

Chapter 4

ECOPHYSIOLOGY OF PLANTS IN DRY ENVIRONMENTS

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1. Introduction

Drought is a meteorological term which indicates a long period when there is not enough rain for the successful growing of crops or replenishment of water supplies. The expression *water stress* is frequently used to indicate the complex series of effects that are triggered in plants by drought. The term *drought stress* is more appropriate to specify when the stress status occurs only over a long period of time. However, because it is often difficult to separate the two phenomena, the definitions of water stress, drought stress and water deficit are frequently used interchangeably. Drought leads to water deficit in the soil and plant tissues, which in turn alters physiological processes and can have ultimate consequences for growth, development and survival of plants. Among the many biochemical and developmental processes that are affected by water stress, decrease of photosynthesis (Bradford and Hsiao, 1982; Flore and Lakso, 1989; Hsiao, 1973), changes in water relations (Brough et al., 1986; Olien and Lakso, 1986), reduction of both cell division and expansion (Hsiao and Acevedo, 1974), abscisic acid (ABA) synthesis (Davies and Zhang, 1991; Zeevaert and Creelman, 1988), and accumulation of sugars (Wang et al., 1995; Wang and Stutte, 1992) play a fundamental role in reducing productivity.

The concept of stress cannot be separated from that of *stress tolerance* (sometimes indicated with the less appropriate term of *stress resistance*), which is the plant's ability to survive in an unfavorable environment. Such ability can derive either from *adaptation* or *acclimation* to the stress condition. Both terms indicate an increase in tolerance and are sometimes erroneously used interchangeably. The difference is in the cause of the increased tolerance: in acclimated plants it is the result of a previous stress condition, while in adapted plants the tolerance is fixed in the genome and arises from selection processes that have occurred over many generations.

On the basis of their mechanisms of adaptation to soil water availability and their water requirements, plants are classified into three general groups. *Hydrophytes* require abundant water supply and flourish where water is almost always present. *Mesophytes* are plants adapted to moist, well-drained conditions (such as most crop plants), whereas *xerophytes* can survive prolonged dry soil conditions. Xerophytes are divided into three categories, *drought avoiders* (ephemerals), *drought resistors* and *drought endurers*. Drought avoiders are annual plants that complete their life cycle during wet periods and survive the dry season as seeds. Usually they are small in size, have high root to shoot ratios and do not need to develop morphological or anatomical adaptations to drought. Drought resistors avoid drought, either by using water conservatively and saving most of the water absorbed by the roots (*water savers*), or by developing structures/mechanisms that allow the uptake of considerable amounts of water and maintain high transpiration rates (*water spenders*). Examples of water savers are succulent plants, such as cacti and agave, which store large volumes of water in fleshy organs (leaves, stem, and roots). These plants have developed numerous adaptations to reduce water loss such as thick cuticles, small leaf size, leaf hairs, sunken stomata, etc. palms and mesquite trees (*Prosopis* sp.) are examples of water spenders, because they use considerable amounts of water. Such water amounts are supplied by the deep or widespread roots, which allow them to explore large volumes of soils. *Drought endurers* are those plants that, due to a series of adaptive mechanisms, can tolerate dehydration, sometime for very

long periods. Mosses, resurrection plant (*Selaginella lepidophylla*), some desert grasses, and sagebrush are examples of drought endurers.

In the present chapter, some of the typical responses of plants to water deficit conditions will be reviewed, with particular attention to the responses taking place in the vegetative tissue of higher plants. Other processes, such as flower and fruit production, seed germination and stress at cellular level will be discussed here only marginally. The present review also includes other types of plant stress frequently associated with drought, such as high temperature and salinity. The last part of the chapter will cover some of the main mechanisms that plants have evolved to cope with these unfavorable conditions. A modeling approach to water stress and its relations with soil moisture dynamics is given in Chapter 3.

2. Water relations

Water makes up between 85 and 90% of the fresh weight of most living herbaceous plants. In higher plants, water is absorbed by roots from soil and then moved to the shoots and leaves through the xylem vessels. The driving force for this movement is the pressure gradients developed by root pressure and by transpiration, which is in turn driven mainly by the difference in water vapor concentration between the leaf and the surrounding air.

The water status of plant tissues, soil, and atmosphere is commonly measured in terms of water potential (Ψ or Ψ_w), which is defined as the free energy per unit volume of water (J m^{-3}). Since energy per unit volume and pressure have the same dimensions, plant and soil water potentials are conventionally expressed in pressure units, such as the pascal (Pa), which is the common measurement unit for water potential (see also Chapter 2). Since the free energy of water is influenced by concentration of dissolved solutes, pressure and gravity, the water potential of a solution can be expressed as the sum of the following components:

$$\Psi = \Psi_0 + \Psi_s + \Psi_p + \Psi_g + \Psi_m + \Psi_v \quad (1)$$

Ψ_0 is the reference potential of pure (no dissolved solutes), free (free of adsorptive forces) water at a specified temperature at sea level exposed to an atmospheric pressure of 101,325 Pa. This component can be ignored, as long as the others are accounted for. Ψ_s is the solute component which indicates that pure water will flow from where the solute potential is higher (i.e., dilute solutions) to where it is lower (i.e., concentrated solutions). Ψ_s is very frequently written as Ψ_π , which is called osmotic potential. Ψ_p is the pressure component which specifies that water under pressure has a higher potential to do work. Ψ_g is the gravitational component. Ψ_m denotes the effects of matric forces on the water potential and Ψ_v is the potential due to humidity (water vapor). Despite the many components of water potential, most of them are rather small in cells (and there is little difference in gravitational potential between a cell and the surrounding environment). Consequently, Eq. (1) can be simplified, to a good approximation, to just the sum of solute and pressure potentials:

$$\Psi = \Psi_s + \Psi_p \quad (2)$$

Because of the complexity of plant and soil water relations, it is not possible to identify a unique index that is indicative of the degree of water deficit to which a plant is subjected. However, leaf or stem Ψ , is probably the most frequently used indicator of plant stress because it accounts for the effects of evaporative demands, the availability of water in the soil, and the hydraulic fluxes within the soil-plant-atmosphere continuum (Andrews et al., 1992). By using Ψ as a stress indicator, water deficit is quantified in terms of the extent to which tissue water content falls below that at full turgor, which is regarded as the optimum water content for growth and development.

Another frequently used indicator of plant water status is relative water content (RWC), which is the water content (on a percentage basis) relative to the water content of the same tissue at full turgor. Clearly, RWC is related to Ψ of the same tissue, although it is affected by stages of growth and by modifications induced by the environment. One disadvantage of using RWC as an indicator of stress is that it is rather insensitive to changes in plant water status when water deficit is not severe.

Even in conditions of adequate soil water supply, nearly all plants are exposed to some degree of water deficit conditions throughout their lives. Whenever the quantity of water loss via transpiration is greater than the amount absorbed by the roots, water in the xylem tissues is subject to a negative pressure (tension). Such a situation occurs regularly during the day, with more or less intensity, and is a function of the environment and of the physiological condition of the plant. Due to genetic variability and the influence of the environment, it is difficult to indicate which thresholds of water potential can be used to indicate levels of water stress. Nevertheless, three degrees of water deficit conditions of a typical mesophyte have been defined (Hsiao, 1973). According to this classification, a plant experiences mild stress when its cellular Ψ is greater than -0.5 MPa, moderate stress when Ψ is between -0.5 and -1.5 MPa and severe stress when Ψ_{cell} is below -1.5 MPa (Hsiao, 1973).

Hydraulic conductivity is a measure of the capability of a medium (in plant physiology, a cell membrane, a tissue, xylem elements, etc.) to transmit water. It is expressed as volume of water per unit area of medium per unit time per unit driving force (i.e., $\text{m}^3 \text{m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$). The loss of hydraulic conductivity experienced by the xylem plays an important role in reducing drought tolerance (Tyree and Sperry, 1989). When the xylem pressure becomes very negative, the column of water that moves in the xylem breaks and becomes air-filled, a phenomenon which is referred to as *cavitation* or embolism (Tyree and Zimmerman, 2002). Consequently, once a xylem vessel element cavitates, the water movement from the roots to the leaves is interrupted and hydraulic conductivity decreases markedly (Vilagrosa et al., 2003). The threshold value of xylem pressure for cavitation is usually defined by the species, as well as the anatomical characteristics of the xylem vessels, such as diameter (Sperry and Tyree, 1988). For example, poplar vessels cavitates at -1.6 MPa whereas those of juniper have a threshold of -3.5 MPa (Lambers et al., 1998). Many species subjected to water deficit have been found to operate very close to the point of cavitation. An effective control of stomatal aperture is important not only to regulate plant water losses, but also to reduce the risk of cavitation by maintaining xylem pressure within a safe range (Jones and Sutherland, 1991). Research conducted on Mediterranean species suggests that a higher resistance to cavitation might contribute to a higher tolerance to water deficit (Pockman and Sperry, 2000). Cavitation is also responsible for a decrease in gas exchange, and in particular of transpiration rate, thus affecting the amount of water that is absorbed by the roots (Cochard et al., 2002; Salleo et al., 2000).

Soil characteristics such as texture can influence plant water relations, thus superimposing on the influence of climate on plant productivity and diversity (see Chapters 2 and 3). When eight shrub species of the Great Basin desert of North America were compared, large differences in plant water relations occurred between sites of similar climate and topography, but with very different soil texture (Sperry and Hacke, 2002). Stem Ψ and vulnerability to cavitation were in fact lower in plants growing in finer-textured (loam) soils loamy versus soils characterized by larger pore spaces (sandy). Rooting depths also tended to be shallower at the loam site. The greater cavitation resistance measured in plants growing in loam soils is likely an adaptation to enhance the water extraction capability of a root system growing in a soil often characterized by lower Ψ . Roots of desert shrubs exposed to soil drought experience more cavitation than stems, suggesting that the root system represents the more limiting component to xylem transport (Kolb and Sperry, 1999; Sperry and Hacke, 2002). This could also represent an advanced evolutionary strategy of enduring or permitting cavitation in a few replaceable roots but preventing it in the stems. Thus, the damage caused by cavitation in roots is not as severe or long lasting as that

caused by stem xylem cavitation. Variation in cavitation resistance within the root system has been observed both spatially and temporally (Hacke et al., 2000; Sperry and Ikeda, 1997). Some desert shrubs are mostly active during the early part of the growing season when soil moisture is more available from rain showers before shedding their leaves and starting a period of drought dormancy. Such drought-deciduous plants appear to have shallow root systems and greater cavitation resistance indicating an enhanced ability to capture water from the upper soil profiles (Sperry and Hacke, 2002). Species differences with respect to cavitation resistance also reflect differences in xylem anatomy and function as well as trade-offs between conductance and safety (Lambers et al., 1998). Although smaller xylem elements can minimize the chances of cavitation, they are also less efficient in hydraulic conductivity.

3. Growth and development

The initial effect of water deficit stress is loss of turgor, with a concomitant reduction in the rate of cell expansion and ultimately, decreased cell size, leaf expansion, stem elongation, seed/fruit size and yield. As water deficit stress develops, competition for water within the plant may occur with the outcome depending upon the stage of growth. Usually, those tissues that are actively growing take precedence over older tissues. Meristems may constitute strong hydraulic sinks because cell division and biosynthesis of new cell materials such as proteins increase the matrix potential (lower Ψ) and establish a strong gradient for water movement to the meristematic tissue. Mesophyll and other photosynthetic tissues are also actively competing for water during mild stress because of the synthesis of new solutes which takes place there. Water may also be directed towards storage organs (e.g., tubers, rhizomes) and other sites where the number of solutes increase, for example due to hydrolysis of starch or other polysaccharides.

The primary effect caused by water deficit conditions on plants is a loss of turgor, which causes reduction in cell size and is accompanied by a reduction in leaf expansion and shoot extension. Reduction in leaf area has the advantage of decreasing the surface area for transpirational water loss. However, a smaller leaf area has the secondary effect of reducing light absorption and photosynthesis.

Under mild to severe water stress conditions, cell structure can be disrupted by dehydration. When the osmotic pressure of a solution is increased above that of the cells, the protoplast starts shrinking, and the plasma membrane separates from the wall protoplast (plasmolysis). This phenomenon mainly occurs in cells that are in contact with solution, such as in epidermal strips, or in root cells of intact plants grown in hydroponics (Munns, 2002). Plasmolysis is unlikely to occur in nature, except in severe conditions, but can be easily induced in the laboratory by immersing a plant cell in a very concentrated solution. Jones (1992) suggested that plasmolysis is rare in normal aerial tissues because it is the cell wall – not the plasma membrane – which supports the negative tensions. The capillary forces at the air-water interface in the cell wall microcapillaries would prevent them from draining and hence plasmolyzing. In the event that plasmolysis does occur, disruption of compartmentation may follow, with the concomitant release of lipases, hydrolases and other enzymes that destroy membranes and other substrates that are normally protected by isolation in compartments. Chloroplasts are also destroyed when they come in contact with vacuolar sap. The capacity to recover from this type of damage varies with the species and represents one of the resistance mechanisms initiated by plants to survive drought. Despite the degree of resistance, all plants have a ‘point of no return’ beyond which recovery is not possible and rehydration may actually aggravate the damage.

Water stress is particularly deleterious to development of reproductive organs. Flowers and fruits have a low frequency of stomata; hence, the driving force for water movement into stomata is smaller compared to leaves. Adequate water supply is, therefore, critically important during reproductive development, as nearly all phases from gamete production to anthesis and seed/fruit expansion are sensitive to water deficits. Water stress during gametogenesis or anthesis can lead

to infertility and seed/fruit abortion. During a mild stress, rapidly transpiring and photosynthesizing leaves can become stronger sinks for water than fruits because of increasing solute accumulation (photoassimilates) in the former. Leaf solute accumulation lowers leaf Ψ , creating a strong gradient for water movement towards them and in severe cases water may actually be resorbed from fruits, thereby inhibiting fruit and seed enlargement.

4. Water deficit stress perception and signaling

Roots are able to detect the early stages of soil drying and many of the shoot responses to soil drying occur before any detectable change in the leaf water status. Although loss of turgor and wilting are the first visible symptoms of soil water availability, there is evidence that a change in water status does not always play the main role in the regulation of plant responses to drought (Davies and Zhang, 1991). In fact, it is now well established that the term water stress does not refer only to situations where water relation parameters are altered. From several studies it has emerged that soil water status, leaf water relations and plant responses are not always correlated (Davies and Zhang, 1991). For these reasons, leaf Ψ is not always representative of ongoing water stress conditions, although it is the most commonly used indicator of shoot water status. Other leaf variables, such as growth rate and stomatal conductance (g_s , reciprocal of the resistance to CO_2 and water vapor transfer between the atmosphere and the internal tissue of the leaf imposed by the stomata), may be more useful indicators of incipient water deficit than water potential or other more commonly used variables. Change in growth and development of plants growing in drying soils can in fact occur even when shoot water relations are not affected. Frequently, depression of gas exchange and of stomatal conductance are detectable at moderate leaf water deficits or even before leaf water status is influenced (Jones et al., 1985).

If changes in water potential are not always involved, plants must have a different type of signal which must communicate the information to the shoot and trigger its responses. Such a signaling system must not be directly associated with a reduction in the flux of water to the shoots and with the change in leaf water status but it has to be responsible for changes in stomatal behavior, leaf initiation and expansion, etc. Tan et al. (1981) reported that tomato plants that had 25% of the root system watered, showed only 20% reduction in transpiration. The authors suggested that tomato roots could adjust their relative absorption capacity for water uptake in response to the transpirational demand.

The ability to regulate stomatal behavior is of vital importance especially for plants growing in water limiting conditions. Consequently, the signaling mechanism allows plants to sense water availability in the soil and promptly develop a response by regulating stomatal aperture, water consumption and adjust leaf water status. When the first indirect pieces of evidence of such signaling system were formulated, scientists needed to find if such a system indeed existed, whether it was composed by just one or more mechanisms, and what its nature was (chemical, electrical, etc.).

Split-root studies have been used to simulate the heterogeneity in the field and study the effects of differential irrigation in herbaceous plants (Williams et al., 1991; Zhang and Kirkham, 1995). Water uptake by one part of the root systems has been shown to be mostly dependent on the localized soil water potential. However, the relationship between soil water potential and water uptake may be affected by variations of the soil water status in other parts of the root system (Simonneau and Habib, 1994). Split root experiments have been widely conducted on plants with commercial importance, such as apple (Gowing et al., 1990), peach (Simonneau and Habib, 1994), grapes (Stoll et al., 2000), sunflower (Masia et al., 1994), barley (Farrar and Minchin, 1991), etc. When apple trees were grown with their root system split between two containers (Gowing et al., 1990), and irrigation was applied only to one container over a three-week period, leaf area was reduced by approximately 50%, due to reduction of both leaf expansion rate and leaf initiation. These responses were obtained even though half of the root

system received optimal supply of water and shoot and leaf water status was not affected. It was concluded that leaf initiation and expansion were somehow inhibited by a signal originated in the dry portion of the root system. Results of the split-root experiments reported above suggest that signaling between roots and shoots must be of a "positive" nature, which means that it is activated by the increase of a substance capable of eliciting a certain physiological response. Although "negative" signals (i.e., substances synthesized in the roots whose transport is completely stopped) have been considered, it is unlikely that they play a key role in the physiological and morphological changes induced by water stress. In order for such substances to be effective, leaves should be sensitive to minor changes in their concentration from very low levels to zero, which is very unlikely to occur (Davies and Zhang, 1991).

Split-root systems are not just experimental tools to study root-to-shoot signaling systems in plants; they are a way to simulate the heterogeneity of soil systems and in particular water availability in the soil. The larger a plant is, the greater the probability that its root system will grow into zones where water is readily available (e.g., deeper in the soil profile) and in others where water is scarce (e.g., more superficial layers). Although there is little information available on water relation profiles along the roots, studies conducted using split-root systems suggest that even if only a few roots are exposed to drying soil, a signal may be generated and translocated to the shoots and leaves. The intensity of such a signal would obviously increase when more and more roots are subjected to dehydration until a threshold is reached and a response is triggered in the canopy. Heterogeneity in plant root systems may be influenced also by size, class, characteristics, age, etc. of the single roots. Zhang and Davies (1989) reported for example that secondary and tertiary roots of maize are much less effective in maintaining tissue turgor when soil dries.

Among the possible chemical signals that may be involved in perception of developing water stress (cytokinins, pH, ion concentrations, etc.), ABA is the most likely (Davies and Zhang, 1991). ABA has long been known to increase considerably in leaves of plants subjected to drought. The marked increase is a consequence of the biosynthesis of this hormone, rather than of a release from storage forms present in the leaves (Dorffling, 1972). There is in fact a considerable body of evidence showing that leaves are not the only source of ABA and that ABA is also synthesized in roots (Cornish and Zeevaart, 1985; Davies and Zhang, 1991). The synthesis of ABA takes place in the root apices, in the non-growing regions, and in the cortex as well in the stele. When roots "sense" the reduction of soil water potential, they start producing ABA, which is then sent to the leaves via xylem flow, thereby functioning as a long distance signaling molecule. It is now widely accepted that stomatal conductance is controlled by the soil water status via ABA. The exact mechanism of action of ABA and its role on stomatal closure is still unclear although we do know that ABA induces efflux of anions and cations from the vacuole to the cytoplasm of guard cells, thus causing their loss of turgor and reduction of stomatal aperture (MacRobbie, 1997). It is not clear, however, if such reaction is caused by changes in ABA concentration in the leaves, by variations of its rate of delivery to the guard cells, or by other reasons, such as ABA-driven fluctuations in xylem pH.

Although leaf water status is often not considered as influencing the response of stomata to ABA, a few studies (Schurr et al., 1992; Tardieu and Davies, 1993; Tardieu et al., 1992) have reported that leaf water potential might have an indirect role in the regulation of the stomatal conductance, via a modification of the stomatal sensitivity to ABA. Moreover, a lower response of the conductance of ABA was observed when water potential was maintained high by pressurizing the root system (Schurr et al., 1992). Another study conducted on sunflower (Gollan et al., 1986) showed that, when leaf water potential and turgor do decline, stomatal conductance can be more closely related to the water status of the soil than to that of the leaves. In contrast to these assumptions, when ABA was removed from the sap of water-stressed wheat, the anti-transpirant activity was still present, which suggested that ABA could not be the only stress signal acting from roots to shoots (Munns and King, 1988).

Among the other types of signals that have been considered, ion and pH gradients may play a role in root-to-shoot communication. It has been reported that the pH of the xylem increase in response to drying soil (Davies and Zhang, 1991). The role of a higher pH could be to favor ABA distribution in the leaves. The variables involved in stomatal regulation are typically distinguished between those involved in the mechanics of stomatal movements and those implicated in the signals triggering these movements (Zeiger et al., 1987). Recently, however, other physiological variables have been proposed. These variables, such as whole-plant hydraulic resistance, soil resistance, root resistance, etc. may affect the hydraulic characteristics of the sap pathway and consequently induce stomatal closure (Cochard et al., 2002).

5. Gas exchange

Stomatal opening/closure provides an important mechanism for plants to regulate movement of gases into or out of leaves. Stomata occupy only about 1 % of the leaf surface area and, in most dicotyledonous plants, they are more numerous on the abaxial leaf surfaces compared to adaxial surfaces, with a stomatal frequency of 50-300 stomata per mm² being more representative for the abaxial leaf surface of most leaves (Nobel, 1999). In contrast, most monocotyledonous plants have approximately equal distribution of stomata on both sides of the leaf. In plants, transpiration (E) is the loss of water that occurs through stomata as the CO₂ needed for photosynthesis is absorbed from the atmosphere. Transpiration is a function of the hydrostatic pressure gradient and of the evaporative demand of the surrounding environment. This evaporative demand depends on the net radiation absorbed by leaves and on the drying power of the air (Nobel, 1999). Existence of a hydrostatic pressure gradient between roots and leaves drives water movement in plants from the soil into roots and ultimately to the atmosphere through leaf transpiration. Although transpiration serves a useful purpose of tissue cooling, excessive transpirational water loss can lead to soil water depletion and tissue dehydration. Stomata can be regulated in response to soil water status (e.g., via ABA) and other environmental cues such as vapor pressure deficit (VPD) and control water loss accordingly. Leaves continuously need to find a compromise between minimizing transpirational water loss and maximizing CO₂ uptake and photosynthesis. Stomata are the ultimate controllers of this delicate balance.

In the previous sections we have seen how water stress influences stomatal aperture, either directly, with the turgor loss of stomatal guard cells (hydropassive closure), or indirectly, with production of ABA or other inhibitory substances (hydroactive closure). In either case, stomatal closure induces a decline in carbon assimilation, with physiological and morphological consequences in both the vegetative and reproductive growth and development. Due to differences in molecular structure and diffusional properties of CO₂ and water, partial stomatal often limits transpiration more than it does CO₂ uptake. Stomatal conductance is in fact only part of the limitation on inward diffusion of CO₂, whereas it is the major limitation on outward diffusion of water vapor (Parkhurst, 1994). Some xerophytes have sunken stomata, an adaptation which increases the resistance to water vapor loss by transpiration. Other adaptations to regulate movement of gases into or out of leaves include small leaf size, thicker cuticles, pubescence, and glaucousness. The differential influence of stomatal closure on CO₂ uptake and water loss has important effects on water use efficiency (WUE). Leaf-level WUE refers to the ratio of photosynthesis (A) to transpiration, whereas WUE of productivity refers to the ratio between biomass accumulation and water used during production of that biomass. Since A and E are both affected by g_s, plant WUE, therefore, also depends on g_s and on factors influencing g_s such as the leaf-air vapor pressure difference (LA VPD, or the difference between the leaf's intercellular air spaces and the bulk air). Temperature also has a pronounced effect on plant WUE, since it affects LA VPD (Lambers et al., 1998).

Of special interest to life in dry environments is the fact that WUE is closely related to the carbon isotope (¹²C or ¹³C) composition of plant tissues. The C isotopic composition of plant

tissues provides an integrated measure of the photosynthetic WUE during the time when the tissue C was assimilated. Plants tend to discriminate against the heavier C isotope, depending on processes such as diffusion or carboxylation (see Farquhar et al., 1989 for a detailed review). High g_s or low A often lead to large discrimination (Δ) against the heavier isotope (^{13}C), resulting in a lower ^{13}C isotope composition ($\delta^{13}\text{C}$) in plant tissues. The consistently close positive correlation between $\delta^{13}\text{C}$ and WUE has led researchers to propose using $\delta^{13}\text{C}$ as a screening index in breeding for high WUE (Hubick and Farquhar, 1987; Meinzer et al., 1990). This follows from ample experimental evidence showing that genotypes which perform best under dry environments (i.e. high WUE) also have higher $\delta^{13}\text{C}$ in their tissues than plants from mesic environments (Smedley et al., 1991).

Most plants (except for CAM plants; see section 7.2.1) keep their stomata closed at night. This allows them to recover from a mild stress thanks to water absorption that continues from the available water supply in the soil. At night, however, water movement is not driven by the negative pressure generated in the xylem by transpiration, because, with stomata being close, transpiration does not take place. In this case, the driving force is caused by the solutes actively accumulated in the xylem sap. Such accumulation induces a decrease in Ψ_s , and thus in Ψ , which drives water movement from the more diluted soil solution to the more concentrated root xylem. Such movement is generally enough to build up a positive hydrostatic pressure (usually between 0.05 and 0.5 MPa) in the xylem which is utilized to rehydrate tissues and repair cavitation.

6. Other stresses associated with drought

Understanding the physiology of plants exposed to environmental stresses can be particularly challenging when the responses derive from different factors. For this reason, in order to better understand the physiological responses of plants to drought, most experiments have been conducted in laboratory, under controlled conditions, with the attempt to maintain non-limiting conditions for all environmental factors except for limiting water availability. In natural habitats, however, such conditions are rare, and it is more common for two or more factors to play a role concurrently to the plant responses. In nature, locations that are subjected to frequent drought periods are frequently associated with levels of irradiance well above the rates required to saturate the photosynthetic processes. High air and soil temperatures are also frequently linked to dry environments, due to the presence of high irradiance levels, low air and soil moisture levels. At the same time, dry environments are often exposed to increasing cellular or soil concentrations of ions or molecules, which, singly or in combination, can cause inhibition of metabolism or physiology (toxicity effects). Such interactions among environmental constraints force plants react to their environment with very complex adaptive responses which influence growth and developmental processes. What follows is a brief review of some of the abiotic plant stresses that are commonly associated with water stress.

6.1. HIGH TEMPERATURE STRESS

The association between drought and high temperature stress is often so close that it is difficult to distinguish the effects of either stress on plant growth and development. In addition, adaptive mechanisms to drought can be truly effective only if they lead to tolerance or avoidance of both stress conditions. The main reason why the two unfavorable conditions are so tangled in nature is because the stress caused by high temperatures is a frequent consequence of limiting soil water availability and of the drought-induced reduction in stomatal aperture. As observed before, when water supply in the soil becomes limiting, ABA is synthesized in the roots and translocated to the leaves where stomatal closure is induced. Although stomatal closure has the advantage of preventing excessive water losses, it also reduces carbon assimilation and induces the cessation of transpirational cooling. The reduction in transpiration causes a rapid rise in leaf temperature (5 °C

and more above air temperature in bright light near midday), which can induce serious damage to the leaves and, if maintained for prolonged period, lead to the death of the plant. The precise temperature threshold for damage to leaves is usually above 45 °C but it is function of several factors, such as species, growing temperature, potential prehardening conditions, environmental factors, etc. Well-hydrated plants can maintain the temperature of their tissues at values close to those of the surrounding air as long as they possess mechanisms to dissipate the heat generated by solar radiation. Plant tissues dissipate heat through three main mechanisms: heat convection, emission of long-wave (infrared) radiation and transpirational cooling. Of the three methods, transpiration is the most effective and, typically, half of the net heat input generated in a leaf by sunlight is dissipated by transpiration (Nobel, 1999). Transpiration dissipates heat because energy is required to break the bonds that hold the molecules of water together in the liquid phase. Consequently, water molecules which leave the walls of the mesophyll cells and are released into the atmosphere have a higher energy than those which are left behind in the leaf tissues. When the plant is well watered, transpiration rate is maximal and the leaf temperature is close to air temperature. On the other hand, when water deficit increases, stomatal aperture and transpiration are reduced and solar energy is no longer dissipated as latent heat for water evaporation, but rather converted into heat, which increases leaf temperature. This explains why, during hot summer days and when the soil water content becomes limiting, leaf temperature becomes higher than air temperature. Inversely, plants receiving an adequate amount of water through their roots have cooler leaves than those that are drought stressed. A relatively new method for the evaluation of plant water status can in fact be the measurement of leaf and canopy temperatures, and the difference between canopy and air temperature can be a tool to detect plant moisture stress (Jackson, 1982) and predict the potential yield of a crop (Tanner, 1963).

Cooling is not the only function of transpiration and water movement in the xylem elements. Water movement from root to shoot is the main avenue for nutrient movement and when transpiration rate decreases, so does nutrient distribution. Nutrient deficiency, however, is seldom a primary impairment for the growth and development of stressed plants because of the reduced metabolism during the unfavorable conditions.

In general, photosynthesis is one of the first processes to be affected by high temperatures. In particular, the chemical nature of the chloroplasts seems to be the reason for the high sensitivity of the electron transport of photosystem II (PS II) to high temperature values. The viscosity of the lipids forming thylakoid membrane changes in fact rapidly when leaf reaches temperatures between 45 and 55 °C, which corresponds to the range within which severe damage to the leaves occurs in almost all plants. At high temperature, there is also decrease in the strength of hydrogen bonds and electrostatic interactions between polar groups of proteins. Consequently, protein structure and functionality are modified with consequences on permeability, biochemistry, etc.

High temperatures affect carbon metabolism not only by reducing photosynthesis but also by accelerating carbon losses. Although both processes are inhibited by high temperatures, the optimal range for respiration in response to temperature is broader than that for photosynthesis. Consequently, as temperature rises, photosynthesis decreases before respiration does. When temperature rises above the temperature compensation point (the temperature value at which the amount of CO₂ released by respiration equals the amount of fixed CO₂), more CO₂ is used as substrate for respiration than what photosynthesis can fix into carbohydrates.

Photorespiration is another process which causes the loss of CO₂ and which is affected by temperatures. Photorespiration occurs when rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), the main enzyme involved in carbon assimilation, reacts with oxygen and, instead of producing two molecules of 3-phosphoglycerate, like it does when it reacts with CO₂, it originates 2-phosphoglycolate and 3-phosphoglycerate, thereby decreasing the efficiency of photosynthesis. As temperature increases, the concentration ratio of CO₂ to O₂ decreases and photorespiration rate increases more relative to the photosynthesis rate. Photosynthesis and

photorespiration occur simultaneously, with loss of CO₂ from those same cells that are fixing CO₂ by the Calvin cycle. This effect is also enhanced by the kinetic properties of rubisco, which has a higher affinity for O₂ at higher temperature (Ku and Edwards, 1978).

6.2. FIRE

Fire is the most extreme case of high temperature stress. Wildfires are often very destructive and are often facilitated by prolonged drought events which create fuel (dead plant material) for such fires. Although wildfires are more often associated with human intervention, they are frequently caused by natural events such as volcanic eruptions and lightning strikes. Wildfires are more common in those regions characterized by a dry season, but can take place practically anywhere in particularly dry years (see Chapter 16).

The type of damage inflicted to plants by fire depends on the temperatures generated in proximity of soil, trunk and canopy. Temperatures are determined by the amount of heat released per unit area which is function of the amount of combustible present per unit area, of the type of combustible (dead wood, conifer needles, etc.), and of environmental factors, such as wind, which help dissipate the heat away from the source. The threshold temperature for plant survival is 60-70 °C, which is a much lower range than the values reached by wildfires near the soil surface (100-150 °C). Most living tissues are thus killed by fire, although the time of exposure to the fire has an influence on the extent of damage as well. Most of the seeds present in the superficial layers of the soil are also killed by the fire. However, frequently dormant seeds present at deeper layers can survive and their germination can actually be stimulated by the fire.

Destructive as fires may be in many dryland environments, they also serve a useful function as being a catalyst for regeneration. Fire plays an important role in accelerating nutrient cycles and their availability to plants. Many non-volatile elements (such as K, P, Ca, etc.) can become more rapidly available after the passage of fire, when they get concentrated in the surface ashes. However, most of the nitrogen present in plants is lost to the atmosphere during fires.

Several plant communities have characteristics that indicate strategies for survival against this sometime devastating environmental feature. Such characteristics include seed dispersal (e.g., *Pinus* spp.) and removal of dormancy triggered by high temperature, trunks protected by thick and fire-repellant bark (e.g., *Quercus suber*), rapid sprouting of new vegetative tissues from epicormic buds (e.g., *Eucalyptus*, *Myrtus*), bulbs, rhizomes, etc. to replaces the tissues damaged by fire. Typical fire-adapted communities include the Mediterranean *maquis*, the Californian *chaparral*, the Australian *kwongan*, and the South African *fynbos*.

6.3. HIGH IRRADIANCE

Areas that are exposed to drought are frequently characterized by cloudless sky, low relative humidity, and scarce vegetation. In such conditions, solar radiation can reach very high levels because less light is intercepted by clouds, water molecules present in the atmosphere or vegetation. For most plants, solar radiation is not only the primary source of energy for photosynthesis, but also acts as a stimulus regulating many growth and developmental processes (e.g., flowering). The direct thermal effects (warming) of solar radiation also allow many metabolic processes to proceed optimally. The thermal effects of solar radiation also help drive evapotranspiration and ultimately water shortages if replenishment from rain and irrigation are inadequate. However, solar radiation can also cause injury and damage, for instance through mutagenesis and through excessive absorption by plants. Very high irradiances can damage the photosynthetic system or leaves in which photosynthetic metabolism has been inhibited by other processes such as water stress or extreme temperatures. Shade-adapted plants are most susceptible to excessive irradiances. Damage can be a result of photooxydation, where bleaching of chlorophyll occurs, or photoinhibition, where no bleaching occurs. Photoinhibition has been

defined as the reversible decrease in photosynthetic capacity induced by the exposure of photosynthetic tissues to high fluxes of photosynthetically active radiation (PAR, 400-700 nm) (Ort, 2001). The decrease in efficiency results largely from changes in photosystem II (PSII), and in particular the D1 protein, although photoinhibition of photosystem I (PSI) has also been reported in some species (Terashima et al., 1994). When excess light hits the protein D1, the excess quanta produced during the light reaction are transferred to the reaction centers instead of being disposed of as heat or fluorescence. This increases the amount of reducing power by creating singlet oxygen or free radicals, which easily damage or destroy the D1 protein itself. If a receptor for this reducing power is present, then the damage can be alleviated. Consequently, the damaged D1 protein has to be removed and replaced with a newly synthesized molecule. If the conditions leading to photoinhibition are persistent during the growing season, the damage can be significant. Photoinhibition can in fact cause decrease in both quantum efficiency and maximum photosynthetic activity. It has been estimated that in natural willow populations the daily depression in carbon assimilation induced by photoinhibition accounts for reduction in biomass of up to 10% (Long et al., 1994). Photoinhibition can be probed by measuring photosynthetic electron transport as oxygen exchange or chlorophyll *a* fluorescence, both of which reflect the electron transfer through the two photosystems (Bolhar-Nordenkamp and Oquist, 1993). Such measurements have shown strong correlations between photoinhibition and the inactivation of the D1 reaction center polypeptide of PS II which binds plastoquinone Q_B (Long et al., 1994). Plants have evolved a variety of mechanisms to avoid high-irradiation damage, for example, leaf movements (heliotropisms), developing highly reflective surfaces (e.g. pubescence), or rapid resynthesis of damaged, plastid pigments and proteins.

C₃ plants, especially those that are more shade-tolerant or more adapted to shady conditions, are more vulnerable to photoinhibition when they are suddenly exposed to high radiation levels. Conversely, sun-adapted plants and plants with C₄ metabolism are less susceptible to this harmful process. Photoinhibition is more common when high radiation levels follow periods characterized by low temperatures. Plant populations living in deserts or at high altitudes, where such climatic conditions are more frequent, are thus more exposed to photoinhibition. In these environments, the depression in photosynthetic rates is even more detrimental due to the limited resources that are available for plant growth.

6.4. SALINITY

Areas exposed to drought are characterized by high soil and plant evaporation rates which lead to depletion of water reserves not replenished by the scarce or absent precipitations. When evaporation exceeds precipitation, soil moisture decreases and salts become more concentrated in the rootzone. Salt accumulation in the soil is a phenomenon that has great repercussions on plant physiology and influence plant communities. Accumulation of salts in the environment induces two types of stresses in plants. First, accumulation of dissolved solutes reduces soil osmotic potential, which lowers the soil water potential (see Chapter 2). This affects the general water balance because plants have to develop an even lower water potential to maintain a downhill potential gradient between roots and soil to preserve water and nutrient uptake. The other consequence of accumulation of salts is the toxic effect caused by high concentration of ions both in the soil and at cellular level. Based on these differences, Levitt (1980) distinguished between salt and ion stresses. Salt stress was defined as the condition of a plant exposed to a salt concentration high enough to lower water potential by 0.05-0.1 MPa; ion stress occurs when the total ion concentration is not high, but the stress is induced by one particular species of ion which can result toxic to plants.

The extent of adaptation varies greatly among species and certain plants have evolved mechanisms for dealing with these stresses. Plants that thrive in the presence of high levels of salts and can complete their reproductive cycle are called *halophytes*, and are usually native to

saline soils. *Salt-tolerant* are defined as those plants which are well adapted to high levels of salt. *Non-halophytes* (sometimes called less correctly *glycophytes*, “sweet plants”) are plants that show toxicity symptoms even at relatively low levels of salt. Another classification distinguishes between plants that tolerate high sodium levels (*natrophilic* plants) and plants are affected by very low levels of soil sodium content (*natrophobic*). Distinction is also made between plants that can live in soil with high calcium content (i.e., in conditions of high pH soil), called *calcicole*, and plants that are more adapted to soil with less calcium content (low pH), known as *calcifuge*.

When referring to the effects of salts in the soil, *sodicity* indicates high concentration of sodium in the soil, whereas *salinity* refers to a high concentration of total salts. The two concepts are often related because soils with a high concentration of salts often have high sodium content. However, the opposite is not always true.

Although there are similarities between the low soil water potential conditions induced by drought and by excess salt content, there are differences in the mechanisms of adaptation initiated by plants (Munns, 2002). During soil desiccation the amount of water available to plants keeps decreasing with time, thus creating ever-decreasing water potentials and continuous renewal of adaptation strategies, such as osmotic adjustment. Conversely, in saline environments, water is usually available and the concentration of salts remains more or less stable, thus creating low, constant water potential conditions.

7. Mechanisms of adaptation

Water stress is not limited to plants living in environments characterized by low soil moisture content. Typically, even well-irrigated plants experience moderate water deficit conditions sometime during the day. This occurs because the evaporative demand, transpiration and evaporation are high and water movement within plants is relatively slow. Consequently, xylem tension increases with the intensity of the stress. Compared to other environmental stresses, such as hypoxia or temperature stresses, drought can take several days, sometime weeks, before it becomes severe enough to affect plant growth. Consequently, if the dehydration process of a plant cell or tissue is sufficiently slow, plants can develop changes in physiological and developmental processes to respond to the unfavorable conditions.

If the water stress condition continues for a prolonged period, however, plants may die of desiccation, unless mechanisms of resistance are initiated. Two main types of mechanism can help reduce the stress condition: 1) prevention or reduction of water loss in certain tissues or organs or 2) increase in rates of absorption and translocation of water. While the first type is usually activated as a quick response to the unfavorable conditions, the second type of reaction typically required anatomical/ morphological adaptations and are thus more efficient for prolonged stresses.

7.1. MORPHOLOGICAL ADAPTATIONS TO WATER STRESS

Most species that have evolved in environments where drought events occur more or less frequently have developed mechanisms of adaptation that enable them to avoid the hottest period of the year. It is quite common for many annual species, such as *Camissonia claviformis*, a drought avoider species native to the Death Valley, California, to complete their reproductive life during the cooler months. A few perennial species, such as in *Euphorbia dendroides*, a Mediterranean shrub, keep their leaves during winter and/or spring and abscise them with the onset of the hot season.

Other species can accurately control leaf angle and leaf folding in order to keep their leaves either parallel (paraheliotropic sun tracking) or perpendicular (diaheliotropic sun tracking) to the direct sunrays. Species with paraheliotropic sun tracking minimize the interception of solar radiation thus maintaining lower temperature. On the other hand, plants with diaheliotropic sun tracking maximize their interception and therefore carbon assimilation, which enables them to

grow rapidly during the favorable conditions (e.g., after rainfall). Increasing the fraction of light that is reflected before it can be absorbed is another method for plants to ensure survival during high temperature stress. In environments with intense solar radiation and high temperatures, it is very frequent for leaves and other organs to be covered with reflective hairs and waxes. Such barriers reduce the amount of energy reaching the vital portions of the plants and decrease the need of evaporative cooling. An example of this type of adaptation can be found in the California desert brittlebush, *Encelia farinosa*, which has green, nearly hairless leaves in winter and develops more pubescent, silvery leaves only when this desert shrub begins to experience water stress during the spring. The new leaves are highly reflective and absorb less than 60% of the intercepted PAR, versus approximately 85% absorption of the non-pubescent leaf type (Ehleringer and Bjorkman, 1978).

As mentioned above, convection of excess heat is another mechanism allowing leaves to dissipate part of the energy derived from interception of solar radiation. Morphological features, such as small, dissected leaves, can reduce boundary layer resistance (r_b), which is the portion of resistance of the transpiration pathway which is caused by the layer of unstirred air surrounding the leaf surface and through which water vapor must diffuse before reaching the turbulent air of the atmosphere. There is a direct relationship between r_b and the thickness of the boundary layer δ , which depends on the wind speed u and leaf size d as

$$\delta \propto \sqrt{\frac{d}{u}}. \quad (3)$$

Consequently, under the same wind conditions, the larger a leaf is, the thicker is δ . With a thick δ , the r_b can be so large that, when the air surrounding the leaf is very still, the layer of unstirred air can be the primary obstacle to transpiration, and therefore to transpirational cooling. This explains why species, such as mesquite, adapted to hot environments, usually have small leaves or dissected leaves with very small leaflets.

Phenological adaptations are often accompanied by modifications at cellular level. Oleander (*Nerium oleander*) is a species native to the Mediterranean basin and very resistant to drought and high temperatures. In this species, a thick cuticle contributes to the reduction of water losses while an increased degree of saturation of fatty acids in membrane lipids makes the membrane less fluid, thus increasing the plant's resistance to high temperatures (Raison et al., 1982).

Conditions of water deficit decrease stomatal aperture and consequently carbon assimilation. Growth of all organs is thus reduced in drought condition. As a result, a reduction in root growth would be expected when the soil water potential declines. Nevertheless, several studies have reported an increase in the proportion of photoassimilates allocated in the root system, associated with water deficit conditions (Buwalda and Lenz, 1992; Hsiao and Jing, 1987). If the stress is not too extreme, plants can maintain root growth in order to increase the absorbing surface and explore bigger soil volume, with consequent increase in the root to shoot ratio. The allocation of photoassimilates for root growth could be interpreted as an investment for following unfavorable periods (Buwalda and Lenz, 1992). This adaptation mechanism is part of what Brouwer (1983) defined as 'functional equilibrium', by which plants respond to a decrease in above-ground resources with increased allocation to shoots, whereas they respond to a decrease in below-ground resources with increased allocation to roots.

7.2. PHYSIOLOGICAL ADAPTATIONS TO WATER STRESS

Loss of turgor induced by drought triggers physiological and biochemical adjustments that are as important for turgor maintenance as morphological adaptations. The importance of elastic and osmotic adjustments for plant resistance to water stress have been highlighted (Schulte and Henry, 1992). *Elastic adjustment* includes physical modifications in the cells, which make them more elastic, thereby facilitating tissue shrinkage during dehydration. *Osmotic adjustment*, or

osmoregulation, is defined as the active accumulation of solutes inside the cell, with the consequent lowering of Ψ_s . This mechanism helps maintaining turgor and water absorption even at low tissue water potentials and prevents mechanical damages to plasma membranes. For mesophytes, the decrease in Ψ_s is usually between 0.2 and 0.8 MPa, but it can be greater in plants adapted to extremely dry conditions. The solutes that are most typically involved in this mechanism are small inorganic ions (mainly K^+), amino acids, organic acids and sugars. They are accumulated preferentially in the vacuoles, in order not to interfere with the structure and functioning of cytosolic enzymes. High solute concentrations can contribute to a greater capacity for turgor maintenance, but the contribution of electrolytes to osmotic adjustment is usually relatively low, if compared with other solutes. Inorganic ions can be toxic and disruptive to organelles, enzymes, and membrane-bound processes, whereas organic ions may serve as more compatible solutes, being tolerated at high concentrations in the cytoplasm (Ahmad et al., 1979; Bieleski, 1982). *Osmolytes* (also referred to as compatible solutes or osmoprotectants) are low molecular weight organic compounds that can be safely synthesized and accumulated in the cytoplasm without interfering with enzyme functions. Many are the plants and bacteria that synthesize osmolytes in response to environmental stresses (Tarczynski et al., 1993). Their main function is to maintain a water potential equilibrium within the cell when solutes are actively accumulated in the vacuole to increase the cell tolerance against abiotic stresses (Tarczynski et al., 1993). The level of many osmolytes increases in fact during the stress and declines when the stress is relieved. Examples of osmolytes are sugar alcohols (sorbitol and mannitol are the two most common ones), the amino acid proline, and glycinebetaine, a quaternary amine. In many species belonging to the *Rosaceae* family, sorbitol is the primary product of photosynthesis and the most actively translocated form of carbohydrate. In apple leaves it has been found to accumulate during drought stress (Bieleski, 1982) and perhaps this compatible solute plays a role in lowering leaf osmotic potential. However, not enough research has been conducted on young leaves, root and stems to determine its importance (Wang et al., 1995). In certain trees, such as apple, mature leaves seem to be able to adjust osmotically, whereas young leaves and tips seem not to be able to do this. The opposite seems to occur in peach, where immature leaves indicate osmotic adjustment and mature leaves do not (Wang et al., 1995). Osmotic adjustment in drought-stressed roots was observed by Ranney et al. (1991) for cherry trees. Numerous results have been obtained on experiments conducted on transgenic tobacco plants where the gene for mannitol was inserted (Tarczynski et al., 1993). Naturally, tobacco is not a mannitol-producer, but transformed plants produced mannitol (up to a maximum concentration of 100 mM) and seemed more tolerant to salinity stress. Genetically modified plants were also able to produce new growth and new leaves, when exposed for 30 days to 250 mM NaCl. There are no assumptions on how accumulation of intracellular mannitol may lead to new growth. The maintained production of roots and leaves, rather than a reallocation of resources, could explain the increased height and weight in transgenic plants (Tarczynski et al., 1993). Trehalose, a non-reducing disaccharide of glucose, is an osmoprotectant which plays an important role in stress protection in a large variety of organisms ranging from bacteria and fungi to invertebrate animals by effectively stabilizing dehydrated enzymes and lipid membranes, thereby protecting biological structures from damage during desiccation. During severe dehydration trehalose can act as a substitute for water, thus maintaining the native folding and biological activity of proteins, and preventing denaturation and aggregation. Despite the wide distribution of trehalose in microorganisms and invertebrates, trehalose had until recently only been found in a few plant species, notably highly desiccation-tolerant, resurrection plants (club mosses *Selaginella lepidophylla* and the angiosperm *Myrothamnus flabellifolius*), so named because of their unique ability to fully recover from a state of almost complete loss of water (Goddijn and van Dun, 1999). These resurrection plants can accumulate trehalose at levels approaching 1% of dry weight under non-stress conditions, whereas the majority of plants do not appear to accumulate easily-detectable amounts of trehalose. However, genes that encode enzymes of trehalose synthesis, i.e., trehalose-6-phosphate

synthase (TPS) and trehalose-6-phosphate phosphatase (TPP), have been recently identified in a number of plants. Transgenic rice and tobacco plants expressing these genes have been shown to exhibit increased drought tolerance (Garg et al., 2002; Pilon-Smits et al., 1998).

As indicated in Figure 1, osmotic adjustment is not one of the first responses to water stress; instead, it follows loss of turgor, stomatal closure and reduction in carbon assimilation rate. However, the role of such mechanism is significant. Leaves that can adjust osmotically can in fact maintain turgor, thus maintaining cell elongation, growth and stomatal aperture at lower water potentials. Osmotic adjustment is a phenomenon that is not limited to leaves. Root cells can accumulate solutes actively into their vacuoles as well. It seems however that the magnitude of the adjustment is smaller in root cells than in leaf cells. The role of osmotic adjustment is the same in root and in leaf cells: reduce Ψ_s to favor water uptake from the surrounding cells (or from the soil, in case of root cells) in order to maintain turgor, cell extension and tissue growth.

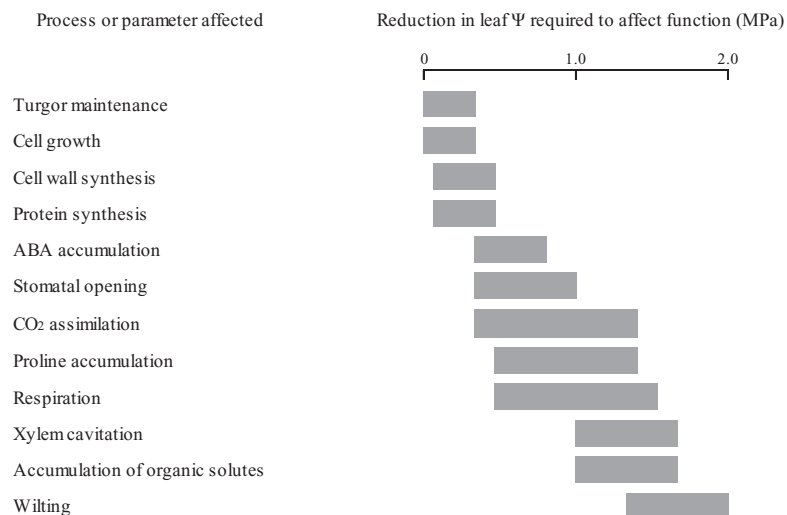


Figure 1. Responses of mesophytic plants to reduction of soil water availability. The horizontal bars indicate the range of lowering of leaf water potential at which symptoms first appear (Modified from Hsiao, 1973).

7.2.1. *C₄* and *CAM* species

Some plant species have developed distinctive modes of photosynthesis, which confer them higher water-use efficiency, thus reducing water needs and increasing their resistance to more arid environments. The group of plants known as *C₄* includes over 8000 species of angiosperms, both monocotyledons and dicotyledons, distributed among 16 different families. A prime example of *C₄* plants is given by Gramineae of warmer regions like sugarcane, maize or tropical grasses. *C₄* plants are so called because the first stable intermediates of photosynthesis present in their leaves are the four-carbon acids malate and aspartate (all other plants are known as *C₃* because 3-phosphoglycerate is the first intermediate of the Calvin cycle). The primary carboxylation process in *C₄* plants is not catalyzed by rubisco, as in *C₃* plants, but by phosphoenolpyruvate (PEP) carboxylase. The *C₄* photosynthesis is made possible by peculiar cells that are present in the leaves. A typical leaf of *C₄* plants is in fact anatomically different from that of a *C₃* plant. While all chloroplast-containing cells (mesophyll cells) in a *C₃* leaf have similar structure, the leaf of a *C₄* plants has two distinct cell types containing chloroplasts: mesophyll and bundle sheath cells

(this particular structure is sometime referred to as Kranz anatomy, from the German word for wreath). After the C_4 acid is formed in the mesophyll cell, it is shuttled to the bundle sheath cells where decarboxylation of the C_4 acid takes place. The CO_2 released is then fixed by rubisco and converted into carbohydrate by the Calvin cycle, just like in C_3 plants. This mechanism allows the creation of a high concentration of CO_2 at the carboxylation site of rubisco, which facilitates carboxylation. In addition, the fact that rubisco is kept in the bundle sheath cells (i.e., not in direct contact with the external atmosphere) results in the elimination of photorespiration. The C_4 cycle is considered a direct adaptation to dry environments because it helps overcome the harmful effects of high temperature on photosynthesis. The suppression of photorespiration, combined with the high affinity that PEP carboxylase has for its substrate (HCO_3^-), enables C_4 plants to maintain photosynthetic and water use efficiencies equal or greater than those of C_3 plants. This allows them to live longer when subjected to water deficit conditions. However, if the stress is prolonged, they too eventually undergo severe damage and death from causes other than starvation.

While C_4 plants separate spatially PEP-carboxylation from rubisco-carboxylation, another group of plants, called CAM (crassulacean acid metabolism) plants, have the two carboxylation processes occurring in the same leaf cell but temporally separated. Plants with CAM photosynthesis have evolved in particularly dry environments and are usually considered as water savers. CAM metabolism was first researched in the 1800's in the Crassulaceae (Crassula, Sedum, Kalanchoe, etc.) family, hence its name. However, we now know that such metabolism is not exclusive to the Crassulaceae family, but it is present in more than 20,000 species of plants. Other families with CAM photosynthesis include Euphorbiaceae, Agavaceae, Cactaceae, and Orchidaceae. The peculiarity of CAM plants is that they can store considerable amounts of water in their shoots by keeping their stomata closed during the hot, dry days and fixing CO_2 during the cool, desert nights, when transpirative forces are lower. As in C_4 plants, the key enzyme for such a process is PEP carboxylase, located in the cytosol, which fixes CO_2 at night to form malate, a four-carbon organic acid, which is temporarily stored in the vacuole. During the day, the stored malate is transported to the chloroplasts where it gets decarboxylated, and the released CO_2 is fixed by rubisco in the Calvin cycle. Thanks to this mechanism, CAM plants have a better WUE than non-CAM plants (C_3) and can reduce water losses by 75-90% (Taiz and Zaiger, 2002).

The common ice plant (*Mesembryanthemum crystallinum*), a plant native to the Namibian Desert, South Africa, is capable of switching from C_3 to CAM metabolism. Such a transition occurs as young plants in their natural habitat experience drought and increasing salinity as the season progresses. This phenomenon could be based on changes in the external conditions alone but indications suggest the shift in metabolism is also part of the plant's developmental program (Cushman and Borland, 2002). *Mesembryanthemum crystallinum* has provided many clues about the mechanisms employed by stress-tolerant plants to survive extreme conditions. A variant of the CAM mechanism, called *CAM-idling*, allows certain CAM plants (or parts of them) to survive for prolonged periods of time with their stomata constantly closed thus losing extremely small amounts of water. Such a phenomenon is possible because the CO_2 that is released by respiration is fixed again into malate by PEP carboxylase. CAM-idling is in most cases the mechanism that allows portions of cacti or other succulents to survive for long periods (up to several months) after detachment from the plant.

8. Conclusions

During the last two- three decades, some of the most relevant concepts in water stress have been highlighted. We now know for example that root signals exert a somewhat dynamic control over stomatal behavior, although the exact modes of action are still extensively being investigated. Current evidence indicates that these signals are chemical in nature, but the potential role of signals of different nature (pH and ion gradients, action potentials, etc.) should not be overlooked,

as these types of signals have important roles in the responses of plants to other stresses (e.g., cold, wounding, attack of pathogens) and in systemic acquired resistance.

Despite the progress obtained in this area of plant science, our understanding of the mechanisms of adaptation is insufficient to predict a plant's response and how these mechanisms may be interacting and integrating. One of the biggest obstacles derives from the fact that it is very difficult to separate the effects of different interrelated stress factors. In addition, we are still not able to assess the importance of one particular physiological or morphological adaptation relative to another.

The capacity that plants have to adjust to the environment is one of their most important attributes. Such ability is fundamental because it permits plants to colonize the most diverse environments. The immense variability of plant responses, however, makes it often difficult to generalize and categorize the different plant responses. This chapter has attempted to provide an overview of some of the typical morphological and physiological mechanisms of adaptation to water stress and to evaluate their importance in the light of plant survival. With water becoming increasingly scarce, it will be extremely valuable to extend our knowledge on water stress physiology. The areas subjected to drought and high temperature conditions are increasing alarmingly. Ecosystems are disappearing due to climate change and diversion of natural waters to human activities. Water has always been one of the major constraints for crop production and ecosystem dynamics (see Chapter 3, 5, 18). The combined expertise of ecologists, breeders, plant physiologists, hydrologists, and biometeorologists is now more than ever before important to better understand these adaptive mechanisms and to select for improved stress tolerance in crop plants.

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