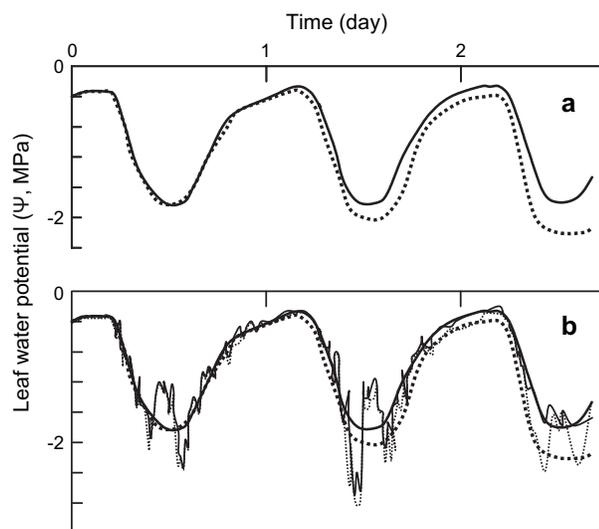


Fig. 2. Schematic illustration of expected time-courses of leaf water potential (ψ_{leaf}) over a period of 3 d following withholding of irrigation at time 0 (dashed lines) and for corresponding irrigated controls (continuous lines). (a) Typical diurnal trends of ψ_{leaf} smoothed by taking 3 h running means. In (b), the expected magnitude of instantaneous variation in ψ_{leaf} , as measured with a pressure chamber, is shown (based on data presented by Jones, 1990).

Drought adaptation in the field usually involves developmental responses occurring over periods of days to weeks. There is little definitive information as to whether it is an integrated measure of water status that provides the controlling signal or whether it may be the maximum daily deficit. In any case, it is difficult to see how the rapidly varying and environmentally extremely sensitive ψ_{leaf} can provide an appropriate long-term signal of stress, especially as it is clear that treatment differences are often smaller than short-term fluctuations in ψ (Fig. 2b; Jones, 1983; Jones *et al.*, 1983), especially in isohydric species. A further difficulty with integration is that, though it is computationally easy to integrate a linear response (e.g. as day-MPa which is analogous to thermal time), physiological responses to water status tend not to be linear (Jones, 1992). An advantage of predawn ψ_{leaf} measurements as a measure of water stress is that they tend to integrate short-term changes in water status. An alternative approach that also smooths out some of the short-term variation in ψ_{leaf} is to use the ψ_{stem} which is the potential of leaves pre-equilibrated in the dark, giving an estimate of ψ_{xylem} ; this can also be better than ψ_{leaf} as an indicator of stress (McCutchan and Shackel, 1992).

Some specific 'case-studies' and water-status measurement in molecular studies

The methods available for measurement of water status relevant to different applications are outlined below and their relative merits summarized in Table 1 for a number of typical experimental situations.

Practical management and irrigation scheduling

Since natural droughts develop over timescales of days to weeks and the developmental or adaptive responses also occur over similar timescales, the most appropriate measures of stress for agronomic purposes are integrated over time and space. This therefore suggests that more integrative soil moisture measurements would be preferred to instantaneous measures such as midday ψ_{leaf} (especially where weather conditions are variable). Predawn ψ_{leaf} and, to a lesser extent, ψ_{stem} may be useful surrogates for ψ_{soil} . Similarly, remote-sensing approaches would have particular advantages because of their capacity to integrate over large areas, although again, those methods such as microwaves that can estimate soil water content may be preferable for agronomic purposes to those (e.g. canopy temperature) that give instantaneous values of crop physiological status.

A capacity for automation is particularly valuable for irrigation scheduling: for example, methods for estimation of soil moisture status based on measurement of soil dielectric constant (time-domain reflectometry, etc.) are particularly suitable for routine recording but usually provide rather poor spatial replication unless large sensor networks are installed. Plant measurements tend to be more difficult to automate; direct measures of water status such as the pressure bomb have never been successfully automated and, although psychrometers can be automated, they tend to be expensive and somewhat unreliable, only giving good data in expert hands. This has led, for agronomic purposes, to a greater emphasis on indirect methods, in spite of their problems, such as dendrometry/morphometrics, thermal sensing of stomatal closure, etc. (Huguet *et al.*, 1992; Naor and Cohen, 2003; Jones, 2004a).

Studies of water movement

Thermodynamic and water potential terminology come into their own when considering water flow in the soil–plant–atmosphere system, as water flow is directly dependent on differences in the appropriate components of water potential (together with the relevant resistances to flow). Most publications on this subject use fully appropriate measures of water status.

Mechanisms of water stress effects on growth and physiology

There is an enormous current interest in improving the drought tolerance of crops. The 'rational' approach is to elucidate existing drought tolerance mechanisms in different plant species or cultivars and to identify the genes involved so that they can be incorporated into improved cultivars. This requires an understanding of the physiological basis of drought tolerance in existing cultivars/wild lines and the variation that exists; in particular, it is necessary to consider the trade-offs between increased

Table 1. A subjective classification of the relative value of different water-status measures for different purposes

More +s indicate greater value, while (–) indicates limited value.

	Water transport	Drought adaptation	Plant breeding/screening	Agronomy and irrigation scheduling
Soil measures				
Soil water content	(–)	+	+	+++
Soil ψ	+++	++	++	+
Plant measures				
Predawn ψ	++	++	+	++
Sucker/stem ψ	++	+	(–)	+
Leaf ψ	+++	(–)	(–)	(–)
Turgor pressure	+++	+++	+	+
RWC	(–)	++	++	++
Stomatal conduct/infrared thermometry	(–)	++	++	++
Morphometry	(–)	+	(–)	+
Comments:	Components of ψ generally needed. Hydraulic potential is particularly relevant for saturated flow in soils.	RWC is often a good surrogate for ψ_P ; predawn ψ is a surrogate for ψ_{soil} .	Critical to have measure of environmental stress: responses such as stomata, ψ_P relate to adaptation.	Response-based measures (e.g. g_s) better than RWC or ψ_{leaf} for isohydric plants; methods need to be easy to use in the field.

survival in dry conditions and the tendency for reduced production. The particular balance between survival and production, and hence the optimal value of specific characters such as stomatal closure or osmotic adjustment, tends to depend critically on the environment in which the plant is to be grown (e.g. terminal drought versus random drought events) as well as on the harvested component of the plant.

As has been pointed out by many authors (Jones *et al.*, 1989; Jones, 1992; Passioura *et al.*, 1993; Kramer and Boyer, 1995; Sinclair and Purcell, 2005) drought tolerance is an emergent property involving a wide range of component processes such as drought escape (e.g. as a result of early maturity), drought avoidance (e.g. through control of water loss or enhanced root uptake), biochemical tolerance of tissue water deficits (favouring survival), and efficiency of water use. Only in rare agricultural situations is survival of drought a key character in agronomic drought tolerance (Sinclair and Purcell, 2005).

In order to understand fully the balance between the different drought-tolerance strategies and their values to plants, it is essential that the experimental protocols include measurements of both environmental stress (e.g. soil moisture and its distribution) and the plant responses (including tissue water status and controls of water uptake and loss such as leaf area and stomatal conductance). Crucially it is also necessary that the drought treatments imposed in experimental systems are similar in intensity to the stresses that occur naturally.

Screening for drought tolerance and plant breeding

In the field: A common approach to identifying drought-tolerant genotypes is to screen for overall yield under dry

conditions. Unfortunately the genotypic rankings and even the location of quantitative trait loci from such screens are very dependent on the range of environments chosen (Hall *et al.*, 2005; Atlin *et al.*, 2006). High-yielding genotypes under a terminal drought scenario, for example, may not perform so well with intermittent drought. For the terminal drought, early maturing genotypes often perform best and therefore may be classified as drought tolerant solely as a result of their phenology, not as a result of any specific 'drought tolerant' physiology. A further complication is that, in many agricultural situations, the most drought-tolerant lines are not necessarily those with the highest potential yields, but those with the most stable yields over a range of water availabilities.

Effective interpretation of field screens in terms of the mechanism giving rise to any apparent drought tolerance (including distinguishing phenological differences and drought escape, drought avoidance, and desiccation tolerance mechanisms) requires the measurement of both the dynamics of soil moisture availability in the rooting zone of each specific cultivar and the corresponding tissue water status changes (at least RWC or preferably both ψ_P and ψ_{π}). In addition, these data need supplementing with information on the control of water loss (e.g. leaf area and stomatal behaviour). In practice, it may not be feasible to obtain such a full data set for all entries in this type of trial, but interpretation, and subsequent incorporation into a breeding programme, will be greatly facilitated by measurements of tissue water status at key developmental stages.

An alternative approach to the discrimination of tolerance mechanisms is to control the water supply to each genotype on the basis of measurements of tissue water status. This facilitates the separation of drought escape and drought tolerance mechanisms. For example, Yue *et al.*

(2006) adjusted sowing dates in a drought screen of different lines of rice so that key growth stages are synchronized, and based individual plant watering regimes on measurements of tissue water status.

Plant breeders have made some progress by combining specific drought-tolerance characters such as improved water use efficiency (Condon *et al.*, 2004) into commercial lines that perform well under specific drought conditions, with some recent advances in understanding the underlying genetics (Masle *et al.*, 2005). The hope is that further improvements in crop drought tolerance can be facilitated by rational combination of appropriate physiological characters. There is unlikely, however, to be a unique ideal combination for these characters for drought tolerance, as many processes can combine in different ways in different genetic backgrounds and environments.

Molecular studies: A large proportion of modern molecular research on drought tolerance attempts to identify those genes whose expression contributes to differences in drought tolerance. Although by no means universal, a substantial proportion of such studies lack any rigorous measurement of either the environmental stress imposed or the resulting tissue water status. Table 2 summarizes the broad types of water-status measures that have been reported in a selection of recent papers (between 2003 and July 2005) where the main objectives have included an analysis of molecular responses to drought. Results are separated into those papers published in some more 'molecular' journals such as *Plant Physiology*, *Plant Molecular Biology*, *The Plant Journal*, and *The Plant Cell* and more 'physiological' journals such as *Journal of Experimental Botany* and *Plant, Cell and Environment*. The summary data in Table 2, where over half the papers in more molecular journals had no measure of water status whatsoever, raise serious concerns.

Where there is a lack of critical information on water status it follows that the experiments are likely to be both difficult to repeat with any certainty and, more importantly, almost certainly limits the value of the information collected. The problems arising from the limited information are of several types, many of which could be avoided by careful experimental design.

- (i) The first common problem is that, notwithstanding the complexity of drought responses and adaptations outlined above, drought tolerance is frequently equated with survival or the proportion of tissue death (Yamaguchi-Shinozaki and Shinozaki, 1994; Cheong *et al.*, 2003; Sugano *et al.*, 2003; Chini *et al.*, 2004; Chen *et al.*, 2005). The widespread use of this measure of drought tolerance probably arises in part because of the ease of conducting severe 'all-or-nothing' experiments, where clear responses are readily detectable, and the perception that, as long as the stress is severe enough, it does not need to be measured.
- (ii) A related point is that assessment of survival requires the desiccation of the tissues to water potentials substantially below those that are commonly observed under typical drought conditions in agriculture and that are known to cause severe yield losses. Although it is rare for any information to be provided on the actual tissue desiccation achieved in experiments, some data are available (Boominathan *et al.*, 2004; Kawaguchi *et al.*, 2004) confirming that the tissue water status achieved (RWC of 49–55%) can be in the range that would normally be expected to lead to severe tissue damage and is much below the normal range in agricultural crops (Barrs, 1968). In both these papers (Boominathan *et al.*, 2004; Kawaguchi *et al.*, 2004) the 'controls' were close to 80% RWC, which is a value that already corresponds, for many plants, to severe dehydration and a water potential of around -3 MPa (Jones and Higgs, 1979); this is well below the value where turgor is commonly lost and growth stops. It could be argued therefore that such experiments can only yield information of limited relevance to the key genes involved in the subtle responses to the mild droughts that are agriculturally more relevant (see also Sinclair and Purcell, 2005).
- (iii) A third common problem is that many studies do not take adequate precautions to ensure that the 'stress' treatments are truly comparable between test lines. For example, it is common to withhold water for an extended but fixed period, and to compare the

Table 2. Use of water-status measures in molecular papers (January 2003 to July 2005) on the subject of water stress or drought tolerance

Articles included in the analysis were those mentioning drought tolerance or water stress where the main topic was subjectively assessed as an investigation into molecular or genetic studies of the effects of drought on gene expression or effects of transgenes on performance under water stress, where n is the number of articles. Note that some papers reported more than one measure of water status.

	n	ψ	RWC	Leaf weight	Soil water	None
<i>Plant Physiology</i> , <i>The Plant Journal</i> , <i>The Plant Cell</i> , <i>Plant Molecular Biology</i>	47	9	8	3	5	26 (55%)
<i>Journal of Experimental Botany</i> , <i>Plant, Cell and Environment</i>	17	5	4	5	7	3 (18%)
Total	64	14	12	8	12	29

response in terms of survival or yellowing of a range of lines, mutants, or transgenics (Cheong *et al.*, 2003; Chini *et al.*, 2004; Chen *et al.*, 2005). Where the lines being compared have differences in leaf size (Chen *et al.*, 2005; De Block *et al.*, 2005) or, less obviously in stomatal conductance, rates of drying may be very different, and thus differences in survival may indicate only differences in water use, not tissue tolerance. In such cases the survivors are unlikely to be particularly productive. Although these differences may be of great interest, it would be of value to have the necessary information to allow dissection of the mechanism(s) involved. A better, more quantifiable, approach could be to apply treatments that ensure comparable tissue water status for the different experimental lines. For example, the use of tissue equilibration at a given water content pioneered by Henson and Quarrie (1981) in their genetic studies of abscisic acid accumulation might be worth further consideration.

Conclusions and recommendations

Although the need for good and appropriate measurements of water status is well recognized for studies of water movement in the soil–plant system and in crop physiology, such measurements are often not made in more molecular studies. An associated problem is that the drought treatments imposed in many molecular studies are often rather unrealistic so that the results may have little relevance to the common objective justification of the work to ‘improve agricultural production’. It would seem that enhanced inputs from environmental plant physiologists could benefit experimental design and enhance the value of the molecular studies for agricultural purposes. In general, although any measure of water status is better than none, a choice of measures including both the environmental stress and the plant water status will substantially enhance the information obtained. What is required for future experiments is a greater use of repeatable protocols that allow researchers to identify specific genes explicitly related to key processes involved in drought tolerance for incorporation into new varieties.

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References

- Alexandre J, Lassalles JP. 1991. Hydrostatic and osmotic-pressure activated channel in plant vacuole. *Biophysical Journal* **60**, 1326–1336.
- Allen RG, Pereria LS, Raes D, Smith M. 1998. *Crop evapotranspiration: guidelines for computing crop water requirements*. FAO 56. Rome, Italy: FAO Land and Water Division.
- Ameglio T, Archer P, Cohen M, Valancogne C, Daudet FA, Dayau S, Cruziat P. 1998. Significance and limits in the use of predawn leaf water potential for tree irrigation. *Plant and Soil* **207**, 155–167.
- Angeles G, Bond B, Boyer JS, *et al.* 2004. The cohesion-tension theory. *New Phytologist* **163**, 451–452.
- Atlin GN, Lafitte HR, Tao D, Laza M, Amante M, Courtois B. 2006. Developing rice cultivars for high-fertility upland systems in the Asian tropics. *Field Crops Research* **97**, 43–52.
- Balling A, Zimmerman U. 1990. Comparative measurements of the xylem pressure of *Nicotiana* plants by means of the pressure bomb and pressure probe. *Planta* **182**, 325–328.
- Barrs HD. 1968. Determination of water deficits in plant tissues. In: Kozlowski TT, ed. *Water deficits and plant growth*. New York, NY: Academic Press, 235–368.
- Bates LM, Hall AE. 1981. Stomatal closure with soil moisture depletion not associated with changes in bulk water status. *Oecologia* **50**, 62–65.
- Boominathan P, Shukla R, Kumar A, Manna D, Negi D, Verma PK, Chattopadhyay D. 2004. Long term transcript accumulation during the development of dehydration adaptation in *Cicer arietinum*. *Plant Physiology* **135**, 1608–1620.
- Boyer JS. 1995. *Measuring the water status of plants and soils*. London: Academic Press.
- Buckingham E. 1907. *Studies in the movement of soil moistures*. US Department of Agriculture Soils Bulletin No. 38.
- Chen ZZ, Hong XH, Zhang HR, Wang YQ, Li X, Zhu JK, Gong ZZ. 2005. Disruption of the cellulose synthase gene, *AtCesA8/IRX1*, enhances drought and osmotic stress tolerance in arabidopsis. *The Plant Journal* **43**, 273–283.
- Cheong YH, Kim KN, Pandey GK, Gupta R, Grant JJ, Luan S. 2003. *Cbl1*, a calcium sensor that differentially regulates salt, drought, and cold responses in arabidopsis. *The Plant Cell* **15**, 1833–1845.
- Chini A, Grant JJ, Seki M, Shinozaki K, Loake GJ. 2004. Drought tolerance established by enhanced expression of the CC-NBS-LRR gene, *ADR1*, requires salicylic acid, *EDS1* and *ABI1*. *The Plant Journal* **38**, 810–822.
- Condon AG, Richards RA, Rebetzke GJ, Farquhar GD. 2004. Breeding for high water-use efficiency. *Journal of Experimental Botany* **55**, 2447–2450.
- Cosgrove DJ, Hedrich R. 1991. Stretch-activated chloride, potassium, and calcium channels coexisting in plasma-membranes of guard-cells of *Vicia faba* L. *Planta* **186**, 143–153.
- Dainty J. 1963. Water relations of plant cells. *Advances in Botanical Research* **1**, 279–326.
- Davies WJ, Zhang J. 1991. Root signals and the regulation of growth and development of plants in drying soil. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**, 55–76.
- De Block M, Verduyn C, De Brouwer D, Cornelissen M. 2005. Poly(ADP-ribose) polymerase in plants affects energy homeostasis, cell death and stress tolerance. *The Plant Journal* **41**, 95–106.
- de Souza CR, Maroco JP, dos Santos TP, Rodrigues ML, Lopes CM, Pereira JS, Chaves MM. 2003. Partial rootzone drying: regulation of stomatal aperture and carbon assimilation in field-grown grapevines (*Vitis vinifera* cv. Moscatel). *Functional Plant Biology* **30**, 653–662.
- Fereres E, Goldhamer DA. 2003. Suitability of stem diameter variations and water potential as indicators for irrigation scheduling of almond trees. *Journal of Horticultural Science and Biotechnology* **78**, 139–144.
- Fuchs EE, Livingston NJ. 1996. Hydraulic control of stomatal conductance in douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] and alder [*Alnus rubra* (Bong.)] seedlings. *Plant, Cell and Environment* **19**, 1091–1098.

- Gardner CMK, Robinson D, Blyth K, Cooper JD.** 2001. Soil water content. In: Smith KA, Mullins C, eds. *Soil and environmental analysis: physical methods*, 2nd edn. New York, NY: Marcel Dekker, 1–64.
- Hall NM, Griffiths H, Corlett JA, Jones HG, Lynn J, King GJ.** 2005. Relationships between water-use traits and photosynthesis in *Brassica oleracea* resolved by quantitative genetic analysis. *Plant Breeding* **124**, 557–564.
- Hartung W, Sauter A, Hose E.** 2002. Abscisic acid in the xylem: where does it come from, where does it go to? *Journal of Experimental Botany* **53**, 27–32.
- Henson IE, Quarrie SA.** 1981. Abscisic acid accumulation in detached cereal leaves in response to water stress. I. Effects of incubation time and severity of stress. *Zeitschrift für Pflanzenphysiologie* **101**, 431–438.
- Higgs KH, Jones HG.** 1991. Water relations and cropping of apple cultivars on a dwarfing rootstock in response to imposed drought. *Journal of Horticultural Science* **66**, 367–379.
- Huguet JG, Li SH, Lorendeau JY, Pelloux G.** 1992. Specific micromorphometric reactions of fruit-trees to water-stress and irrigation scheduling automation. *Journal of Horticultural Science* **67**, 631–640.
- Idso SB, Jackson RD, Pinter PJ, Reginato RJ, Hatfield JL.** 1981. Normalizing the stress-degree-day parameter for environmental variability. *Agricultural Meteorology* **24**, 45–55.
- James JJ, Alder NN, Muhling KH, Lauchli AE, Shackel KA, Donovan LA, Richards JH.** 2006. High apoplastic solute concentrations in leaves alter water relations of the halophytic shrub, *Sarcobatus vermiculatus*. *Journal of Experimental Botany* **57**, 139–147.
- Jones HG.** 1972. *Effects of water stress on photosynthesis*. Canberra: Australian National University.
- Jones HG.** 1973a. Photosynthesis by thin leaf slices in solution. 2. Osmotic stress and its effects on photosynthesis. *Australian Journal of Biological Sciences* **26**, 25–33.
- Jones HG.** 1973b. Estimation of plant water status with the beta-gauge. *Agricultural Meteorology* **11**, 345–355.
- Jones HG.** 1983. Estimation of an effective soil-water potential at the root surface of transpiring plants. *Plant, Cell and Environment* **6**, 671–674.
- Jones HG.** 1990. Physiological-aspects of the control of water status in horticultural crops. *Hortscience* **25**, 19–26.
- Jones HG.** 1992. *Plants and microclimate*, 2nd edn. Cambridge: Cambridge University Press.
- Jones HG.** 1997. Stomatal control of photosynthesis and transpiration. *Journal of Experimental Botany* **49**, 387–398.
- Jones HG.** 2004a. Application of thermal imaging and infrared sensing in plant physiology and ecophysiology. *Advances in Botanical Research* **41**, 107–163.
- Jones HG.** 2004b. Irrigation scheduling: advantages and pitfalls of plant-based methods. *Journal of Experimental Botany* **55**, 2427–2436.
- Jones HG, Flowers TJ, Jones MB.** 1989. *Plants under stress*. Cambridge: Cambridge University Press.
- Jones HG, Higgs KH.** 1979. Water potential–water content relationships in apple leaves. *Journal of Experimental Botany* **30**, 965–970.
- Jones HG, Luton MT, Higgs KH, Hamer PJC.** 1983. Experimental control of water status in an apple orchard. *Journal of Horticultural Science* **58**, 301–316.
- Jones HG, Sutherland RA.** 1991. Stomatal control of xylem embolism. *Plant, Cell and Environment* **14**, 607–612.
- Kacperska A.** 2004. Sensor types in signal transduction pathways in plant cells responding to abiotic stressors: do they depend on stress intensity? *Physiologia Plantarum* **122**, 159–168.
- Kaiser WM.** 1982. Correlations between changes in photosynthetic activity and changes in total protoplast volume in leaf tissue from hygro-, meso-, and xerophytes under osmotic stress. *Planta* **148**, 538–545.
- Kawaguchi R, Girke T, Bray EA, Bailey-Serres J.** 2004. Differential mRNA translation contributes to gene regulation under non-stress and dehydration stress conditions in *Arabidopsis thaliana*. *The Plant Journal* **38**, 823–839.
- Kirkham MB.** 2004. *Principles of soil and plant water relations*. Burlington, MA: Elsevier Academic Press.
- Kramer PJ, Boyer JS.** 1995. *Water relations of plants and soils*. London: Academic Press.
- Levitt J.** 1972. *Responses of plants to environmental stresses*. New York, NY: Academic Press.
- Lew RR.** 1996. Pressure regulation of the electrical properties of growing *Arabidopsis thaliana* root hairs. *Plant Physiology* **112**, 1089–1100.
- Lynn BH, Carlson TN.** 1991. Simulating transpiration plateaus: the importance of leaf water potential. *Ecological Modelling* **58**, 199–208.
- Marshall TJ, Holmes JW, Rose CW.** 1996. *Soil physics*, 3rd edn. Cambridge: Cambridge University Press.
- Masle J, Gilmore SR, Farquhar GD.** 2005. The ERECTA gene regulates plant transpiration efficiency in *Arabidopsis*. *Nature* **436**, 866–870.
- McCutchan H, Shackel KA.** 1992. Stem water potential as a sensitive indicator of water stress in prune trees (*Prunus domestica* L. cv. French). *Journal of the American Society for Horticultural Science* **117**, 607–611.
- Mullins C.** 2001. Matric potential. In: Smith KA, Mullins C, eds. *Soil and environmental analysis: physical methods*, 2nd edn. New York, NY: Marcel Dekker, 65–93.
- Munns R, Passioura JB, Guo J, Chazen O, Cramer GR.** 2000. Water relations and leaf expansion: importance of time scale. *Journal of Experimental Botany* **51**, 1495–1504.
- Naor A, Cohen S.** 2003. Sensitivity and variability of maximum trunk shrinkage, midday stem water potential, and transpiration rate in response to withholding irrigation from field-grown apple trees. *Hortscience* **38**, 547–551.
- Nobel PS.** 1999. *Physicochemical and environmental plant physiology*, 2nd edn. London: Academic Press.
- Passioura JB.** 1988. Changing concepts regarding plant water relations: response. *Plant, Cell and Environment* **11**, 569–571.
- Passioura JB, Condon AG, Richards RA.** 1993. Water deficits, the development of leaf area and crop productivity. In: Smith JAC, Griffiths H, eds. *Water deficits: plant responses from cell to community*. Oxford: Bios, 253–264.
- Ramahaleo T, Alexandre J, Lassalles JP.** 1996. Stretch activated channels in plant cells: a new model for osmoelastic coupling. *Plant Physiology and Biochemistry* **34**, 327–334.
- Remorini D, Massai R.** 2003. Comparison of water status indicators for young peach trees. *Irrigation Science* **22**, 39–46.
- Roderick ML.** 2001. Viewpoint: on the use of thermodynamic methods to describe water relations in plants and soil. *Australian Journal of Plant Physiology* **28**, 729–742.
- Roderick ML.** 2005. Plant-water relations and the fibre saturation point. *New Phytologist* **168**, 25–37.
- Schulte PJ.** 1992. The units of currency for plant water status. *Plant, Cell and Environment* **15**, 7–10.
- Simonneau T, Habib R.** 1991. The use of tree root suckers to estimate root water potential. *Plant, Cell and Environment* **14**, 585–591.
- Sinclair TR, Ludlow MM.** 1985. Who taught plants thermodynamics: the unfulfilled potential of plant water potential. *Australian Journal of Plant Physiology* **12**, 213–217.

- Sinclair TR, Purcell LC.** 2005. Is physiological perspective relevant in a 'genocentric' age? *Journal of Experimental Botany* **56**, 2777–2782.
- Slatyer RO.** 1967. *Plant–water relationships*. London: Academic Press.
- Slatyer RO, Taylor SA.** 1960. Terminology in plant–soil–water relationships. *Nature* **187**, 922–924.
- Sperry JS, Adler FR, Campbell GS, Comstock JP.** 1998. Limitation of plant water use by rhizosphere and xylem conductance: results from a model. *Plant, Cell and Environment* **21**, 347–359.
- Spomer LA.** 1985. Techniques for measuring plant water. *Hort-Science* **20**, 1021–1028.
- Sugano S, Kaminaka H, Rybka Z, Catala R, Salinas J, Matsui K, Ohme-Takagi M, Takatsuji H.** 2003. Stress-responsive zinc finger gene *zpt2-3* plays a role in drought tolerance in petunia. *The Plant Journal* **36**, 830–841.
- Tardieu F, Simonneau T.** 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *Journal of Experimental Botany* **49**, 419–432.
- Tomos AD, Leigh RA.** 1999. The pressure probe: a versatile tool in plant cell physiology. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 447–472.
- Townend J, Reeve MJ, Carter A.** 2001. Water release characteristics. In: Smith KA, Mullins C, eds. *Soil and environmental analysis: physical methods*, 2nd edn. New York, NY: Marcel Dekker, 95–140.
- Ursprung A, Blum G.** 1916. Über die verteilung des osmotischen wertes in der pflanze. *Berichte der Deutschen Botanischen Gesellschaft* **34**, 88–104.
- Wilkinson S, Davies WJ.** 2002. ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Plant, Cell and Environment* **25**, 195–210.
- Yamaguchi-Shinozaki K, Shinozaki K.** 1994. A novel *cis*-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *The Plant Cell* **6**, 251–264.
- Yue B, Xue W, Xiong L, Yu X, Cui K, Jin D, Xing Y, Zhang Q.** 2006. Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance. *Genetics* **172**, 1213–1228.