

# Plant Water Relations: Absorption, Transport and Control Mechanisms

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## 1. Introduction

Although water is abundant on Earth - covering 71% of the total surface - its distribution is not uniform and can easily cause restrictions in availability to vegetal production. At global scale, these restrictions are easily observed in dry climates and can appear in other regions which do not currently experience drought, as provided by the future backdrop of climate change (IPCC, 2007).

The influences of water restriction on losses in the production and distribution of vegetation on the terrestrial surface are significantly larger than all other losses combined which are caused by biotic and abiotic factors (Boyer, 1985). This striking effect of water on plants emerges from its physiological importance, being an essential factor for successful plant growth, involving photosynthesis and several other biochemical processes such as the synthesis of energetic composites and new tissue. Therefore, in order to characterise the growth and productive behaviour of plant species it is essential to have an understanding of plant water relations, as well as the consequences of an inadequate water supply. Broadly, the water state of a plant is controlled by relative rates of loss and absorption, moreover it depends on the ability to adjust and keep an adequate water status. This will be considered throughout this chapter.

## 2. Absorption and water flow through plants

Independent of the species, plants require from the soil a water volume that overcomes its metabolic necessities. Through the transpiration process plants transmit to the atmosphere the majority of the water absorbed from soil (generally around 90%). From this perspective, it is noted that the plant water requirements are defined primarily by the atmosphere evapotranspirative demand, which is a predominately passive process. Figuratively, and with some caveats, we can compare a plant water flow with the principles of oil flow in the wick of an old fashion lampion (Fig. 1).

When it is fired, the oil that is burned on the upper extremity of the wick is quickly replaced by new one that is situated just below, and so on - following the physical forces of interaction between liquid and tissue - until reaching the level of the fuel reservoir, in the basal extremity of the wick. Applying this example to the plant, the burning of oil can be analogous to the

process of the loss of water vapour through their leaves, i.e. the *transpiration*, which is caused by the pressure gradient of vapour between tissue saturated with water from the leaves and air, the “dry” atmosphere. The variations in this pressure gradient of the vapour will define the *evaporative demand* of the environment where the plant is. In the other extreme - where it represents the liquid reservoir of the lampion - we have water content present in the soil. In this scene, it is noted that the water flow through the plant is dependent on the energy formed by the gradient of the water content that is established between the soil and the atmosphere. However, we will see throughout this chapter that plants, unlike our lampion, can and must modulate this gradient in order to survive the wide variations of water availability between types of soils, weather and seasons.

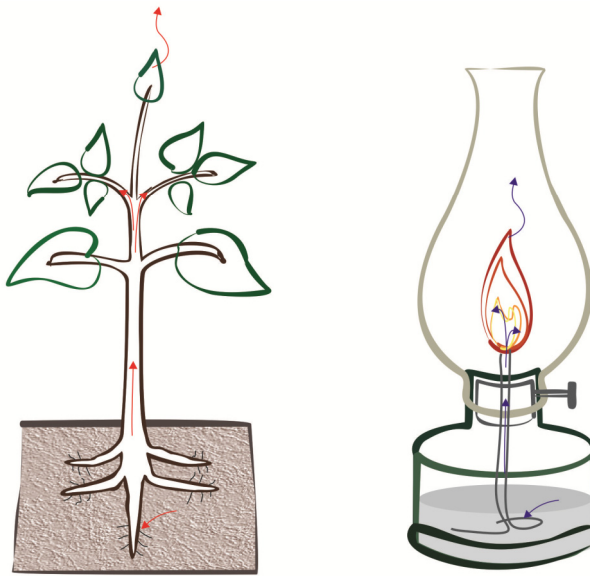


Fig. 1. Schematic representation of water flow through the plant (arrows), by analogy with the oil flow through the wick of an old fashion lampion.

## 2.1 Water potential

The water content in the soil, plants and atmosphere is usually described as *water potential* ( $\Psi_w$ ). This is based on the relation between the water content in the part of a system and pure water at the same temperature and atmospheric pressure, measured in pressure units (megapascal-MPa or bars-Bar). By definition, the potential of free pure water at atmospheric pressure and at a temperature of 25°C corresponds to 0 (zero) MPa. The contrast in the water potential between two points invariably determines the direction of water transport in a system. More precisely, the water potential represents all the water pressure in a given system and it is the sum of osmotic potential ( $\Psi_{\pi}$ ), matrix potential ( $\Psi_m$ ), hydrostatic pressure or the turgor potential ( $\Psi_p$ ) and the gravitational potential ( $\Psi_g$ ).

The osmotic potential ( $\Psi_{\pi}$ ) is the chemical potential of water in a solution due to the presence of dissolved substances (solutes). This is always negative because the water moves

from one point with a lower concentration of solutes (for example, pure water) to a point with a higher concentration. So, the higher concentration of the solutes at a point which makes the system more negative will be the osmotic potential in this place. The water potential can also be influenced by a charged surface - mainly by soil components and cell walls - which compose the influence of the matrix potential ( $\Psi_m$ ). In the soil, this influence of the matrix is so great that water potential is assumed negligible and therefore equivalent to the matrix potential. Concerning the potential of hydrostatic pressure ( $\Psi_p$ ), it is noted that this component of the water potential can be positive or negative and it refers to the physical pressure that water exerts on a given system. For example, if we observe a turgid cell of a root cortex or a leaf mesophyll, the hydrostatic pressure is positive. However, in a xylem vessel subjected to a stressful condition - in a transpiring plant - this component of hydrostatic pressure is negative. Finally, we should emphasise that the gravitational potential ( $\Psi_g$ ) - ignored in most cases - is very important in studies of the water potential of tree species, where plant height exerts a great influence on water flow. Considering that this gravitational component fluctuates at a rate of 0.1 MPa for every 10 meters of vertical displacement, it is suggested to consider if when plant height is 10 m or more.

## 2.2 Water dynamics in soil-plant-atmosphere system

From these components of water potential we return to our lampion scheme (Fig. 1) and show how the potential can vary over the continuum soil-plant-atmosphere, exposing the control points of each step of water flow from the soil to the atmosphere.

### 2.2.1 Soil water

The water potential in soil affects water reservoir and its availability for plants, hence it has a large impact on plant growth and production. Furthermore, the soil water content exerts a great influence on some physical and chemical properties of soil, such as the oxygen content, which interferes with root breathing, microbial activity and soil chemical status. Water potential is directly dependent on soil physical characteristics, and varies with time and space, depending on soil water balance. That balance is determined by input (rain, irrigation) and output of the soil (drainage, evaporation and root absorption). It is noteworthy that the amount of rain affecting soil water reservoir is only the *effective precipitation*. This is the amount of precipitation that is actually added and stored in the soil. For example, during drier periods less than 5 mm of daily rainfall would not be considered effective, as this amount of precipitation would likely evaporate from the surface before soaking into the ground.

It is important to emphasise that behaviour of water into soil differs from that in a pot, like the oil in the lampion reservoir (Fig. 1). That is, soil water interacts with the matrix and solutes, and it is under pressure or tension, resulting in various energy states, relative to free water (Kirkham, 2005). With regard to the physiological aspect, it is important to point out that the water content in soil is associated with three terms: *field capacity*, the *permanent wilting point* and the *available water content*.

The term "field capacity" corresponds to the maximum water content that a given soil can retain by capillarity, after saturation and gravity drainage, and it is conventionally estimated as the water content when the matrix potential is -0.03 MPa (-0.3 Bar). In spite of the great applicability of this term to irrigation management, field capacity has been recognized as an

imprecise term due to theoretical advances and precise irrigation techniques. It is because the capillary soil water constantly (even slowly) decreases (due to evaporation from soil surface or drainage losses) and never stabilises (Fig. 2), it turns the soil water potential decreases while the matrix potential increases. This is most evident with medium and fine texture soils (for example, those rich in clay and organic matter), which maintain a significant drainage rate over a long time. Therefore, there is no real and unique value for accurately characterising the field capacity of a given soil. Furthermore, the continuous drainage can induce an overestimation of the water consumption of the plant. Despite these uncertainties, the term *field capacity* is still useful for a qualitative understanding - rather than a quantitative understanding - of the water behaviour of a particular soil, providing an estimate of the maximum limit of water accumulation. It is noteworthy that the inaccuracy of the field capacity determination occurs mainly when analysis takes place on samples in the laboratory, which can be countered with evaluations directly in the soil, with specific sensors and considering together all characteristics of each site. In general, clay soils or those with higher content of organic matter (upper to 5% of organic matter) present a higher soil water holding capacity (average field capacity ranging from 35 to 40% vol). In contrast, sandy soils have a lower water holding capacity and field capacity typically ranges from 10-15% vol. It is important to observe that *field capacity* cannot be regarded as a maximum limit of the water available to plants, due to the fact that plants also use free water that is in contact with the roots at the moment of soil drainage.

The *wilting point* (WP) is another important parameter in soil water dynamics as it dramatically affects plant physiology. This term is also known as the *permanent wilting point*, and can be defined as the amount of water per unit weight (or volume) of soil that is so tightly retained by the soil matrix that roots are unable to absorb causing the wilting of plant. In other words, it corresponds to the water potential of soil under which plants cannot maintain turgor pressure, even if a series of defence mechanisms have been triggered (e.g. increased ABA synthesis, stomatal closure, osmotic adjustment, leaf fall) (For more details see the Chapter by Mastrangelo et al.).

Similarly with FC, the value of water content in a soil at WP is not a unique and precise value despite it is conventionally measured at -1.5 MPa (-15 Bar) (Fig. 2). The WP is influenced by the physical and chemical characteristics of soil, but also by the plant species considered. This is because various plant species differ in their ability to deal with low soil water content due to differences in roots anatomy and depth, osmotic adjustment capacity and other defence drought mechanisms.

Conventionally, the *wilting point* is estimated as the water content when the matrix potential of the soil is -1.5 MPa (-15 bar). Nevertheless, some species of plants can absorb water from soil at a potential much smaller than this limit. For example olive trees can set a water potential gradient between dry soil (-3 MPa) and leaf (-7 MPa) (Dichio et al., 2006). Similarly, *Larrea divaricata* may absorb water at -6.0 MPa soil water potential (Kirkham, 2005). Another species of the same genus of desert plant (*Larrea tridentata*) can survive with soil water potentials up to -11.5 MPa, maintaining the photosynthetic activity of leaves within the range between -5 and -8 MPa (Fitter & Hay, 2002). These examples serve to explain that the permanent wilting point does not exclusively depend on the soil but also on the plant species. At the *permanent wilting point*, the water potential of soil tends to be less than or equal to the osmotic potential of the plant, which is extremely low in plants adapted to dry environments.

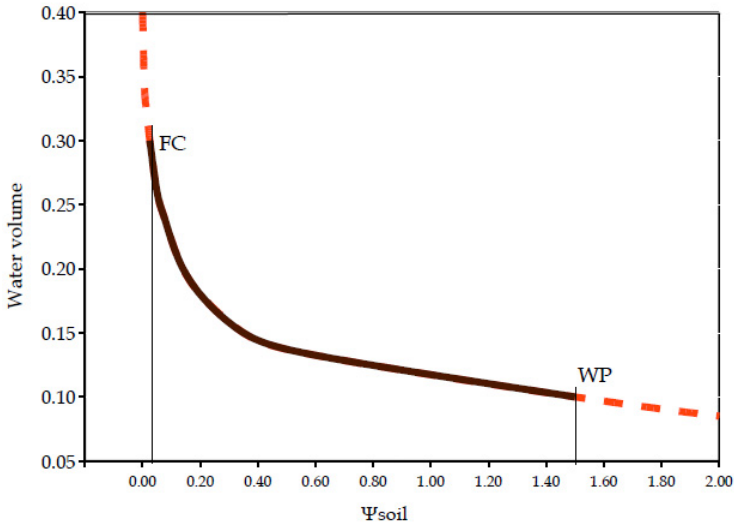


Fig. 2. Variation of the matrix of the water potential of soil ( $\Psi_{\text{soil}}$ , negative values) in relation to water volume ( $\text{cm}^3.\text{cm}^{-3}$  of soil), characterising the limits of the field capacity (FC,  $-0.03$  MPa) and wilting point (WP,  $-1.5$  MPa) of a given soil. The curve was generated from results obtained by Santos, H.P., in Bento Gonçalves-RS, Brazil. 2010.

The indiscriminate use of a fixed value to estimate *field capacity* (FC) and the *permanent wilting point* (WP) can generate false interpretations. However, this reference to the water content in the soil is essential for calculating the *available water content* (AWC) for the plants. The AWC is calculated considering the soil volume explored by roots and the % of water content determined as the difference between FC and WP. Due to this interval of water availability, one may assume that water could be absorbed by the roots with the same facility in the range between FC and WP. For some plants this may be true, given that the energy to extract water from the soil is small, compared to the energy needed to transport the water from the root system to the atmosphere. However, with the reduction of soil water potential, there is also a reduction in its hydraulic conductivity (i.e. water moves slowly in the soil), limiting the water absorption capacity of the roots. In this scene - and for a majority of crops - the yields are reduced if the water content in the soil approaches the *wilting point*. Thus, the available water content should be considered as a relative value and, for the same soil water potential, it may have different proportions of accessibility, depending on the ability of each species to exploit or capture available water.

### 2.2.2 Water absorption by the roots

As was pointed out in Figure 1, the water flow of a plant is primarily controlled by the transpiration rate. In this flow system it is essential indeed that there are no limitations on water absorption by the root system. As the roots absorb water, there is a reduction in the water potential in the soil that is in contact with the roots (rhizosphere). This process establishes a water potential gradient between the rhizosphere and a neighbouring region of the soil which presents a higher water potential and which coordinates the water movement

towards the roots of a transpiring plant (Fig. 3). This water movement in the soil occurs mainly through mass flow due to the fact that the water filled micropores of the soil are interconnected. Therefore, water flows from soil to root at a rate depending on the water potential gradient between soil and plant which is affected by plant water need, hydraulic conductivity of the soil, soil type and soil water content. Sandy soils have higher conductivity due to greater porosity, but they also retain less water in relation to clay soils or soils rich in organic matter.

At *field capacity*, water is initially removed from the centre of the largest pores (spaces  $\geq 50$  nm, that are too large to have any significant capillary force) between the soil particles, maintaining the water next to the particles due to adhesive forces. The reduction in water content causes a drastic decrease in soil hydraulic conductivity, because the water is replaced by air in the spaces between the soil particles (Fig. 3). Thus, the water movement in the soil is limited to the periphery of soil pores, which can promote restrictions in the hydraulic conductivity to the root surface and reach the *permanent wilting point* (discussed previously).

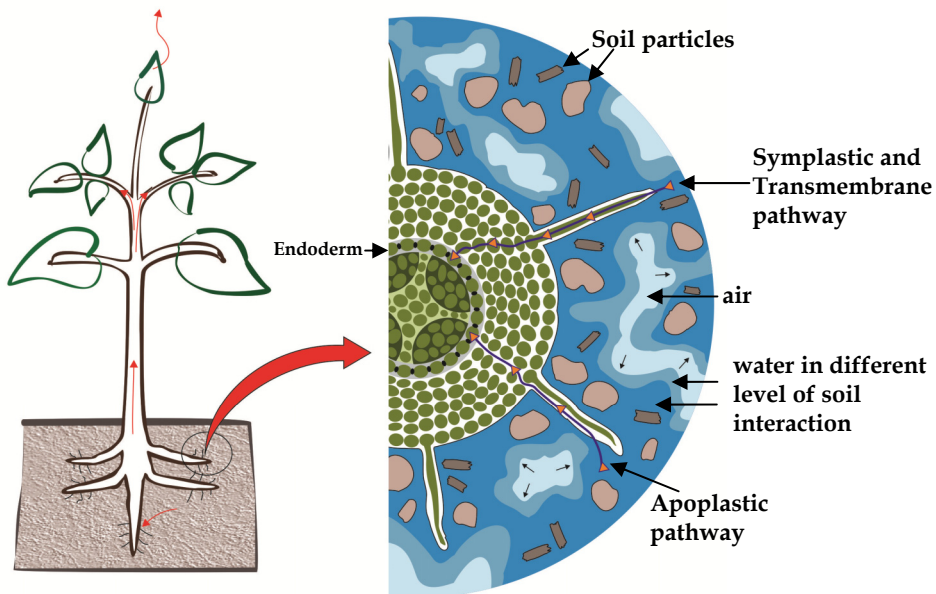


Fig. 3. Detail of the rhizosphere. Note the water adsorbed in soil particles. As the water is absorbed by the roots, the open spaces filled with air increase (small arrows). During absorption, water can flow by symplastic, apoplastic and transmembrane pathways (detailed in the text) to the endoderm cells, where the Casparian strip is present (represented by black points between the endoderm cells).

The water absorption by the roots is related to its surface directly in contact with soil. Thus, longer and younger (less suberised) roots with more root hairs are essential for increasing the contact surface and improve the water absorption capacity of the soil (Fig. 3). Moreover,

the distribution and proportion of the roots is very important for meeting the water demand of a plant. In humid regions, as tropical rain forest, plants usually do not require very extensive root systems (i.e. root:shoot ratio  $< 0.15$ , Abdala et al., 1998), because a small volume of soil can meet the demands of transpiration. In addition, the water absorbed from that small soil volume is frequently (and easily) replenished by rainfall. This condition in turn induces a reduction of the root:shoot ratio. On the other hand, in dry regions, the plants invest more in their roots, increasing the root:shoot ratio such that the roots can represent up to 90% of a plant biomass in some species of a desert climate, such as observed in some species from open areas of the Bana woodland in southern Venezuela (i.e. root:shoot  $> 5$ , Bongers et al., 1985) and from savanna in Brazil (Abdala et al., 1998). It is important to note that the use of this root:shoot relation in the classification of plants with respect to their *habitat* must be made with caution. In many species, a higher investment in roots is more related to the accumulation of reserves and not specifically to an increased root surface for water absorption (e.g. *Manihot spp.*). A higher investment in roots can also support a process called *hydraulic lift*, when the roots translocate the water from the soil positions with a greater water potential (for example, deeper) to soil positions with a less negative water potential. This process promotes a *hydraulic redistribution* (Burgess & Bleby, 2006) in the soil independently from plant transpiration, because it occurs when the stomata are closed (e.g. at night in  $C_3$  and  $C_4$  plants, and during the day in CAM - Crassulacean Acid Metabolism - plants).

With the water reaching the roots, the absorption process is directly dependent on the water potential gradient between the rhizosphere and the root xylem. There are two ways to establish this gradient, characterised by two absorption processes: 1) *osmotically driven absorption*, common in plants with low transpiration activity; and 2) *passive absorption*, which dominates in plants with high transpiration activity. The osmotically driven absorption occurs in plants under conditions of heat and non-limiting water availability in the soil, but with a restricted capacity of transpiration (for example, without leaves or with a limited vapour pressure deficit). In these cases, there is an accumulation of solutes in xylem vessels (for example, sucrose by degradation of starch reserves in the roots), reducing the xylem water potential in relation to the soil water potential (Kramer & Boyer, 1995). This condition results in water absorption and an increase in root pressure, which is itself responsible for the guttation that means the leaf water output through the hydathodes (pores located at the margins of the leaves) (Fig. 4). Moreover, increase in root pressure also promotes the water exudation in lesions of branches, easily observed in some species (e.g. exudation on branches of grape and kiwifruit plants after pruning in early spring).

In passive absorption and with an increasing rate of transpiration, the tension in the xylem vessels increases, indicating a predominance of the pressure potential influence upon the osmotic potential through the establishment of the water potential gradient between the root xylem and the rhizosphere. Under these conditions, the roots become a passive absorption organ, where the water is sucked into a mass flow promoted by the transpiration activity of aerial parts of the plant. A grapevine, for example, which during its annual growth and production cycle transpires between 650 to 900 mm of water, in accordance to environmental conditions where it is growing, and this volume corresponds to about 85% of all its absorbed water (Mullins et al., 1992).



Fig. 4. Detail of a leaf of a wheat plant (*Triticum aestivum* L.) presenting guttation in the morning. Photo: Ana Cláudia Pedersen.

The water intake in the roots can follow three ways into the root tissue in relation to the route of the epidermis to the endoderm of the root, called *radial water transport* (Fig. 3): 1) *apoplastic*, where the water moves through the intercellular spaces and does not pass through any membranes, exclusively occupying the continuous network of the cell walls; 2) *symplastic*, where the water moves exclusively from one cell to another through plasmodesmata connections; and 3) *transmembrane*, which corresponds to a mixed path between the first two, where the water goes in one direction through the root tissue, entering (symplastic) and exiting (apoplastic) cells. The relative importance of these pathways is still a cause of much discussion, but there is some evidence for the suggestion that plants displaying low transpiration activity predominantly witness symplastic transport, while those displaying high transpiration activity witness a greater proportion of apoplastic transport (Boyer, 1985; Steudle, 2001). Another important detail in relation to these different pathways is relevant only in the outer layers of the root tissue, because in the endoderm the water apoplastic flow is limited due to the Casparian strip (Fig. 3). In this hydrophobic barrier, the radial and transverse endodermal cell walls are impregnated with lignin, suberin, structural wall proteins and wax. Note that in many plants this barrier also occurs in the epidermal cells, forming a double layered hydrophobic barrier in the roots (Enstone et al., 2003). It is important to note that the Casparian strip does not always establish a barrier that is totally impermeable to water and solutes coming from the soil. This can be observed in - for example - the development of young roots where pericycle growth can break parts of the endoderm and allow free access to water until the reconstitution of the tissue.

With regard to water absorption control in the roots, plants also present a family of membrane water transporter proteins (water-channel proteins), called *aquaporins*. These proteins have a critical role in water absorption, reducing the resistance to the water flow along the transcellular path. The number of these proteins available for the root surface is variable throughout the day, being higher during the photoperiod due to the higher



demands of photo-transpiration. The *aquaporins* are controlled by many **endogenous and exogenous** factors of the roots, such as pathogens, phosphorylation, pH, solute gradient, temperature and all environment factors that interfere in hydraulic conductance along the water flow by the plant (Chaumont et al., 2005; Maurel et al., 2008).

### 2.2.3 Ascension of water through the plant: Vascular system

The presence of plants outside the water environment - among other factors - has been related to the evolution of the vascular system, which allows for the speedy upward movement of water to meet the demand of transpiration from the leaves. Water supply through cells by diffusion (difference in chemical gradient) alone is not able to maintain the hydration of a perspiring canopy plant. The need for a vascular system is more evident when we observe the hydraulic dynamic of a tree during a hot day, which demands a large flow of water (for example, 200 to 400 liters day<sup>-1</sup>) to fit a transpiring surface that is situated along elevated positions, and in some species is higher than 100 meters (e.g. *Sequoiadendron gigantea*).

The water flows from the roots to the shoot of the plant through the xylem. The general mechanism to explain this upward movement of water is the *cohesion-tension theory*, which was proposed in the late 19th century. Basically, this theory holds that the water evaporated in leaves establishes a tensile strength in the xylem, where the hydrogen bonds provide a continuous intermolecular attraction (cohesion) between the water molecules from the leaf to the root (Fig. 1). Thus, the water column in the xylem lumen is driven out of a region with a higher water potential, i.e. from the root and the stem, to a region with a lower water potential, as the leaves, and finally toward to the air that can reach very low water potential (e.g. -100 MPa, at 50% of air relative humidity).

Recently, the cohesion theory has been questioned as a result of assessments of tension in xylem vessels, which do not present a direct relation with tension values measured on leaves through pressure chambers. Furthermore, it is assumed that the hydrophobic interaction between the internal walls of the xylem and the sap composition (lipids, proteins, polysaccharides etc.) prevents the development of a tensile strength larger than 1 MPa (Zimmerman et al., 2004), which is smaller than the estimated tension of rising water in a 30 m high tree (3 MPa). However, despite these questions, many studies argue that the fundamentals of the *cohesion-tension theory* are still valid for explaining the water flow in the continuous soil-plant-atmosphere (Richter, 2001; Steudle, 2001; Cochard, 2002; Tyree, 2003). These elements support the idea that the water column of leaves to the roots provides an auto-regulation mechanism between the process of loss and absorption of water by the plants. Therefore, although the importance of the *cohesion-tension theory* has been neglected by some critics, this mechanism is considered to be essential for the survival of plants during the transpiration process, i.e. the loss of water.

With rising water in the trunk, in addition to pressure force, there is also capillarity strength in the vessels. In a perspiring plant, the water moves continuously from the xylem bundles to the intercellular spaces in the leaves, where the water potential is lower. Due to capillarity strength, water which evaporates through leaf stomata is replaced by the water contained in the lumen of the vascular bundles. In physiological temperatures (25°C), the cohesive forces between the water molecules are sufficient to prevent the

disruption of the water column. This tension and the capillarity forces present in the vascular bundles also present resistance to the water flow along the plant by two major ways: 1) the inherent properties of the xylem flow and 2) the geometric aspects of the xylem conduits (*vessel elements* and *tracheids*). In this respect, it is notable that plants with the *vessel elements* of xylem can present a significantly lower hydraulic resistance than plants with *tracheids* (Tyree & Zimmermann, 2002). As such, the xylem diameter has a great influence on the hydraulic conductivity or water flow ( $J_v$ , mm s<sup>-1</sup>), according to the Hagen-Poiseuille equation which describes the transport of fluids in ideal capillaries:

$$J_v = (\pi R^4 \Delta \Psi) / 8 \eta L \quad (1)$$

In this equation,  $\Delta \Psi$  is the difference in the water potential (MPa) between two points of observation throughout the capillary,  $R$  is the radius of the capillary (mm) with a determined length  $L$  (mm), through which occurs a flow with a constant viscosity  $\eta$  (1,002 x 10<sup>-3</sup> Pa second<sup>-1</sup>, at 20°C) (Nobel, 2009). Accordingly, this equation shows that  $J_v$  is proportional to the fourth power of the vessel conductor diameter (the xylem). According to this logic, a trunk that presents few xylem vessels with large diameter has a greater  $J_v$  than a trunk with the same xylem area distributed in a larger number of vessels of smaller diameter. In general, xylem vessels with larger diameter are also longer. Xylem vessel diameter is also variable throughout the growth season, being larger in those vessels formed early in the growing cycle. This makes growth rings visible in transversal cuts of tree species. The vessels diameter is an important factor in preventing cavitation or embolism (formation of air bubbles by breaking the water column under high tensile strength, such as values close to -30 MPa), with thinner vessels being less susceptible to such a water column breakage. Generally, the cross-sectional area of vascular bundles is proportional to the transpiring leaf surface. This may be observed in plants adapted to arid environments, which have thinner vessels and a small transpiring surface, as reduced root:shoot ratio.

#### 2.2.4 Leaf water and transpiration

Returning to our lampion scheme (Fig. 1), we emphasise that the leaves are the final frontier of the water flow in the continuous soil-plant-atmosphere system. In leaf mesophyll there is an extensive system of intercellular spaces - present in cell walls - which correspond to the internal surface of water contact with the air. By this interface between the cell walls and the intercellular spaces is established a water potential gradient, mobilising the water by the cell walls from the final extremities of the xylem bundles. This water flow by the cell walls occurs in an analogous way to the principles of water movement through the soil matrix, and the water interacts with cellulose microfibrils and other hydrophilic components of the cell wall (Fig. 5). Due to the high surface tension and as a result of water evaporation in the surface of the cell walls which are in contact with the air in the intercellular spaces, it is established that the tensile strength is transmitted to the xylem. Therefore, it is the tensile strength that drives the upward flow of the water column from the root and is produced in the internal evaporation process in the leaves. The maintenance of this process - as a result - depends on the output (in the atmosphere) of water vapour present in the intercellular spaces. As the leaf cuticle represents a barrier to water outlet - allowing on average only 5% of water permeability - the water vapour moves from leaf intercellular spaces to the atmosphere predominantly through stomatal diffusion. This process of the loss of water

vapour by the leaves is called *transpiration* and corresponds to the majority (90%) of the volume of water absorbed by plants.

Transpiration has a number of positive effects (e.g. helps with mineral transport and leaf cooling) however it also may contribute to induce water stress when soil dry.

In the *continuum* soil-plant-atmosphere of water flow, there are two major factors determining the water potential of a plant: 1) the water potential of the soil, which characterises the water supply; and 2) transpiration, which defines the loss of water. The plant, which is an intermediate in this process, may regulate the water potential gradient between the soil and the atmosphere primarily through the regulation of stomatal conductance.

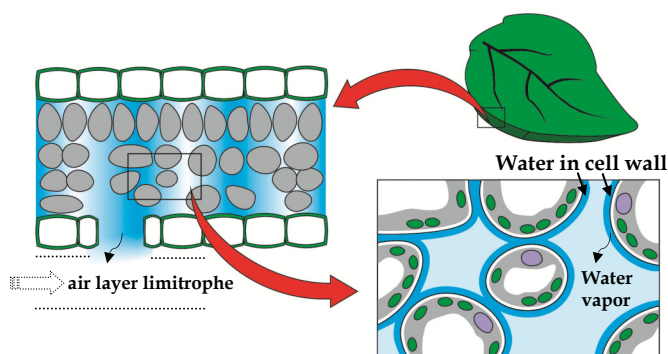


Fig. 5. Leaf water. The evaporation from the cell walls of mesophyll should be noted. The water vapour escapes through the stomatal opening and this flow is directly influenced by the boundary layer of air.

The stomata have a quick and fine control of the water relations of a plant, coordinating the control of the water potential gradient between the leaf and the air. In this interface of the leaf with the environment, it is important to note that small changes in the relative humidity of the air are reflected in major changes in the water potential gradient, which requires a stomatal control so as to maintain the water stability of the plant. A simple variation from 100% to 99% of the relative humidity already corresponds to a decrease of -1.36 MPa in the water potential of the air. This decrease becomes more evident in the water potential of the air in average (80%) and in extreme (50%) conditions of relative humidity, which respectively provide values of -14 and -93.6 MPa at 20°C (Nobel, 2009). If we compare the water potentials of the air with the average water potential of a mesophyte plant (-0.5 MPa), the high gradient always determines that the water is diffused from the leaves to the air (Fig. 4). Throughout this water route between the leaf and the air there are two components that can exert resistance to the diffusion process: 1) *stomatal resistance*, which is coordinated by the stomatal opening; and 2) *resistance of the air boundary layer*, which is located closest to the leaf surface (Fig. 5) and it is directly influenced by wind speed. The higher the speed of the wind, the greater is the frequency of air renewal in this layer surrounding the leaf,

restricting diffusion resistance for the maintenance of a major gradient of the water potential. Morphological and anatomical variations among leaves can interfere with the speed of displacement of this thin layer of air, restricting the rate of transpiration in dry environments. Among these modifications, we highlight the presence of hair, the stomata located at the lower surface of the leaf, and the shape and size of the leaves. Although these changes interfere directly with the transpiration rate, they do not exercise a variable and instantaneous control in relation to the ambient conditions, as is done by the stomata.

During the day, great changes occur in the water potential along the soil-plant-atmosphere system. Initially, let us consider a mesophyte plant in a constant atmospheric condition of 75% relative humidity at 20°C (-39 MPa of air water potential) and soil without water restriction (at *field capacity*). During the nocturnal period, the transpiration is virtually nil, by stomatal closure, promoting an equilibrium between soil, root and leaf water potentials leading the potential gradient to be near zero (Fig. 6). With the first rays of the sun, at dawn, the stomata opens, allowing the water diffusion of the leaf (transpiration) and, as a consequence, reducing the leaf water potential.

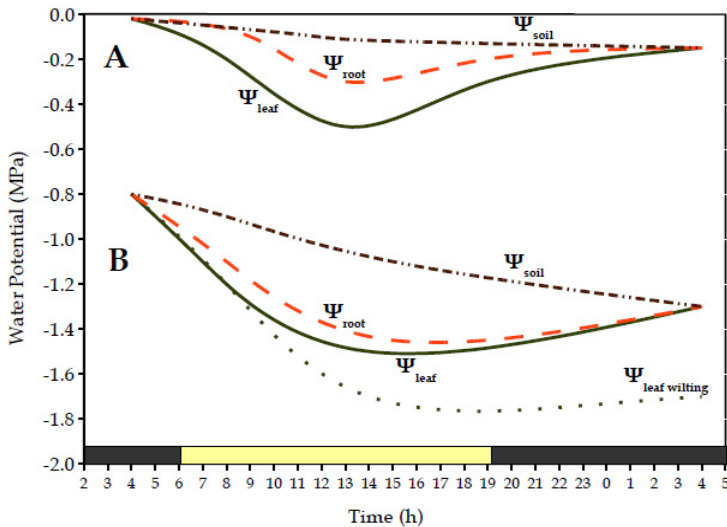


Fig. 6. Schematic daily variation of soil ( $\Psi_{\text{soil}}$ ), root ( $\Psi_{\text{root}}$ ) and leaf ( $\Psi_{\text{leaf}}$ ) water potential of a plant well hydrated (A) and under water restriction (B).

When leaf water potential does not equilibrate with  $\Psi_{\text{soil}}$  at the end of day (case B), the permanent wilting point ( $\Psi_{\text{leaf wilting}}$ ) is reached.

As a result - and with a delay that depends on the cohesion-tension forces of water and the water column size between the leaf and root - it begins the reduction of the root water potential. This reduction is slight (on average -0.3 MPa), due to high water availability of the soil. The reduction of the water potential in the plant reaches the minimum limits during the hottest times of the day, forcing the stomata to close for small intervals for extreme cases of transpiration demand (Fig. 6A). At dusk, these variations of the water potential in the leaf and the root are reversed.

In considering a plant under the same atmospheric conditions, but with severe water restriction (a soil water potential near to the permanent wilting point), the water potentials of the leaf and root necessarily reach values that are more negative for the sustainability of the water flow by the plant. At high limits of negative potential, the differences among the water potential of the leaf and root are smaller, but with great difference in relation to the soil water potential (Fig. 6B). If this condition persists, there will be a decrease in the turgor pressure of the leaves, causing temporary leaf wilting, which is recomposed during the nocturnal period. Mesophytic plants can tolerate this reduction in soil moisture up to the limit of -1.5 MPa, while some xerophyte plants can reach limits of -5.5 MPa (Nobel, 2009).

### 3. Physiological and biochemical aspects of water in plants

In a plant cell, the water is predominantly located in the vacuole and represents the majority of the mass of growing tissue (on average 90%). This predominance is due to the importance that this universal solvent represents in the physiological and biochemical processes of a plant cell. At the cellular level - due to its polar structure - the water acts on the dissolution and mobilisation of ions and organic metabolites, such as amino acids, proteins, carbohydrates and hormones. These water properties are also crucial for the flow between parts of the plant, acting directly in the transportation of nutrients, carbohydrates and hormones. The water acts in membrane integrity and the support of herbaceous plants through the cells turgor pressure, which varies between 1 and 5 MPa. This turgor pressure is also essential in the expansion process of cell walls and in growing tissues. This subsection will expose some physiological and biochemical aspects of the water relations in plants.

#### 3.1 Water deficit and its effects on plant growth

When a plant is under water deficit, responses at physiological, biochemical and molecular scale are triggered (Shao et al., 2008). Physiological responses are linked to a condition of recognition of stress by the root system, turgor changes and water potential and, consequently stomatal conductance, internal CO<sub>2</sub> concentration and photosynthetic activity decrease. In biochemical terms, there will be a decrease in the photochemical activity of photosynthesis, rubisco enzyme activity and the accumulation of secondary metabolites linked to stress (such as glutathione and polyamines). From a molecular perspective, several genes expressed under stress conditions are activated, such as genes linked to the biosynthesis of abscisic acid and the synthesis of specific proteins.

As the cells hydration is reduced and the plant goes into a condition of water deficit, abscisic acid and solutes increase in the plant, especially in the root system (the increase in solutes occurs in a relative manner, due to water reduction). These factors will reduce stomatal conductance and, consequently, photosynthetic activity which ultimately will result in a reduction in the synthesis of proteins and cell walls, as well as a decrease in the rate of cell expansion (Taiz & Zeiger, 2004). The sum of these responses to water deficit contribute to explain the reduction of plant growth.

#### 3.2 Elements that define water demand

Basically, according the cohesion-tension theory already described, the water demand of plants is generated by a gradient at the top of the plant which generates negative hydrostatic

pressure that “suck” soil water through the plant into the atmosphere. Two main environmental factors will determine this *evaporative demand*: wind speed and solar radiation (Chavarria et al., 2009). Thus, those plants that exist in environments with high winds (e.g. 20 km h<sup>-1</sup> or more) or intense solar radiation (e.g. 2500 μE m<sup>-2</sup> s<sup>-1</sup> of photosynthetically active radiation) will suffer a greater water loss to the atmosphere. These plants need to make use of water control mechanisms in order to tolerate these environmental conditions. Plants can suffer morphological and anatomical alterations and osmotic regulation to improve stomatal resistance and increase water absorption through the root system.

### 3.3 Mechanisms of water status regulation

#### 3.3.1 Morphological and anatomical characteristics associated with water control

Plants live with a constant dilemma, namely to undergo photosynthesis while preventing water loss. Every time plants open their stomata to allow the influx of carbon dioxide, they lose water by diffusion.

When plants achieved their “*terrestrial status*” and left behind the conditions of *algae*, they required several changes to adapt to this new environment. They acquired a root system for physical support and absorption of water and nutrients, vascularity for the movement of water and photoassimilates, and a stomatal complex and a wax layer for the regulation of water loss to the atmosphere.

It is known that intense solar radiation is accompanied by elevated temperatures, which can cause severe physical damage to leaves and lead to senescence and leaf abscission or, on small scale, reduce carbon assimilation. This situation forces plants to adopt strategies to minimise such negative effects.

In order to adjust the balance between water availability and atmospheric demand, plants can reduce the size and number of leaves. The organisation of the mesophyll and leaf dimensions is modified by water restrictions, and can provide a strategy for plants to affect the stomatal conductance and CO<sub>2</sub> diffusion (Evans et al., 2009). Plants which need greater efficiency in water use (μmol CO<sub>2</sub> fixed / H<sub>2</sub>O transpired) have a strategic demand to retain higher CO<sub>2</sub> concentrations inside. Characteristics of leaf anatomy related to water deficit are reduction of thickness, higher cell density and smaller intercellular spaces, all of which try to mitigate the problems of excessive water loss (Chartzoulakis et al., 2002). The reduction in cell size and, consequently, the reduction of tissues are associated with the turgor decrease of cells (Ogbonnaya et al., 1998). However, smaller cells may stay more *turgid* when compared to larger cells, having better capacity to tolerate conditions of water restriction (Burghardt et al., 2008). These changes in cell size result in an increased internal surface to of CO<sub>2</sub> exchange per leaf area, seeking to maintain the photosynthetic rate with reduced stomatal conductance (Ennajeh et al., 2010; Syvertsen et al., 1995).

The relation of the root with shoots is also strongly modified due to conditions of water unavailability. One way that plants use to keep their water status stable is by reducing the growth of the shoot, in order to reduce leaf area and water loss to the atmosphere. At the same time, plants can invest in the growth of the root system, in order to increase the soil volume explored and in turn water absorption. All these mechanisms occur through hormonal changes as initiated by abscisic acid signals. Nevertheless, later on it will be the

effects of interactions between auxins, cytokines and gibberellins which define the relation between root and shoot.

Anatomically, the change in vascular diameter may be a response to water deficit conditions and tends to decrease under water deficit (Kutlu et al., 2009). By reducing the radius of vessels, xylem conductivity is reduced (increased resistance) according to equation 1. There are some situations of high *evaporative demand* which increase the tension in the xylem vessels, causing a disruption of the water column and the formation of air bubbles by embolism (Tyree & Sperry, 1989). The disruption of the water column can also occur under conditions where the water freezes inside the plant.

Another mechanism which protects the leaf from attack by insects and helps the plant to avoid water loss is the presence of leaf trichomes (Molina-Montenegro et al., 2006). Trichomes reduce water loss by: 1) reducing the arrival of solar radiation on the leaf surface; and 2) reducing the interference of wind on the boundary layer, which reduces the differences in water potential between the leaf and the atmosphere (Ehleringer, 1984; Vogelmann, 1993).

Leaf cuticle presents variations in anatomical position and chemical composition. Chemically, these cuticles are characterised by two specific groups of lipids: 1) cutin, which forms the support structure of the membrane; and 2) waxes deposited on the external surface - called *epicuticular waxes* - and also strongly dispersed within the matrix of the cutin (below the surface) called *cuticular waxes* (Devine et al., 1993). The most important function of epicuticular waxes is to avoid leaf water loss to the environment. In addition, they reduce leaf nutrient loss, prevent excessive solar radiation, pathogenic microorganisms, cooling, wind damage and physical abrasion (Vigh et al., 1981; Mendgen, 1996; Barnes & Cardoso-Vilhena, 1996; Scherbatskoy & Tyree, 1990; Kerstiens, 1996; Eglinton & Hamilton, 1967). Epicuticular waxes may be amorphous, have a flat format covering the entire surface area of the leaf, or have the shape of a crystal or blade. Involved in the formation of these waxes are alkanes, esters, ketones and alcohols (Shepherd et al., 1995).

An important aspect of plant morphology is the density of leaf veins. Angiosperms average 8 mm of vein per mm<sup>2</sup> of leaf area, while non-angiosperms have consistently averaged close to 2 mm mm<sup>-2</sup> throughout 380 million years of evolution (Boyce et al., 2009). This was an important ecological strategy for the increment of photosynthesis, especially under conditions of higher temperatures and transpiration rates.

The most important anatomical tools to optimise the plant water use is the stomatal complex. Variations in opening, size and position of the stomata in the leaf help plants grow under conditions of water deficit (Larcher, 1995).

### 3.3.2 Stomata metabolism

Stomatal complexes are present in green stems, flowers and fruits, averaging 30 to 400 per square millimetre. They are formed by an opening, called a *pore*; two *guard cells* that are responsible for opening and closing the pore; and in some situations neighbouring cells called *subsidiary cells*, whose function is to support the guard cells. The stomata liberate into the atmosphere around 95% of the water that exits from the leaves and causes 90% of gas exchange (in this case, also involving carbon dioxide), while only 5% of the water output is

through the cuticle. There are two types of stomata morphology: kidney-shaped and grass-like, sized between 5 and 15  $\mu\text{m}$  wide and 20  $\mu\text{m}$  long (Fig. 7).

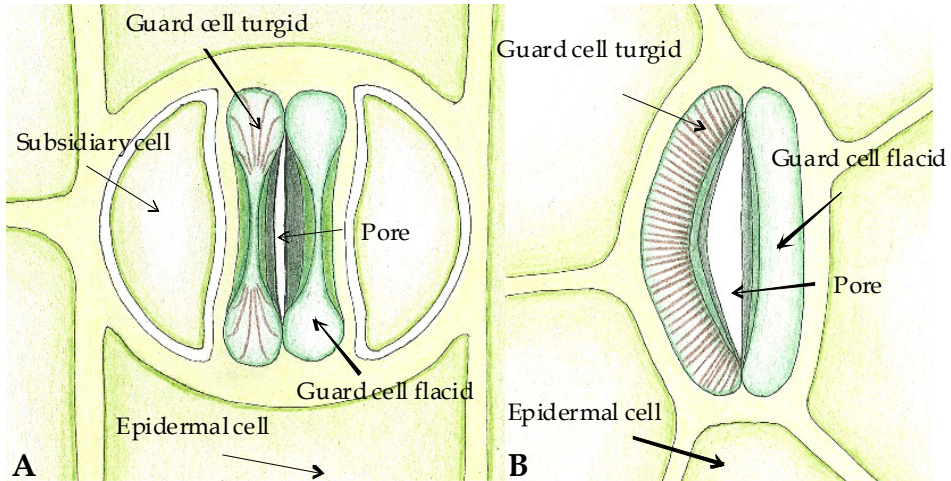


Fig. 7. Stomatal complexes: grass-like (A) and kidney-shaped (B), with the left guard cells turgid (open pore) and the right flacid (closed pore).

The stomata works as a hydraulic valve regulated by water. When turgid, the stomatal pore opens, and when flaccid due to water loss it closes (Fig. 7). In the early morning, light in the blue band (440-490nm) indicates the arrival of solar radiation and possibility of photosynthetic activity. Thus, the plant opens the stomatal pores to allow influx of carbon dioxide. The first theory about stomatal opening control was called the "starch-sugar hypothesis," and it was widely accepted during the early 1940s. This hypothesis suggested that the hydrolysis of starch in soluble sugars decreased the osmotic potential of cell, promoting water absorption and stomatal opening. However, that theory lost strength as advances were made by studies of potassium movement in the guard cells. Currently, three modes covering the osmoregulation processes of guard cells are accepted: 1) the influx of potassium and chloride through proton pump activation and the synthesis of malate from starch breakdown; 2) sucrose synthesis by starch hydrolysis; and 3) sucrose synthesis by carbon dioxide fixation due to photosynthetic activity.

The blue light signalling process occurs through receptor pigments (phototropins). Thereafter, the opening process occurs by the reduction in the osmotic potential of guard cells by potassium input, consuming ATP by ATPase located in the plasmatic membrane. This ATPase release protons inside the guard cells, causing a variation in pH around 0.5 to 1, which enables membrane hyperpolarisation and, consequently, the opening of channels favours potassium absorption. The resulting osmotic gradient causes water movement towards these cells. Therefore, the guard cells become turgid and the stomatal pore opens, due to the action of cellulose microfibrils (Fig. 7). However, it is important to emphasise that chloride and malate also contribute to the establishment of the osmotic gradient in guard cells exposed to blue light.



When solar radiation begins to decrease this affects the photochemical phase reducing levels of ATP and NADPH<sup>+</sup> resulting in losses for the biochemical phase. Internally, the carbon dioxide content begins to rise as they are not being used in the carboxylation process in the Calvin-Benson cycle (C<sub>3</sub>). At the same time, calcium ions play an important role when they enter the guard cells, causing solute output and decreasing the osmotic potential of these cells, making the stomata close.

An atmosphere enriched with carbon dioxide can favour the photosynthetic activity of plants with the C<sub>3</sub> mechanism. However, excessive carbon availability will result in stomatal closure in some species, even with a C<sub>3</sub> mechanism (Ainsworth & Rogers, 2007). An example of natural CO<sub>2</sub> enriched conditions, where the plants can be submitted to excessive concentrations of carbon dioxide, occurs near volcanic activity (Miglietta & Raschi, 1993).

### 3.3 Hormonal and molecular responses in different water conditions

The plants that display a higher production capacity, due to the morphological, physiological and metabolic changes of their organs and cells, tend to present higher demands on available resources and, consequently, possess greater vulnerability to conditions of water restriction. However, there are several strategies for the adaptation to dry environments that can be considered as a tool for progress in overcoming limiting conditions on growth and production. Generally, some plants can accumulate water to delay or escape such stress conditions, while others can deal with the stress through decreased metabolic activity (Bartels, 2005). The effect of water restriction depends on the degree and duration of the stress, the stage of plant development, the genotypic ability of the species and environmental interactions. In recent years, and mainly due to climate changes, several studies have looked to understand the biochemical and molecular basis of water stress (Yokota et al., 2006; Xoconostle-Cázares et al., 2010). Water stress can influence a plant on several levels, with cell expansion and growth being the first processes to respond to water limitation. With the gradual increase of stress, other processes are also affected, such as photosynthesis and allocation of assimilates. At the cellular level, membranes and proteins can be damaged with the increment of reactive oxygen species or peroxidation. These responses are common with other abiotic stresses, such as the effects of salinity and low temperatures, resulting in the synthesis of a similar group of proteins (Artlip & Wisniewski, 2002).

Tolerance to water deficit can be manifested in four ways: 1) the seasonal adjustment of growth to avoid stressful conditions; 2) morphological adaptations, such as an increase in the root:shoot ratio, a reduction in the leaf area and wax accumulation on the leaf surface; 3) physiological adaptations, such as stomatal responses and leaf abscission; and 4) metabolic changes. Among the metabolic changes of adaptation, osmotic adjustment represents the most common change and results from the accumulation of certain metabolites.

The root has been generally accepted as the organ which acts on the perception of water stress, although so far there is no knowledge about how the cells in roots perceive the soil moisture content. However, when the plant water potential is influenced by the water restriction of soil or saline stress, the stomata must respond quickly to avoid water deficit. Signalling between the root and stomata is carried out by abscisic acid (ABA), which has been considered to be a "*plant hormone of stress*" due to its participation in the signalling

networks of other factors of stress (Artlip & Wisniewski, 2002). ABA is synthesized from carotenoid by the synthesising enzyme of ABA (zeaxanthin epoxidase, 9-cis-epoxycarotenoid dioxygenase and aldehyde oxidase) which is induced in the root apex or in the parenchyma cells of vascular bundles by water or saline stress. After the synthesis of ABA in the roots, it is transported through the xylem to the leaves alone or conjugated with glucose (the latter being more appropriate for transport over long distances). The proportion of each form of transport (alone or conjugated) is variable between species (Sauter et al., 2002). Once in the leaf, conjugated ABA is hydrolysed into its free form by the apoplastic enzyme  $\beta$ -D-glucosidase, inducing stomatal closure through a signalling system in the guard cells of chloroplasts (Yokota et al., 2006).

In addition to this long distance signalling between the roots and the stomata, recent studies also point out that the leaves must act as sensors of relative humidity in order to avoid desiccation. This is clear from observations of the extremely quick closure of stomata with increments in the vapour pressure gradient between the leaf and the air, even when there is adequate water availability in the soil (Assmann et al., 2000). Recent works expose the possibility that leaf sensors of relative humidity are located in or near their own stomata guard cells (Yokota et al., 2006). In addition, there is genetic evidence that leaf sensitivity to relative humidity (RH) is related to ABA metabolism, exerting a hormonal effect over a short distance. A recent study on genetic selection, based on infrared thermal imaging, identified two genes (*OST1* and *ABA2*) that are directly involved in the signalling route of RH sensing in guard cells (Xie et al., 2006). *OST1* codes a protein kinase that is involved with stomata closure, while *ABA2* codes an enzyme involved in ABA biosynthesis. This reinforces the involvement of ABA as a mediator in a signalling network of guard cells, which can be shared between different stimuli to control stomata closure (Yokota et al., 2006).

ABA has been related to quantitative and qualitative variations in the gene expression and protein synthesis stimulated by water stress (Artlip & Wisniewski, 2002). Meanwhile, it is notable that some of the proteins that are *de novo* synthesized do not appear in the responses to the application of abscisic acid (ABA) and these signals/response routes to the water stress have ABA-dependent and ABA-independent routes (Yokota et al., 2006). In ABA-independent routes, the signal molecule provided by the roots is still unknown. Bioinformatics analysis has promoted advances in the identification of several factors of transcription that are induced by water deficit, classified in six major groups: AP2/ERF (APETALA2/ethylene-response factor); bZIP (Basic leucine-zipper protein); MYB/MYC Zinc-finger protein; CDT-1; NAC and Dreb (Xoconostle-Cázares et al., 2010). In ABA-dependent routes, the promoters of genes containing a *cis*-sequence of six nucleotides are known as the *ABA response element* (ABRE). The genes' expression of ABA-dependence is activated by the AREB/ABF link - a transcription factor of the type bZIP - on the ABRE sequence. Further, in a gene ABA-dependent RD22 expression there is involvement with the transcription factors MYB and MYC, and they are related to the final stages of responses to drought that are ABA dependent (Yokota et al., 2006). The promoters of genes related with responses to drought also have an alternative regulatory sequence of nine nucleotides called a *dehydration response element* (DRE). The DREs are involved in the ABA-independent expressions of genes that are induced under drought conditions. The *trans*-factors to the *cis*-elements are CBF/DREB1 and DREB2, which are expressed transiently after the detection of

drought and thereby stimulate the targeted genes involved in drought tolerance (Taiz & Zeiger, 2002; Yokoda et al., 2006).

Genes that are stimulated by drought can be categorised into two groups: 1) coding genes of those proteins responsible for protecting cells and organs against stress; and 2) coding genes of those proteins necessary for signals' translation and regulation of gene expression. The proteins of the first group act directly on membrane functions, the maintenance of water potential, proteins' protection and oxidative stress control. Stands out in this group the family of the *Embryogenesis Abundant protein* (LEA), which is formed by five types of proteins based on the structural domain and which are suspected of acting to protect the cell membrane (Taiz & Zeiger, 2002). Beyond that, and by their hydrophilic properties, they act in water retention and prevent the crystallisation of other proteins and molecules during drying. Within the LEA family itself, the D-11/RAB/Dehydrins group stands out, whose function has been related with the stabilisation of proteins and membranes. This group presents a wide distribution between plant species, and can be considered to be an alternative to the constitutive defence against rapid changes in the *water status* of tissue (Artlip & Wisniewski, 2002). The *aquaporins* family represents another important protein in cell protection against water stress, facilitating water absorption by the plasmatic membrane. Its importance in water relations was recently evidenced by the differential accumulation of aquaporins in relation to the degree of drought tolerance in varieties of beans (Montalvo-Hernández et al., 2008). With regard to membrane protection and the water status of the plant we may also highlight lipid transfer proteins (LTPs) that catalyse the transfer of several classes of phospholipid and glycoproteins for deposits in cell walls or between membrane vesicles (Kader, 1997). The results with LTPs show that these proteins are induced during different conditions of stress and that they can act to increase membrane fluidity, decreasing water loss by increasing tissue impermeability, and as a physical barrier to biotic stress (Treviño & O'Connell, 1998; Maghuly et al., 2009; Gong et al., 2010).

Another protein family has been associated with responses to drought, namely Heat Shock Proteins (HSPs), which are widely distributed in nature. These proteins are known as molecular chaperones, acting in the folding and assembly of functional proteins and in the removal of non-functional proteins, facilitating the recovery of cellular functions after stress. Several HSPs - classified according to their molecular weight - are induced in conditions of water and saline stress, such as HSP70 (the DnaK family), the chaperones GroEL and HSP60, HSP90 and HSP100 and the small HSP (sHSP) (Alamillo et al., 1995; Campalans et al., 2001, Wang et al., 2004). Within these proteins, there is the cyclophilin, which is a chaperone protein with systemic properties and which is highly induced during water stress, conferring multiple tolerances to abiotic stress (Gottschalk et al., 2008; Sekhar et al., 2010). During conditions of stress, the recycling of macromolecules which lose their function to maintain cellular homeostasis is essential. In this process, and under conditions of water stress, an increase of protease activity has been observed (Campalans et al., 1999; Seki et al., 2001), which is important in the destruction of denatured proteins and in the recycling of amino acids necessary to synthesize proteins for water deficit responses. In addition, the ubiquitin and polyubiquitin proteins are also induced with water restriction, both of which act marking proteins for proteolytic degradation (Campalans et al., 1999, Barrera-Figueroa et al., 2007).

### 3.4 Osmotic regulation

As discussed above, the osmotic regulation process of stomatal opening occurs by the movement of solutes - called *osmolytes* - thereby influencing water movement between the cells. This water movement has several purposes in relation to cell hydration inside plants, such as the stomatal opening and higher water absorption via root system. Several compounds that work in the osmotic regulation of plants are known: carbohydrates (sucrose, sorbitol, mannitol, glycerol, arabinitol, pinitol) (Hare et al., 1998), nitrogen compounds (proteins, betaine, glutamate, aspartate, glycine, choline, putrescine, 4-gamma aminobutyric acid) (Kinnersley & Turano, 2000) and organic acids (malate and oxalate) and so on (Sairam & Tyagi, 2004).

From the standpoint of thermodynamics, there are concepts relating to water potential that are elementary in the explication of water movement. In turgid cells the water potential ( $\Psi_w$ ) is composed by pressure ( $\Psi_p$ ) and osmotic ( $\Psi_{\pi}$ ) potentials. The greatest possibility of interference in the  $\Psi_w$  of plant cells is by varying the amount of internal solutes. Thus, osmotic regulation is a process in which variation of the amount of solutes - and consequently in osmotic potential - interferes in water movement.

The higher the concentration of solutes in a solution, the greater will be the disorder of the system (entropy). Furthermore, this condition leads to more negative osmotic potential and, consequently, to a more negative water potential. The water inside the plant flows from the sites with higher water potentials to those with lower ones. Thus, an increase in solute concentration in the cell favours the entry of water.

The solutes that plants use to decrease the water potential and control the water flow must have a low molecular weight. Among these solutes, the best-studied is potassium ( $K^+$ ), the lack of which is related to tissue dehydration. However, most references point out that the main role of potassium within the osmoregulation process is the regulation of stomata opening (Läuchli, 1984; Hsiao & Läuchli, 1986). It also acts in water absorption by the roots, in transpiratory control and in the cells' ability to resist low temperatures (Grewal & Singh, 1980). In particular, under conditions of potassium deficit, sugars from starch hydrolysis become more relevant in osmotic regulation than in the process of stomatal opening (Poffenroth et al., 1992).

The proline amino acid also stands out as an osmotic regulator, and is linked to stress both by water deficit and salinity (Molinari et al., 2004; Zhu et al., 2005). Nevertheless, proline does not only exert the function of osmoregulation in plant cells during periods of water deficit, but it can also protect against the activity of free radicals, regulate the pH in the cytoplasm, protect against the denaturation of macromolecules and also act as a source of carbon and nitrogen under conditions of stress (Sharma and Dietz, 2006; Vanrensburg et al., 1993; Sivakumar et al., 2000; Díaz et al., 1999). Another compound with importance in osmotic regulation is glycinebetaine, which, like proline, does not just exert or exercise its function in osmotic potential. These compounds also act in the reduction of reactive oxygen species (ROS) produced under conditions of stress in plants. With the process of tissue dehydration, there is production of ROS, such as the singlet oxygen ( $^1O_2$ ), the superoxide radical ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and the hydroxyl radical ( $OH^-$ ) (Liu & Huang, 2000). Reactive oxygen species (ROSs) are produced from oxygen metabolism and play an important role as indicators in the stress process of water deficit. Among these are oxygen

ions, peroxides and free radicals, and these compounds will cause oxidative stress in cells and prejudice their operation.

## 4. Practical aspects of measuring plant water status

### 4.1 Pressure chamber

The most widely used tool for determining water potential is the pressure chamber (Scholander et al., 1965). The measurement is made on the section between a stem or a branch and a vegetative part, which is placed inside a pressure chamber filled with an inert gas (Fig. 8).

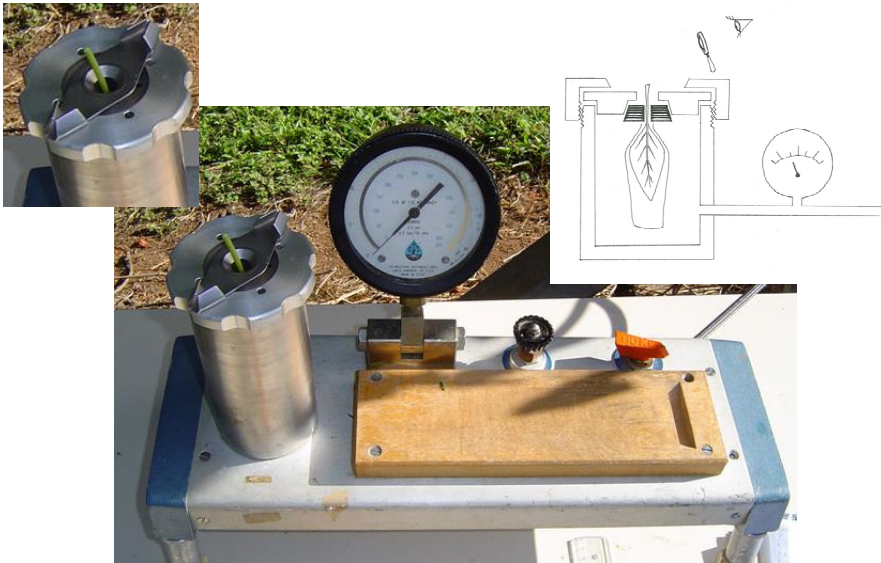


Fig. 8. Pressure chamber used to determine water potential in plants. Photo: Geraldo Chavarria.

After leaf preparation, leaf bagging and chamber sealing, the chamber pressure is increased gradually, resulting in a force inverse to the entry of water, in order to expel water through the observed section of the xylem. Generally, the evaluation of the potential requires the use of a magnifying glass. The pressure exercised to expel water is considered the pressure which is retained in the cells. This technique is used in research for the characterisation of the *water status*, but it may be useful in some crops as a tool for determining the appropriate time for irrigation. Furthermore, some works also point to a high correlation between leaf temperature measured with an infrared thermometer and water potential in the leaf measured with a pressure chamber (Lafitte & Courtois, 2002).

### 4.2 Porometry

Another technique widely used to measure the water availability in plants is that of stomatal diffusive resistance, using a device called a *porometer*, since the main route of gas exchange

between the plant and atmosphere occurs through the stomata. The resistance to water diffusion by the stomata is measured in both sides of the leaf. The equipment may evaluate four different processes: 1) mass flow (air forced through the leaf); 2) vapour diffusion (dry air pumped into the chamber, which is equipped with a sensitive device to detect variations in humidity inside the chamber); 3) maintenance flow (dry air pumped into the chamber and steadily retained, where the flow changes are used to calculate resistance to stomatal diffusion); and 4) state of equilibrium (to monitor the time necessary for the occurrence of equilibrium by applying a dry air flow in the chamber attached to the leaf) (Fig. 9).



Fig. 9. Stomatal conductance determination using a porometer. Photo: Ana Cláudia Pedersen.

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## 6. References

Abdala, G.C.; Caldas, L.S.; Haridasan, M.; Eiten, G. (1998). Above and belowground organic matter and root:shoot ratio in cerrado in central Brazil. *Brazilian Journal of Ecology*, Vol. 2, No. 2, pp. 11-23.

- Ainsworth, E. A. & Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising (CO<sub>2</sub>): mechanisms and environmental interactions. *Plant, Cell and Environment*, Vol. 30, N° 1, pp. 258-270.
- Alamillo, J.; Almogura, C.; Bartels, D. & Jordano, J. (1995). Constitutive expression of small heat shock proteins in vegetative tissues of the resurrection plant *Craterostigma plantagineum*. *Plant and Molecular Biology*, Vol.29, pp. 1093-1099, ISSN 0167-4412
- Artlip, T.S. & Wisniewski, M. E. (2002). Induction of Proteins in Response to Biotic and Abiotic Stresses, In: *Handbook of Plant and Crop Physiology*. M. Pessaraki, (Ed.), 657-679, Marcel Dekker Inc., ISBN 0-8247-0546-7, New York.
- Assmann, S.M.; Snyder, J.A. & Lee, Y.R.J. (2000). ABA-deficient (aba1) and ABA-insensitive (abi1-1, abi2-1) mutants of *Arabidopsis* have a wild-type stomatal response to humidity. *Plant Cell and Environment*, Vol.23, No. 4, pp. 387-395, ISSN 1365-3040
- Barnes, J.D. & Cardoso-Vilhena, J. (1996). Interactions between electromagnetic radiation and cuticle. In: *Plant cuticles: an integrated functional approach*. G. Kerstiens, (Ed.), 157-174, Bios Scientific Publishers, ISBN 1-85996-130-4, Lancaster
- Barrera-Figueroa, B.; Pena-Castro, J.; Acosta-Gallegos, J.A.; Ruiz-Medrano, R. & Xoconostle-Cazares, B. (2007). Isolation of dehydration-responsive genes in a drought tolerant common bean cultivar and expression of a group 3 late embryogenesis abundant mRNA in tolerant and susceptible bean cultivars. *Functional Plant Biology*, Vol. 34, No. 4, pp. 368-381, ISSN 1445-4408
- Bartels, D. & Sunkar, R. (2005). Drought and salt tolerance in plants. *Critical Reviews in Plant Sciences*, Vol.24, No. 1, pp. 23-58, ISSN 1549-7836
- Bartels, D. (2005). Desiccation tolerance studied in the resurrection plant *Craterostigma plantagineum*. *Integrative and Comparative Biology*, Vol. 45, No. 5, pp. 696-701, ISSN 1540-7063
- Bongers, F.; Engelen, D.; Klinge, H. (1985). Phytomass structure of natural plant communities on spodosols in southern Venezuela: the Bana woodland, *Vegetatio*, Vol.63, pp. 13-34.
- Boyce, C.K.; Brodrick, T.J.; Feild, T.S. & Zwieniecki, M.A. (2009). Angiosperm leaf vein evolution was physiologically and environmentally transformative. *Proceedings of the Royal Society B*, Vol. 276, pp. 1771-1776, ISSN 1471-2954
- Boyer, J.S. (1985). Water transport. *Annual Review of Plant Physiology*, Vol.36, pp. 473-516, ISSN 0066-4294
- Burgess, S. & Bleby, T. (2006). Redistribution of soil water by lateral roots mediated by stem tissues. *Journal of Experimental Botany*, Vol. 57, No. 12, pp. 3283-3291, ISSN 002-0957
- Burghardt, M.; Burghardt, A.; Gall, J.; Rosenberger, C. & Riederer, M. (2008). Ecophysiological adaptations of water relations of *Teucrium chamaedrys* L. to the hot and dry climate of xeric limestone sites in Franconia (Southern Germany). *Flora*. Vol. 203, pp. 3-13, ISSN 0367-2530
- Campalans, A.; Messegue, R.; Goday, A. & Pagès, M. (1999). Plant responses to drought, from ABA signal transduction events to the action of the induced proteins. *Plant Physiology and Biochemistry*, Vol. 37, No. 5, pp. 327-340, ISSN 0981-9428
- Campalans, A.; Pagès, M. & Messegue, R. (2001). Identification of differentially expressed genes by the cDNA-AFLP technique during dehydration of almond (*Prunus amygdalus*). *Tree Physiology*, Vol. 21, No. 10, pp. 633-643, ISSN 0829-318X

- Chartzoulakis, K.; Patakasb, A.; Kofidisc, G.; Bosabalidisc, A. & Nastoub, A. (2002). Water stress affects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars. *Scientia Horticulturae*, Vol. 95, pp. 39-50, ISSN 0304-4238
- Chaumont, F.; Moshelion, M. & Daniels, M.J. (2005). Regulation of plant aquaporin activity. *Biology of the Cell*, Vol. 97, No. 10, pp. 749-764, ISSN 0248-4900
- Chavarria, G.; Cardoso, L.S.; Bergamaschi, H.; Santos, H.P. das; Mandelli, F. & Marodin, G.A.B. (2009). Microclimate of vineyards under protected cultivation. *Ciência Rural*, Vol. 39, No. 7, pp. 2029-2034, ISSN 0103-8478
- Cochard, H. (2002). A technique for measuring xylem hydraulic conductance under high negative pressures. *Plant, Cell and Environment*, Vol. 25, No. 6, pp. 815-819, ISSN 1365-3040
- Devine, M.; Duke, O. S. & Fedtke, C. (1993). *Physiology of Herbicide Action*. Englewood Cliffs, NJ: P.T.R Prentice-Hall.
- Díaz, P.; Borsani, O. & Monza, J. (1999). Acumulación de prolina en plantas en respuesta al estrés osmótico. *Agrociencia*, Vol. 3, N° 1, pp. 1-10.
- Dichio, B.; Xiloyannis, C.; Sofo A. Montanaro G. (2006). Osmotic regulation in leaves and roots of olive tree (*Olea europaea* L.) during water deficit and rewatering. *Tree Physiology*, 26:179-185.
- Eglinton, G. & Hamilton, R.J. (1967). Leaf epicuticular waxes. *Science*, Vol. 156, No. 3780, p. 1322-1335, ISSN 0036-8075
- Ehleringer, J.R. (1984). Ecology and ecophysiology of leaf pubescence in North American desert plants. In: *Biology and Chemistry of Plant Trichomes*, E. Rodriguez, P.L. Healy & I. Mehta, (Eds.), 113-132, Plenum Press, ISBN 030-6413-93-0, New York.
- Ennajeh, M.; Vadel, A.M, Cochard, H. & Khemir, H. (2010). Comparative impacts of water stress on the leaf anatomy of a drought-resistant and a drought-sensitive olive cultivar. *Journal of Horticultural Science & Biotechnology*, Vol. 85, No. 4, pp. 289-294, ISSN 1462-0316
- Enstone, D.E.; Peterson, C.A. & Ma, F. (2003). Root endodermis and exodermis : structure, function, and responses to the environment. *Journal of Plant Growth Regulation*, Vol. 21, No. 4, pp. 335-351, ISSN 0721-7595
- Evans, J.R.; Kaldenhoff, R.; Genty, B. & Terashima, I. (2009). Resistances along the CO<sub>2</sub> diffusion pathway inside leaves. *Journal of Experimental Botany*. Vol. 60, pp. 2235-2248.
- Faludi-Daniel, A. (1981). Stomatal behaviour and cuticular properties of maize leaves of different chilling-resistance during cold treatment. *Physiologia Plantarum*, Vol.24, pp. 287-290, ISSN 0031-9317
- Fitter, A. & Hay, R. (2002). *Environmental Physiology of Plants*, Academic Press, ISBN 0-12-257766-3, San Diego, California
- Gong, P.; Zhang, J.; Li, H.; Yang, C.; Zhang, C.; Zhang, X.; Khurram, Z.; Zhang, Y.; Wang, T.; Fei, Z. & Ye, Z. (2010). Transcriptional profiles of drought-responsive genes in modulating transcription signal transduction, and biochemical pathways in tomato. *Journal of Experimental Botany*, Vol. 61, No. 13, pp. 3563-3575, ISSN 0022-0957
- Gottschalk, M.; Dolgener, E.; Xoconostle-Cazares, B.; Lucas, W.J.; Komor, E. & Schobert, C. (2008). *Ricinus communis* cyclophilin: functional characterisation of a sieve tube protein involved in protein folding. *Planta*, Vol. 228, No. 4, pp. 687-700, ISSN 0032- 0935



- Grewal, J.S. & Singh, S.N. (1980). Effect of potassium nutrition on frost damage and yield of potato plants on alluvial soils of the Punjab (India). *Plant Soil*, Vol. 57, pp. 105-110
- Hare, P.D.; Cress, W.A. & Staden, J.V. (1998). Dissecting the roles of osmolyte accumulation during stress. *Plant, Cell and Environment*, Vol. 21, pp. 535-553, ISSN 1365-3040
- Hsiao, T.C. & Läuchli, A. (1986). Role of potassium in plant-water relations. In: *Advances in Plant Nutrition*, B. Tinker & A. Läuchli (Eds.), Vol. 2, 281-311, Praeger Scientific, ISBN 0-27592-069-0, New York
- IPCC (August 2011). Intergovernmental Panel of Climatic Change WGII, fourth assessment report, 12/08/2011. Available from [http://www.ipcc.ch/publications\\_and\\_data/publications\\_and\\_data\\_reports.htm](http://www.ipcc.ch/publications_and_data/publications_and_data_reports.htm)
- Kader, J.C. (1997). Lipid-transfer proteins: a puzzling family of plant proteins. *Trends in Plant Science*, Vol. 2, No. 2, pp. 66-70, 1360-1385
- Kerstiens, G. (1996). Diffusion of water vapour and gases across cuticles and through stomatal pores presumed closed. In: *Plant cuticles: an integrated functional approach*. G. Kerstiens, (Ed.), 121-134, Bios Scientific Publishers, ISBN 1-85996-130-4, Lancaster
- Kinnersley, A.M. & Turano, F.J. (2000). Gamma aminobutyric acid (GABA) and plant responses to stress. *Critical Reviews in Plant Sciences*, Vol. 19, pp. 479-509, ISSN 1549-7836
- Kirkham, M.B. (2005). Principles of soil and plant water relations. Elsevier Academic Press, ISBN 0-12-409751-0, San Diego, California.
- Kramer, P.J. & Boyer, J.S. (1995). *Water relations of plants and soils*. Elsevier Academic Press, ISBN 0-12-425060-2, San Diego, California
- Kutlu N, Terzi R, Tekeli C, Senel G, Battal P & Kadioglu A (2009). Changes in anatomical structure and levels of endogenous phytohormones during leaf rolling in *Ctenanthe setosa*. *Turkish Journal of Biology*, Vol. 33, pp. 115-122, ISSN 1300-0152
- Lafitte, H. R.; Courtois, B. (2002) Interpreting cultivar x environment interactions for yield in upland rice: assigning value to drought-adaptive traits. *Crop Science*, Vol. 42, No. 1, pp. 1409-1420, ISSN 0011-183X
- Larcher W (1995). *Physiological Plant Ecology*. Springer-Verlag, ISBN 978-3540435167, Berlin.
- Läuchli, A. (1984). Mechanism of nutrient fluxes at membranes of the root surface and their regulation in the whole plant. In: *Roots, Nutrient and Water Influx, and Plant Growth*, S.A. Barber; D.R. Bouldin (Eds.), 1-25, ASA Special Publication, ISBN 0891180826, Madison
- Levine, L.H.; Richards, J.T. & Wheeler, R.M. (2009). Super-elevated CO<sub>2</sub> interferes with stomatal response to ABA and night closure in soybean (*Glycine max*). *Journal of Plant Physiology*, Vol.166, No. 9, pp. 903-913, ISSN 0176-1617
- Liu X.; Huang B (2000). Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. *Crop Science*, Vol. 40, N<sup>o</sup>1, pp. 503-510.
- Maghuly, F.; Borroto-Fernandez, E.G.; Khan, M. A.; Herndl, A.; Marzban, G. & Laimer, M. (2009). Expression of calmodulin and lipid transfer protein genes in *Prunus incisa* x serrula under different stress conditions. *Tree Physiology*, Vol. 29, No. 3, pp. 437-444, ISSN 0829-318X
- Marschner, H. (1995). *Mineral nutrition of higher plants*. (2Ed). Academic Press, ISBN 0-12-4735428, Michigan

- matter and root:shoot ratio in cerrado in central Brazil. *Brazilian Journal of Ecology*, Vol. 2, No. 2, pp. 11-23.
- Maurel, C.; Verdoucq, L.; Luu, D. & Santoni, V. (2008). Plant aquaporins: membrane channels with multiple integrated functions. *Annual Review of Plant Biology*, Vol. 59, pp. 595-624, ISSN 0066-4294
- Mendgen, K. (1996). Fungal attachment and penetration. In: *Plant cuticles: an integrated functional approach*. G. Kerstiens, (Ed.), 175-188, Bios Scientific Publishers, ISBN 1-85996-130-4, Lancaster
- Miglietta, F. & Raschi, A. (1993). Studying the effect to elevated CO<sub>2</sub> in the opening a naturally enriched environment in central Italy. *Vegetatio*, Vol. 104, No. 105, pp. 391-400, ISSN 0042-3106
- Molina-Montenegro, M.A.; Ávila, P.; Hurtado; Valdivia, A.I. & Gianoli, E. (2006). Leaf trichome density may explain herbivory patterns of *Actinote* sp. (Lepidoptera: Archaeidae) on *Liabum mandonii* (Asteraceae) in a montane humid forest (Nor Yungas, Bolivia). *Acta Oecologica*, Vol. 30, pp. 147-150, ISSN 1146-609X
- Molinari, H.B.C.; Marur, C.J.; Bepalhok Filho, J.C.; Kobayashi, A.K.; Pileggi, M.; Leite Júnior, R.P.; Pereira, L.F.P. & Vieira, L.G.E. (2004). Osmotic adjustment in transgenic citrus rootstock Carrizo citrange (*Citrus sinensis* Osb x *Poncirus trifoliata* L. Raf) overproducing proline. *Plant Science*, Vol. 167, pp. 1375-81, ISSN 0168-9452
- Montalvo-Hernández, L.; Piedra-Ibarra, E.E.; Gómez-Silva, L.; Lira-Carmona, R.; Acosta-Gallegos, J.A.; Vazquez-Medrano, J.; Xoconostle-Cázares, B. & Ruíz-Medrano, R. (2008). Differential accumulation of mRNAs in drought-tolerant and susceptible common bean cultivars in response to water deficit. *The New Phytologist*, Vol. 177, No. 1, pp. 102-113, ISSN 0028-646X
- Mullins, G.M.; Bouquet, A.; Williams, L.E. (1992). *Biology of the grapevines*. Cambridge University Press, New York. 239 p.
- Nobel, P.S. (2009). *Physicochemical and environmental plant physiology*. (4th Ed). Elsevier, ISBN 978-0-12-374143-1, London
- Ogbonnaya, C.I.; Nwalozie, M.C.; Roy-Macauley, H. & Annerose, D.J.M. (1998). Growth and water relations of Kenaf (*Hibiscus cannabinus* L.) under water deficit on a sandy soil. *Industrial Crops and Products*, Vol. 8, pp. 65-76, ISSN 0926-6690
- Poffenroth, M.; Green, D.B. & Tallman, G. (1992). Sugar concentrations in guard cells of *Vicia faba* illuminated with red or blue light. *Plant Physiology*, Vol. 98, pp. 1460-1471, ISSN 0032-08894
- Richter, H. (2001). The cohesion theory debate continues: the pitfalls of cryobiology. *Trends in Plant Science*, Vol. 6, No. 10, pp. 456-457, ISSN 1360-1385
- Sairam, R.K. & Tyagi, A. (2004). Physiology and molecular biology of salinity stress tolerance in plants. *Current Science*, Vol. 86, pp. 407-421, ISSN 0011-3891
- Sauter, A.; Dietz, K.J. & Hartung, W. (2002). A possible tress physiological role of abscisic acid conjugates in root-to-shoot signalling. *Plant Cell and Environment*, Vol. 25, No. 2, pp. 223-228, ISSN 1365-3040
- Scherbatskoy, T. & Tyree, M.T. (1990). Kinetics of exchange of ions between artificial precipitation and maple leaf surfaces. *New Phytologist*, Vol. 114, pp. 703-712, ISSN 0028-646X

- Scholander, P.F.; Hammel, H.T.; Hemmingsen, E.A. & Bradstreet, E.D. (1965). Hydrostatic pressure and osmotic potentials in leaves of mangroves and some other plants. *Proceedings of the National Academy Science*, Vol. 51, N° 1, pp.119-125.
- Sekhar, K.; Priyanka, B.; Reddy, V.D. & Rao, K.V. (2010). Isolation and characterization of a pigeonpea cyclophilin (CcCYP) gene, and its over-expression in Arabidopsis confers multiple abiotic stress tolerance. *Plant Cell and Environment*, Vol. 33, No. 8, pp. 1324-1338, ISSN 1365-3040
- Seki, M.; Narusaka, M.; Abe, H.; Kasuga, M.; Yamaguchi-Shinozaki, K.; Carninci, P.; Hayashizaki, Y. & Shinozaki, K. (2001). Monitoring the Expression Pattern of 1300 Arabidopsis Genes under Drought and Cold Stresses by Using a Full-Length cDNA Microarray. *Plant Cell*, Vol. 13, No. 1, pp.61-72, ISSN 1040-4651
- Shao, H.B.; Chu, L.Y.; Jaleel, C.A. & Zhao, C.X. (2008). Water-deficit stress-induced anatomical changes in higher plants. *Comptes Rendus Biologies*, Vol. 331, No. 3, pp. 215-25, ISSN 1631-0691.
- Sharma, S.S. and K.J. Dietz, 2006. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *Journal of Experimental Botany*. Vol. 57, pp. 711-726.
- Shepherd, T.; Robertson, G.W.; Griffiths, D.W.; Birch, A.N.E. & Duncan, G. (1995). Effects of environment on the composition of epicuticular wax from kale and swede. *Phytochemistry*, Vol. 40, pp. 407-417, ISSN 0031-9422
- Sivakumar, P.; Sharmila, P.; Saradhi, P.P. (2000). Proline alleviates salt-stress induced enhancement in Rubisco oxygenase activity. *Biochem Biophys Res Commun*, Vol. 279, N° 1, pp. 512-5.
- Steudle, E. (2001). The cohesion-tension mechanism and the acquisition of water by plant roots. *Annual Review of Plant Physiology and Plant Molecular Biology*, Vol. 52, pp. 847-875, ISSN 1040-2519
- Syvertsen, J.P.; Lloyd, J.; Mcconchie, C.; Kriedemann, P.E. & Farquhar, G.D. (1995). On the relation between leaf anatomy and CO<sub>2</sub> diffusion through the mesophyll of hypostomatous leaves. *Plant Cell and Environment*, Vol. 18, pp. 149-157, ISSN 1365-3040
- Taiz, L. & Zeiger, E. (2002). *Plant physiology*. (3rd Ed.). Sinauer Associates, ISBN 0-87893-823-0, Sunderland.
- Treviño, M.B. & O'Connell, M.A. (1998). Three drought-responsive members of the nonspecific lipid-transfer protein gene family in *Lycopersicon pennellii* show different developmental patterns of expression. *Plant Physiology*, Vol. 116, No. 4, pp. 1461-1468, ISSN 0032-0889
- Tyree, M.T. & Sperry, J.S. (1989). Vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Physiology and Plant Molecular Biology*, Vol. 40, pp. 19-38, ISSN 1040-2519
- Tyree, M.T. & Zimmermann, M.H. (2002). *Xylem Structure and the Ascent of Sap*. (2Ed). Springer series in wood science, Springer-Verlag, ISBN 3-540-43354-6, Berlin
- Tyree, M.T. (2003). Plant hydraulics: the ascent of water. *Nature*, Vol. 423, No. 6943, pp. 923-923, ISSN 0028-0836
- Vanrensburg, L.; Kruger, G.H.J.; Kruger, R.H. (1993). Proline accumulation the drought tolerance selection: its relationship to membrane integrity and chloroplast ultra structure in *Nicotiana tabacum* L. *Journal of Plant Physiology*, Vol. 141, N°1, pp.188-94.

- Vigh, L.; Horváth, I.; Farkas, T.; Mustardy, L.A. & Vogelmann, T.C. (1993). Plant-tissue optics. *Annual Review of Plant Physiology and Plant Molecular Biology*, Vol. 44, pp. 231–251, ISSN 1040-2519
- Vogelmann, T.C. (1993). Plant tissue optics. *Annual Review Plant Physiological and Plant Molecular biology*. Vol. 44, pp. 231-251.
- Wang, W.X.; Vinocur, B.; Shoseyov, O. & Altman, A. (2004). Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends in Plant Science*, Vol. 9, No. 5, pp. 244-252, ISSN 1360-1385
- Xie, X.; Wang, Y.; Williamson, L.; Holroyd, G.H.; Tagliavia, C.; Murchie, E.; Teobald, J.; Knight, M.R.; Davies, W.J.; Leyser, H.M. & Hetherington, A.M. (2006). The identification of genes involved in the stomatal response to reduced atmospheric relative humidity. *Current Biology*, Vol. 16, No. 9, pp. 882-887, ISSN 0960-9822
- Xoconostle-Cázares, B.; Ramírez-Ortega, F.A.; Flores-Elenes, L. & Ruiz-Medrano, R. (2010). Drought tolerance in crop plants. *American Journal of Plant Physiology*, Vol. 5, No. 5, pp. 241-256, ISSN 0176-1617
- Yokota, A.; Takahara, K & Akashi, K. (2006). Water Stress. In: *Physiology and Molecular Biology of Stress Tolerance in Plants*, K.V. M. Rao; A.S. Raghavendra & K.J. Reddy (Eds.), 15-39, Springer, ISBN-10 1-4020-4224-8, Netherlands
- Zhu, X.; Gong, H.; Chen, G.; Wang, S. & Zhang, C. (2005). Different solute levels in two spring wheat cultivars induced by progressive field water stress at different developmental stages. *Journal of Arid Environments*, Vol. 62, pp. 1-14, ISSN 0140-1963
- Zimmermann, U.; Schneider, H; Wegner, L.H. & Haase, A. (2004). Water ascent in tall trees: does evolution of land plants rely on a highly metastable state? *New Phytologist*, Vol. 162, No. 3, pp. 575-615, ISSN 1469-8137