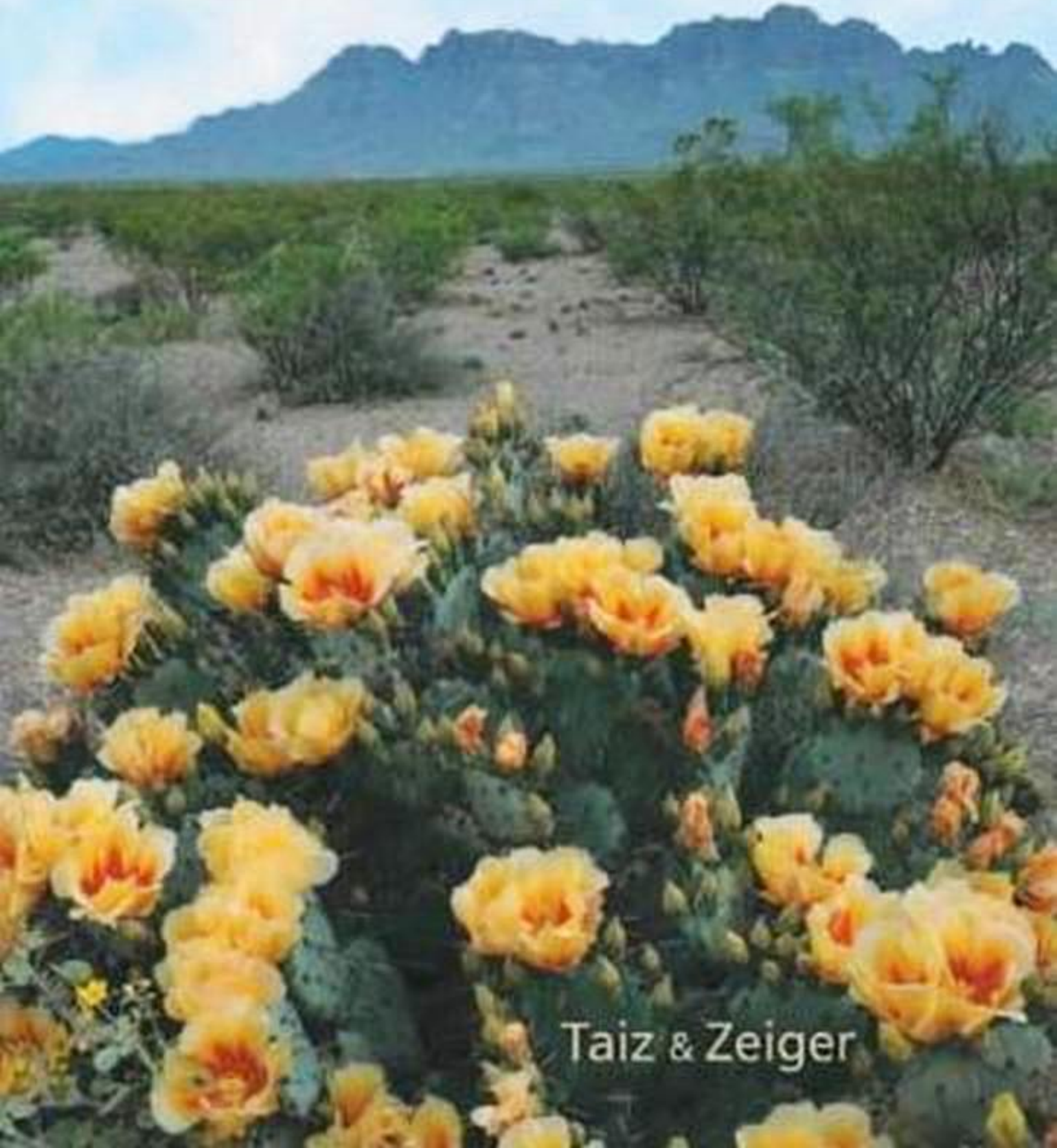
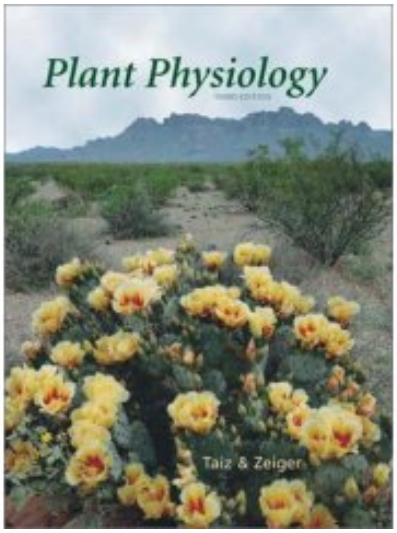
Plant Physiology

THIRD EDITION



Taiz & Zeiger



Plant Physiology, 3rd ed

by Lincoln Taiz and Eduardo Zeiger

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Book Description

With this Third Edition, the authors and contributors set a new standard for textbooks in the field by tailoring the study of plant physiology to virtually every student—providing the basics for introductory courses without sacrificing the more challenging material sought by upper-division and graduate-level students.

Key pedagogical changes to the text will result in a shorter book. Material typically considered prerequisite for plant physiology courses, as well as advanced material from the Second Edition, will be removed and posted at an affiliated Web site, while many new or revised figures and photographs (now in full color), study questions, and a glossary of key terms will be added. Despite the streamlining of the text, the new edition incorporates all the important new developments in plant physiology, especially in cell, molecular, and developmental biology.

The Third Edition's interactive Web component is keyed to textbook chapters and referenced from the book. It includes WebTopics (elaborating on selected topics discussed in the text), WebEssays (discussions of cutting-edge research topics, written by those who did the work), additional study questions (by chapter), additional references, and suggestions for further reading.

Book Info

Plant Physiology textbook covers the transport and translocation of water and solutes, biochemistry and metabolism, and growth and development. Twenty-three scientists contributed to the text.

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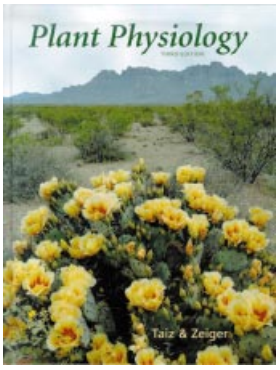

Plant physiology. 3rd edn.
L. Taiz and E. Zeiger.
Sunderland: Sinauer Associates. $104·95. 690 pp.

Plant physiology is part of the essential core curriculum every botanist has to master. As usually non-motile organisms that are, in most cases, fixed to a single locality for their entire lifetime, plants have special needs to cope with widely disparate, and often highly changeable environmental conditions. Physiological adaptations play as great a role in the evolutionary struggle for life of a plant as morphological ones.

Plant physiology by Taiz and Zeiger (and a plethora of contributing expert authors) is a well-received, established textbook aimed at students taking introductory courses in the field. One's first impression of the book is one of excellent craftsmanship: from the eye-catching cover, to the quality of the paper and print, this third edition of *Plant physiology* is not only comprehensive, it is attractive. A single encounter will turn the first-time user into a potential buyer. The book is subdivided into 25 chapters, grouped into three larger sections (water, metabolism and development) that cover the major topics of modern plant physiology. All topics are treated in a very balanced way, with approximately equal weight being lent to each. Starting with the basics of each subject, the reader is taken to the very forefront of current knowledge. The writing style is succinct and lucid throughout, and the text is arranged in a two-column format that is very reader-friendly. Specific topics are easy to find using the detailed table of contents or index.

In the light of the explosive growth of our understanding of physiological processes in plants resulting from technological advances in the field of molecular biology, it is an amazing achievement to find that the authors have managed to keep the book's length to a 'mere' 690 pages. That this has not been achieved at the expense of including recent literature is borne out throughout the book: figures 19–41, for example, have been adopted from a 2001 publication. The extensive reference lists that conclude each chapter also demonstrate how up-to-date this third edition is, with a large proportion of the references dating from the last 5 years. The transfer of the apprentice from the textbook to the forefront research literature is greatly facilitated in this way. A glossary giving a brief explanation of many technical terms reinforces this impression.

An outstanding feature of this textbook is the large number of crisp figures, most of them in full colour. Although also rendering the figures aesthetically pleasing, the use of colour usually serves a didactic purpose (which may well be its primary cause). I found none of the figures to be overladen with detail nor of inappropriate (microscopically small or inflated) size. Full marks for this!

Plant Physiology is a modern textbook with a refreshing style and layout. The overall impression is one of a well-thought-out teaching aid. The authors/editors have achieved a remarkable feat in bringing it up-to-date without allowing any dead wood to accumulate (a symptom of ageing that unfortunately befalls the majority of textbooks as they advance through numerous editions). Let's hope they will be able to retain this phoenix-like rejuvenating potential in future editions. In its third edition, *Plant physiology* successfully defends its position in the top league of botanical textbooks. It is excellently produced, attractive and fun to use. It can even make an aged botanist wish he were an undergraduate student again!

Thomas Lazar

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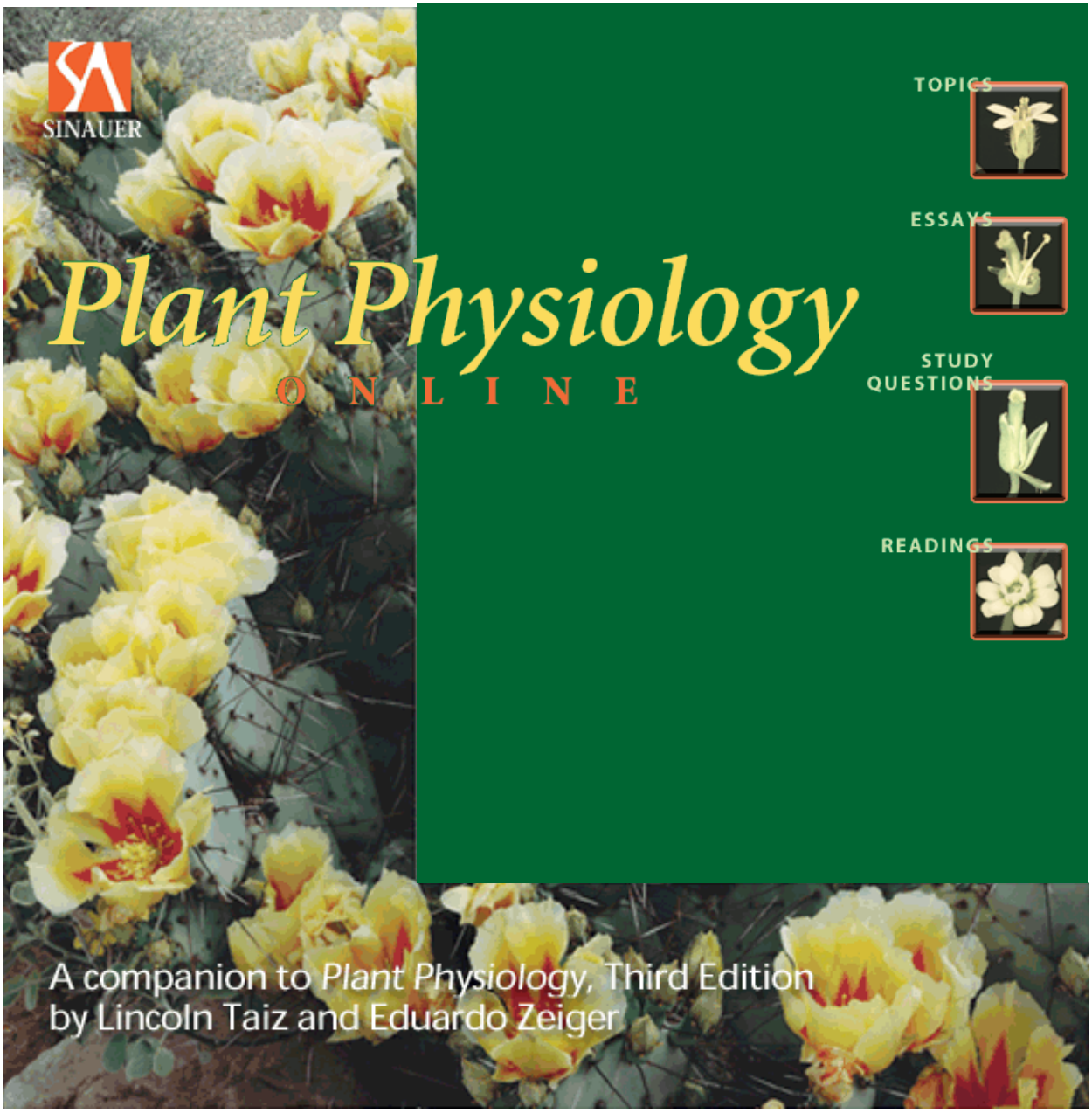


Chapter

25

Stress Physiology

IN BOTH NATURAL AND AGRICULTURAL CONDITIONS, plants are frequently exposed to environmental stresses. Some environmental factors, such as air temperature, can become stressful in just a few minutes; others, such as soil water content, may take days to weeks, and factors such as soil mineral deficiencies can take months to become stressful. It has been estimated that because of stress resulting from climatic and soil conditions (abiotic factors) that are suboptimal, the yield of field-grown crops in the United States is only 22% of the genetic potential yield (Boyer 1982).

In addition, stress plays a major role in determining how soil and climate limit the distribution of plant species. Thus, understanding the physiological processes that underlie stress injury and the adaptation and acclimation mechanisms of plants to environmental stress is of immense importance to both agriculture and the environment.

The concept of plant stress is often used imprecisely, and stress terminology can be confusing, so it is useful to start our discussion with some definitions. **Stress** is usually defined as an external factor that exerts a disadvantageous influence on the plant. This chapter will concern itself with environmental or abiotic factors that produce stress in plants, although biotic factors such as weeds, pathogens, and insect predation can also produce stress. In most cases, stress is measured in relation to plant survival, crop yield, growth (biomass accumulation), or the primary assimilation processes (CO_2 and mineral uptake), which are related to overall growth.

The concept of stress is intimately associated with that of **stress tolerance**, which is the plant's fitness to cope with an unfavorable environment. In the literature the term *stress resistance* is often used interchangeably with *stress tolerance*, although the latter term is preferred. Note that an environment that is stressful for one plant may not be stressful for another. For example, pea (*Pisum sativum*) and soybean (*Glycine max*) grow best at about 20°C and 30°C, respectively. As temperature increases, the pea shows signs of heat stress much sooner than the soybean. Thus the soybean has greater heat stress tolerance.

If tolerance increases as a result of exposure to prior stress, the plant is said to be **acclimated** (or hardened). Acclimation can be distinguished from **adaptation**, which usually refers to a *genetically* determined level of resistance acquired by a process of selection over many generations. Unfortunately, the term *adaptation* is sometimes used in the literature to indicate acclimation. And to add to the complexity, we will see later that gene expression plays an important role in acclimation.

Adaptation and acclimation to environmental stresses result from integrated events occurring at all levels of organization, from the anatomical and morphological level to the cellular, biochemical, and molecular level. For example, the wilting of leaves in response to water deficit reduces both water loss from the leaf and exposure to incident light, thereby reducing heat stress on leaves.

Cellular responses to stress include changes in the cell cycle and cell division, changes in the endomembrane system and vacuolization of cells, and changes in cell wall architecture, all leading to enhanced stress tolerance of cells. At the biochemical level, plants alter metabolism in various ways to accommodate environmental stresses, including producing osmoregulatory compounds such as proline and glycine betaine. The molecular events linking the perception of a stress signal with the genomic responses leading to tolerance have been intensively investigated in recent years.

In this chapter we will examine these principles, and the ways in which plants adapt and acclimate to water deficit, salinity, chilling and freezing, heat, and oxygen deficiency in the root biosphere. Air pollution, an important source of plant stress, is discussed in **Web Essay 25.1**. Although it is convenient to examine each of these stress factors separately, most are interrelated, and a common set of cellular, biochemical, and molecular responses accompanies many of the individual acclimation and adaptation processes.

For example, water deficit is often associated with salinity in the root biosphere and with heat stress in the leaves (resulting from decreased evaporative cooling due to low transpiration), and chilling and freezing lead to reductions in water activity and osmotic stress. We will also see that plants often display cross-tolerance—that is, tolerance to one stress induced by acclimation to another. This behavior implies that mechanisms of resistance to several stresses share many common features.

WATER DEFICIT AND DROUGHT RESISTANCE

In this section we will examine some drought resistance mechanisms, which are divided into several types. First we can distinguish between **desiccation postponement** (the ability to maintain tissue hydration) and **desiccation tolerance** (the ability to function while dehydrated), which are sometimes referred to as drought tolerance at high and low water potentials, respectively. The older literature often uses the term *drought avoidance* (instead of *drought tolerance*), but this term is a misnomer because drought is a meteorological condition that is tolerated by all plants that survive it and avoided by none. A third category, **drought escape**, comprises plants that complete their life cycles during the wet season, before the onset of drought. These are the only true "drought avoiders."

Among the desiccation postponers are water savers and water spenders. *Water savers* use water conservatively, preserving some in the soil for use late in their life cycle; *water spenders* aggressively consume water, often using prodigious quantities. The mesquite tree (*Prosopis* sp.) is an example of a water spender. This deeply rooted species has ravaged semiarid rangelands in the southwestern United States, and because of its prodigious water use, it has prevented the reestablishment of grasses that have agronomic value.

Drought Resistance Strategies Vary with Climatic or Soil Conditions

The water-limited productivity of plants (Table 25.1) depends on the total amount of water available and on the water-use efficiency of the plant (see Chapters 4 and 9). A plant that is capable of acquiring more water or that has higher water-use efficiency will resist drought better. Some plants possess adaptations, such as the C_4 and CAM modes of photosynthesis that allow them to exploit more arid environments. In addition, plants possess acclimation mechanisms that are activated in response to water stress.

Water deficit can be defined as any water content of a tissue or cell that is below the highest water content exhibited at the most hydrated state. When water deficit develops slowly enough to allow changes in developmental processes, water stress has several effects on growth, one of which is a limitation in leaf expansion. Leaf area is important because photosynthesis is usually proportional to it. However, rapid leaf expansion can adversely affect water availability.

TABLE 25.1
Yields of corn and soybean crops in the United States

	Crop yield (percentage of 10-year average)		
Year	Corn	Soybean	
1979	104	106	
1980	87	88	Severe drought
1981	104	100	
1982	108	104	
1983	77	87	Severe drought
1984	101	93	
1985	112	113	
1986	113	110	
1987	114	111	
1988	80	89	Severe drought

Source: U.S. Department of Agriculture 1989.

If precipitation occurs only during winter and spring, and summers are dry, accelerated early growth can lead to large leaf areas, rapid water depletion, and too little residual soil moisture for the plant to complete its life cycle. In this situation, only plants that have some water available for reproduction late in the season or that complete the life cycle quickly, before the onset of drought (exhibiting drought escape), will produce seeds for the next generation. Either strategy will allow some reproductive success.

The situation is different if summer rainfall is significant but erratic. In this case, a plant with large leaf area, or one capable of developing large leaf area very quickly, is better suited to take advantage of occasional wet summers. One acclimation strategy in these conditions is a capacity for both vegetative growth and flowering over an extended period. Such plants are said to be *indeterminate* in their growth habit, in contrast to *determinate* plants, which develop preset numbers of leaves and flower over only very short periods.

In the discussions that follow, we will examine several acclimation strategies, including inhibited leaf expansion, leaf abscission, enhanced root growth, and stomatal closure.

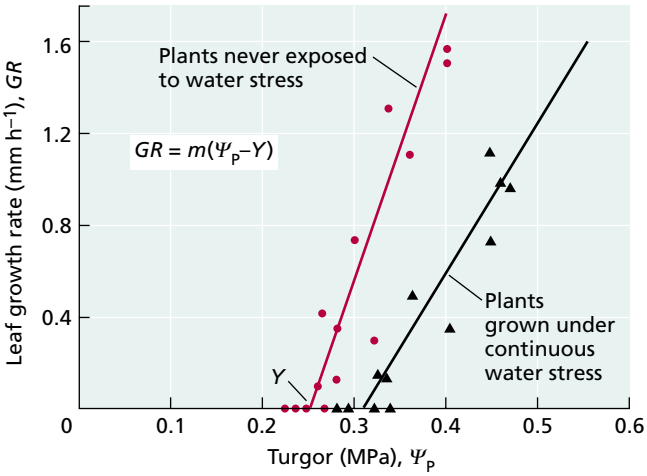


FIGURE 25.1 Dependence of leaf expansion on leaf turgor. Sunflower (*Helianthus annuus*) plants were grown either with ample water or with limited soil water to produce mild water stress. After rewatering, plants of both treatment groups were stressed by the withholding of water, and leaf growth rates (GR) and turgor (Ψ_p) were periodically measured. Both decreased extensibility (m) and increased threshold turgor for growth (Y) limit the leaf's capacity to grow after exposure to stress. (After Matthews et al. 1984.)

Decreased Leaf Area Is an Early Adaptive Response to Water Deficit

Typically, as the water content of the plant decreases, its cells shrink and the cell walls relax (see Chapter 3). This decrease in cell volume results in lower turgor pressure and the subsequent concentration of solutes in the cells. The plasma membrane becomes thicker and more compressed because it covers a smaller area than before. Because turgor reduction is the earliest significant biophysical effect of water stress, turgor-dependent activities such as leaf expansion and root elongation are the most sensitive to water deficits (Figure 25.1).

Cell expansion is a turgor-driven process and is extremely sensitive to water deficit. Cell expansion is described by the relationship

$$GR = m(\Psi_p - Y) \qquad (25.1)$$

where GR is growth rate, Ψ_p is turgor, Y is the yield threshold (the pressure below which the cell wall resists plastic, or nonreversible, deformation), and m is the wall extensibility (the responsiveness of the wall to pressure).

This equation shows that a decrease in turgor causes a decrease in growth rate. Note also that besides showing that growth slows down when stress reduces Ψ_p, Equation 25.1 shows that Ψ_p need decrease only to the value of Y, not to zero, to eliminate expansion. In normal conditions, Y is usually only 0.1 to 0.2 MPa less than Ψ_p, so small decreases in water content and turgor can slow down or fully stop growth.

Water stress not only decreases turgor, but also decreases m and increases Y. Wall extensibility (m) is normally greatest when the cell wall solution is slightly acidic. In part, stress decreases m because cell wall pH typically rises during stress. The effects of stress on Y are not well understood, but presumably they involve complex structural changes of the cell wall (see Chapter 15) that may not be readily reversed after relief of stress. Water-deficient plants tend to become rehydrated at night, and as a result substantial leaf growth occurs at that time. Nonetheless, because of changes in m and Y, the growth rate is still lower than that of unstressed plants having the same turgor (see Figure 25.1).

Because leaf expansion depends mostly on cell expansion, the principles that underlie the two processes are similar. Inhibition of cell expansion results in a slowing of leaf expansion early in the development of water deficits. The smaller leaf area transpires less water, effectively conserving a limited water supply in the soil over a longer period. Reduction in leaf area can thus be considered a first line of defense against drought.

In indeterminate plants, water stress limits not only leaf size, but also leaf number, because it decreases both the number and the growth rate of branches. Stem growth has been studied less than leaf expansion, but stem growth is probably affected by the same forces that limit leaf growth during stress.

Keep in mind, too, that cell and leaf expansion also depend on biochemical and molecular factors beyond those that control water flux. Much evidence supports the view that plants change their growth rates in response to

stress by coordinately controlling many other important processes such as cell wall and membrane biosynthesis, cell division, and protein synthesis (Burssens et al. 2000).

Water Deficit Stimulates Leaf Abscission

The total leaf area of a plant (number of leaves × surface area of each leaf) does not remain constant after all the leaves have matured. If plants become water stressed after a substantial leaf area has developed, leaves will senesce and eventually fall off (Figure 25.2). Such a leaf area adjustment is an important long-term change that improves the plant's fitness in a water-limited environment. Indeed, many drought-deciduous, desert plants drop all their leaves during a drought and sprout new ones after a rain. This cycle can occur two or more times in a single season. Abscission during water stress results largely from enhanced synthesis of and responsiveness to the endogenous plant hormone ethylene (see Chapter 22).

Water Deficit Enhances Root Extension into Deeper, Moist Soil

Mild water deficits also affect the development of the root system. Root-to-shoot biomass ratio appears to be governed by a functional balance between water uptake by the root and photosynthesis by the shoot (see Figure 23.6). Simply stated, *a shoot will grow until it is so large that water uptake by the roots becomes limiting to further growth*; conversely, *roots will grow until their demand for photosynthate from the shoot equals the supply.* This functional balance is shifted if the water supply decreases.

As discussed already, leaf expansion is affected very early when water uptake is curtailed, but photosynthetic activity is much less affected. Inhibition of leaf expansion reduces the consumption of carbon and energy, and a greater proportion of the plant's assimilates can be distributed to the root system, where they can support further root growth. At the same time, the root apices in dry soil lose turgor.

All these factors lead to a preferential root growth into the soil zones that remain moist. As water deficits progress, the upper layers of the soil usually dry first. Thus, plants commonly show a mainly shallow root system when all soil layers are wetted, and a loss of shallow roots and proliferation of deep roots as water in top layers of the soil is depleted. Deeper root growth into wet soil can be considered a second line of defense against drought.

Enhanced root growth into moist soil zones during stress requires allocation of assimilates to the growing root tips. During water deficit, assimilates are directed to the fruits and away from the roots (see Chapter 10). For this reason the enhanced water uptake resulting from root growth is less pronounced in reproductive plants than in vegetative plants. Competition for assimilates between roots and fruits is one explanation for the fact that plants are generally more sensitive to water stress during reproduction.

FIGURE 25.2 The leaves of young cotton (*Gossypium hirsutum*) plants abscise in response to water stress. The plants at left were watered throughout the experiment; those in the middle and at right were subjected to moderate stress and severe stress, respectively, before being watered again. Only a tuft of leaves at the top of the stem is left on the severely stressed plants. (Courtesy of B. L. McMichael.)

Stomata Close during Water Deficit in Response to Abscisic Acid

The preceding sections focused on changes in plant development during slow, long-term dehydration. When the onset of stress is more rapid or the plant has reached its full leaf area before initiation of stress, other responses protect the plant against immediate desiccation. Under these conditions, stomata closure reduces evaporation from the existing leaf area. Thus, stomatal closure can be considered a third line of defense against drought.

Uptake and loss of water in guard cells changes their turgor and modulates stomatal opening and closing (see Chapters 4 and 18). Because guard cells are located in the leaf epidermis, they can lose turgor as a result of a direct loss of water by evaporation to the atmosphere. The decrease in turgor causes stomatal closure by **hydropassive closure**. This closing mechanism is likely to operate in air of low humidity, when direct water loss from the guard cells is too rapid to be balanced by water movement into the guard cells from adjacent epidermal cells.

A second mechanism, called **hydroactive closure**, closes the stomata when the whole leaf or the roots are dehydrated and depends on metabolic processes in the guard cells. A reduction in the solute content of the guard cells results in water loss and decreased turgor, causing the stomata to close; thus the hydraulic mechanism of hydroactive closure is a reversal of the mechanism of stomatal opening. However, the control of hydroactive closure differs in subtle but important ways from stomatal opening.

Solute loss from guard cells can be triggered by a decrease in the water content of the leaf, and abscisic acid (ABA) (see Chapter 23) plays an important role in this

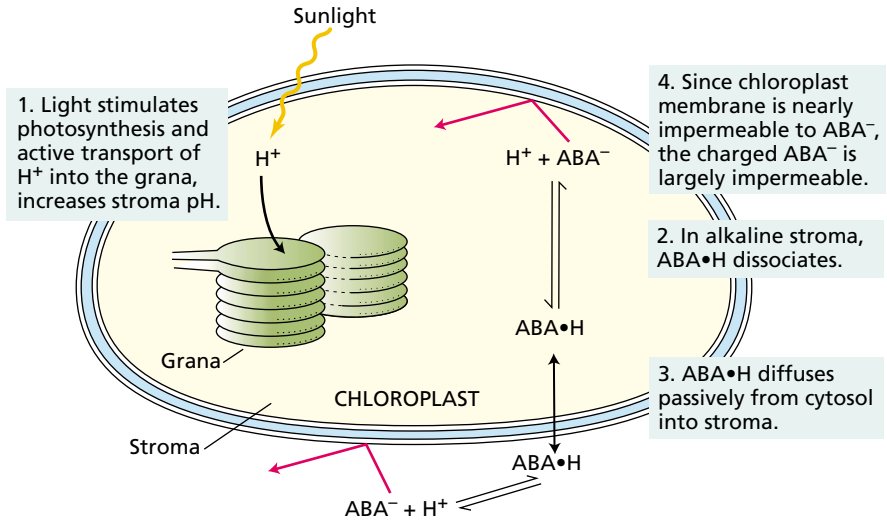


FIGURE 25.3 Accumulation of ABA by chloroplasts in the light. Light stimulates proton uptake into the grana, making the stroma more alkaline. The increased alkalinity causes the weak acid ABA•H to dissociate into H^+ and the ABA^- anion. The concentration of ABA•H in the stroma is lowered below the concentration in the cytosol, and the concentration difference drives the passive diffusion of ABA•H across the chloroplast membrane. At the same time, the concentration of ABA^- in the stroma increases, but the chloroplast membrane is almost impermeable to the anion (red arrows), which thus remains trapped. This process continues until the ABA•H concentrations in the stroma and the cytosol are equal. But as long as the stroma remains more alkaline, the total ABA concentration (ABA•H + ABA^-) in the stroma greatly exceeds the concentration in the cytosol.

process. Abscisic acid is synthesized continuously at a low rate in mesophyll cells and tends to accumulate in the chloroplasts. When the mesophyll becomes mildly dehydrated, two things happen:

1. Some of the ABA stored in the chloroplasts is released to the apoplast (the cell wall space) of the mesophyll cell (Hartung et al. 1998). The redistribution of ABA depends on pH gradients within the leaf, on the weak-acid properties of the ABA molecule, and on the permeability properties of cell membranes (Figure 25.3). The redistribution of ABA makes it possible for the transpiration stream to carry some of the ABA to the guard cells.
2. ABA is synthesized at a higher rate, and more ABA accumulates in the leaf apoplast. The higher ABA concentrations resulting from the higher rates of ABA synthesis appear to enhance or prolong the initial closing effect of the stored ABA. The mechanism of ABA-induced stomatal closure is discussed in Chapter 23.

Stomatal responses to leaf dehydration can vary widely both within and across species. The stomata of some dehydration-postponing species, such as cowpea (*Vigna unguiculata*) and cassava (*Manihot esculenta*), are unusually responsive to decreasing water availability, and stomatal conductance and transpiration decrease so much that leaf water potential (Ψ_w; see Chapters 3 and 4) may remain nearly constant during drought.

Chemical signals from the root system may affect the stomatal responses to water stress (Davies et al. 2002). Stomatal conductance is often much more closely related to soil water status than to leaf water status, and the only plant part that can be directly affected by soil water status is the root system. In fact, dehydrating only part of the root system may cause stomatal closure even if the well-watered portion of the root system still delivers ample water to the shoots.

When corn (*Zea mays*) plants were grown with roots trained into two separate pots and water was withheld from only one of the pots, the stomata closed partially, and the leaf water potential increased, just as in the dehydration postponers already described. These results show that stomata can respond to conditions sensed in the roots. Besides ABA (Sauter et al. 2001), other signals, such as pH and inorganic ion redistribution, appear to play a role in long-distance signaling between the roots and the shoots (Davies et al. 2002).

Water Deficit Limits Photosynthesis within the Chloroplast

The photosynthetic rate of the leaf (expressed per unit leaf area) is seldom as responsive to mild water stress as leaf expansion is (Figure 25.4) because photosynthesis is much less sensitive to turgor than is leaf expansion. However, mild water stress does usually affect both leaf photosynthesis and stomatal conductance. As stomata close during early stages of water stress, water-use efficiency (see Chapters 4 and 9) may increase (i.e., more CO_2 may be taken up per unit of water transpired) because stomatal closure inhibits transpiration more than it decreases intercellular CO_2 concentrations.

As stress becomes severe, however, the dehydration of mesophyll cells inhibits photosynthesis, mesophyll metabolism is impaired, and water-use efficiency usually decreases. Results from many studies have shown that the relative effect of water stress on stomatal conductance is significantly larger than that on photosynthesis. The response of photosynthesis and stomatal conductance to water stress can be partitioned by exposure of stressed

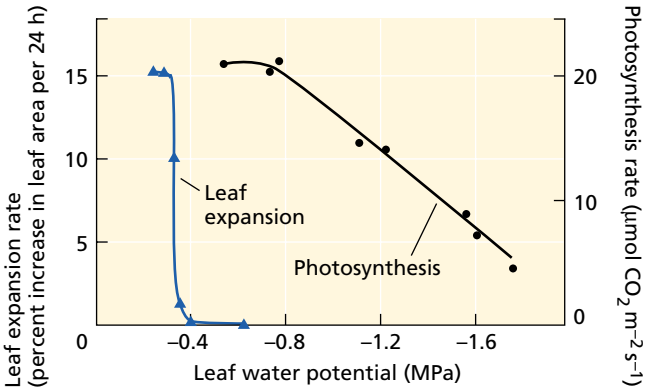


FIGURE 25.4 Effects of water stress on photosynthesis and leaf expansion of sunflower (*Helianthus annuus*). This species is typical of many plants in which leaf expansion is very sensitive to water stress, and it is completely inhibited under mild stress levels that hardly affect photosynthetic rates. (After Boyer 1970.)

leaves to air containing high concentrations of CO_2. Any effect of the stress on stomatal conductance is eliminated by the high CO_2 supply, and differences between photosynthetic rates of stressed and unstressed plants can be directly attributed to damage from the water stress to photosynthesis.

Does water stress directly affect translocation? Water stress decreases both photosynthesis and the consumption of assimilates in the expanding leaves. As a consequence, water stress indirectly decreases the amount of photosynthate exported from leaves. Because phloem transport depends on turgor (see Chapter 10), decreased water potential in the phloem during stress may inhibit the movement of assimilates. However, experiments have shown that translocation is unaffected until late in the stress period, when other processes, such as photosynthesis, have already been strongly inhibited (Figure 25.5).

This relative insensitivity of translocation to stress allows plants to mobilize and use reserves where they are needed (e.g., in seed growth), even when stress is extremely severe. The ability to continue translocating assimilates is a key factor in almost all aspects of plant resistance to drought.

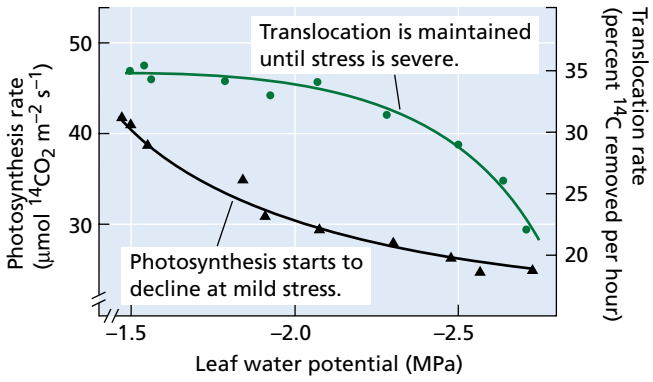


FIGURE 25.5 Relative effects of water stress on photosynthesis and translocation in sorghum (*Sorghum bicolor*). Plants were exposed to $^{14}CO_2$ for a short time interval. The radioactivity fixed in the leaf was taken as a measure of photosynthesis, and the loss of radioactivity after removal of the $^{14}CO_2$ source was taken as a measure of the rate of assimilate translocation. Photosynthesis was affected by mild stress, whereas, translocation was unaffected until stress was severe. (After Sung and Krieg 1979.)

Osmotic Adjustment of Cells Helps Maintain Plant Water Balance

As the soil dries, its matric potential (see **Web Topic 3.3**) becomes more negative. Plants can continue to absorb water only as long as their water potential (Ψ_w) is lower (more negative) than that of the soil water. Osmotic adjustment, or accumulation of solutes by cells, is a process by which water potential can be decreased without an accompanying decrease in turgor or decrease in cell volume. Recall Equation 3.6 from Chapter 3: $\Psi_w = \Psi_s + \Psi_p$. The change in cell water potential results simply from changes in solute potential (Ψ_s), the osmotic component of Ψ_w.

Osmotic adjustment is a net increase in solute content per cell that is independent of the volume changes that result from loss of water. The decrease in Ψ_s is typically limited to about 0.2 to 0.8 MPa, except in plants adapted to extremely dry conditions. Most of the adjustment can usually be accounted for by increases in concentration of a variety of common solutes, including sugars, organic acids, amino acids, and inorganic ions (especially K^+).

Cytosolic enzymes of plant cells can be severely inhibited by high concentrations of ions. The accumulation of ions during osmotic adjustment appears to be restricted to the vacuoles, where the ions are kept out of contact with enzymes in the cytosol or subcellular organelles. Because of this compartmentation of ions, other solutes must accumulate in the cytoplasm to maintain water potential equilibrium within the cell.

These other solutes, called **compatible solutes** (or compatible osmolytes), are organic compounds that do not interfere with enzyme functions. Commonly accumulated compatible solutes include the amino acid proline, sugar alcohols (e.g., sorbitol and mannitol), and a quaternary amine called glycine betaine. Synthesis of compatible solutes helps plants adjust to increased salinity in the rooting zone, as discussed later in this chapter.

Osmotic adjustment develops slowly in response to tissue dehydration. Over a time course of several days, other changes (such as growth or photosynthesis) are also taking place. Thus it can be argued that osmotic adjustment is not an independent and direct response to water deficit, but a result of another factor, such as decreased growth rate.

Nonetheless, leaves that are capable of osmotic adjustment clearly can maintain turgor at lower water potentials than nonadjusted leaves. Maintaining turgor enables the continuation of cell elongation and facilitates higher stomatal conductances at lower water potentials. This suggests that osmotic adjustment is an acclimation that enhances dehydration tolerance.

How much extra water can be acquired by the plant because of osmotic adjustment in the leaf cells? Most of the extractable soil water is held in spaces (filled with water and air) from which it is readily removed by roots (see Chapter 4). As the soil dries, this water is used first, leaving behind the small amount of water that is held more tightly in small pores.

Osmotic adjustment enables the plant to extract more of this tightly held water, but the increase in total available water is small. Thus the cost of osmotic adjustment in the leaf is offset by rapidly diminishing returns in terms of water availability to the plant, as can be seen by a comparison of the water relations of adjusting and nonadjusting species (Figure 25.6). These results show that osmotic adjustment promotes dehydration tolerance but does not have a major effect on productivity (McCree and Richardson 1987).

Osmotic adjustment also occurs in roots, although the process in roots has not been studied so extensively as in leaves. The absolute magnitude of the adjustment is less in roots than in leaves, but as a percentage of the original tissue solute potential (Ψ_s), it can be larger in roots than in leaves. As with leaves, these changes may in many cases increase water extraction from the previously explored soil only slightly. However, osmotic adjustment can occur in the root meristems, enhancing turgor and maintaining root growth. This is an important component of the changes in root growth patterns as water is depleted from the soil.

Does osmotic adjustment increase plant productivity? Researchers have engineered the accumulation of osmoprotective solutes by conventional plant breeding, by physiological methods (inducing adjustment with controlled water deficits), and through the use of transgenic plants expressing genes for solute synthesis and accumulation. However, the engineered plants grow more slowly, and they are only slightly more tolerant to osmotic stresses. Thus the use of osmotic adjustment to improve agricultural performance is yet to be perfected.

Water Deficit Increases Resistance to Liquid-Phase Water Flow

When a soil dries, its resistance to the flow of water increases very sharply, particularly near the *permanent wilting point*. Recall from Chapter 4 that at the permanent wilting point (usually about –1.5 MPa), plants cannot regain turgor pressure even if all transpiration stops (for more details on the relationship between soil hydraulic conductivity and soil water potential, see **Figure 4.2.A in Web Topic 4.2**). Because of the very large soil resistance to water flow, water delivery to the roots at the permanent wilting point is too slow to allow the overnight rehydration of plants that have wilted during the day.

Rehydration is further hindered by the resistance within the plant, which has been found to be larger than the resistance within the soil over a wide range of water deficits (Blizzard and Boyer 1980). Several factors may contribute to the increased plant resistance to water flow during drying. As plant cells lose water, they shrink. When roots shrink, the root surface can move away from the soil particles that hold the water, and the delicate root hairs may be damaged. In addition, as root extension slows during soil drying, the outer layer of the root cortex (the hypodermis) often becomes more extensively covered with suberin,

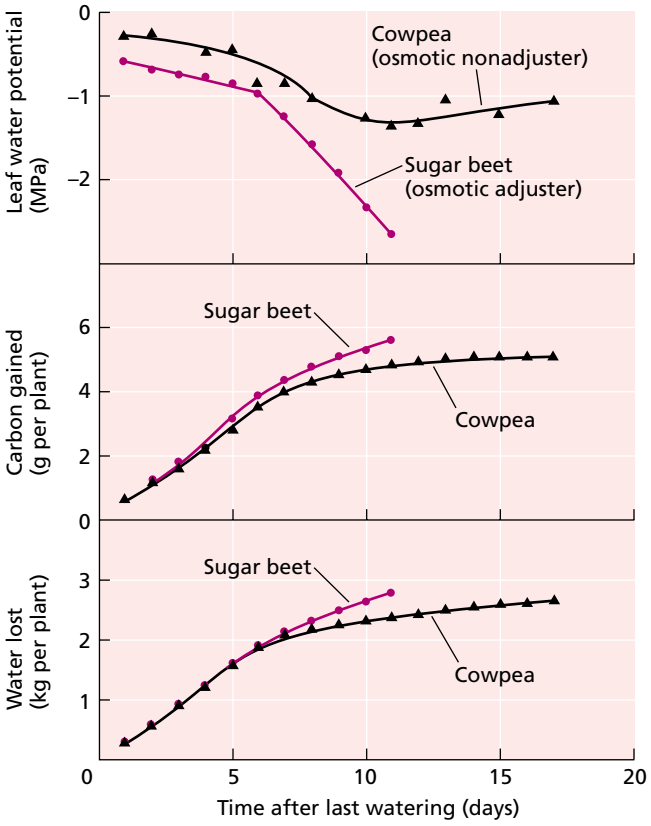


FIGURE 25.6 Water loss and carbon gain by sugar beet (*Beta vulgaris*), an osmotically adjusting species, and cowpea (*Vigna unguiculata*), a nonadjusting species that conserves water during stress by stomatal closure. Plants were grown in pots and subjected to water stress. On any given day after the last watering, the sugar beet leaves maintained a lower water potential than the cowpea leaves, but photosynthesis and transpiration during stress were only slightly greater in the sugar beet. The major difference between the two plants was the leaf water potential. These results show that osmotic adjustment promotes dehydration tolerance but does not have a major effect on productivity. (After McCree and Richardson 1987.)

a water-impermeable lipid (see Figure 4.4), increasing the resistance to water flow.

Another important factor that increases resistance to water flow is *cavitation*, or the breakage of water columns under tension within the xylem. As we saw in Chapter 4, transpiration from leaves "pulls" water through the plant by creating a tension on the water column. The cohesive forces that are required to support large tensions are present only in very narrow columns in which the water adheres to the walls.

Cavitation begins in most plants at moderate water potentials (–1 to –2 MPa), and the largest vessels cavitate first. For example, in trees such as oak (*Quercus*), the large-diameter vessels that are laid down in the spring function as a low-resistance pathway early in the growing season, when ample water is available. As the soil dries out during the summer, these large vessels cease functioning, leaving the small-diameter vessels produced during the stress period to carry the transpiration stream. This shift has long-lasting consequences: Even if water becomes available, the original low-resistance pathway remains nonfunctional, reducing the efficiency of water flow.

Water Deficit Increases Wax Deposition on the Leaf Surface

A common developmental response to water stress is the production of a thicker cuticle that reduces water loss from the epidermis (cuticular transpiration). Although waxes are deposited in response to water deficit both on the surface and within the cuticle inner layer, the inner layer may be more important in controlling the rate of water loss in ways that are more complex than by just increasing the amount of wax present (Jenks et al. in press).

A thicker cuticle also decreases CO_2 permeability, but leaf photosynthesis remains unaffected because the epidermal cells underneath the cuticle are nonphotosynthetic. Cuticular transpiration, however, accounts for only 5 to 10% of the total leaf transpiration, so it becomes significant only if stress is extremely severe or if the cuticle has been damaged (e.g., by wind-driven sand).

Water Deficit Alters Energy Dissipation from Leaves

Recall from Chapter 9 that evaporative heat loss lowers leaf temperature. This cooling effect can be remarkable: In Death Valley, California—one of the hottest places in the world—leaf temperatures of plants with access to ample water were measured to be 8°C below air temperatures. In warm, dry climates, an experienced farmer can decide whether plants need water simply by touching the leaves because a rapidly transpiring leaf is distinctly cool to the touch. When water stress limits transpiration, the leaf heats up unless another process offsets the lack of cooling. Because of these effects of transpiration on leaf temperature, water stress and heat stress are closely interrelated (see the discussion of heat stress later in this chapter).

Maintaining a leaf temperature that is much lower than the air temperature requires evaporation of vast quantities of water. This is why adaptations that cool leaves by means other than evaporation (e.g., changes in leaf size and leaf orientation) are very effective in conserving water. When transpiration decreases and leaf temperature becomes warmer than the air temperature, some of the extra energy in the leaf is dissipated as sensible heat loss (see Chapter 9). Many arid-zone plants have very small leaves, which minimize the resistance of the boundary layer to the transfer of heat from the leaf to the air (see Figure 9.14).

Because of their low boundary layer resistance, small leaves tend to remain close to air temperature even when transpiration is greatly slowed. In contrast, large leaves have higher boundary layer resistance and dissipate less thermal energy (per unit leaf area) by direct transfer of heat to the air.

In larger leaves, leaf movement can provide additional protection against heating during water stress. Leaves that orient themselves away from the sun are called *paraheliotropic*; leaves that gain energy by orienting themselves normal (perpendicular) to the sunlight are referred to as *diaheliotropic* (see Chapter 9). Figure 25.7 shows the strong effect of water stress on leaf position in soybean. Other factors that can alter the interception of radiation include wilting, which changes the angle of the leaf, and leaf rolling in grasses, which minimizes the profile of tissue exposed to the sun.

Absorption of energy can also be decreased by hairs on the leaf surface or by layers of reflective wax outside the cuticle. Leaves of some plants have a gray-white appearance because densely packed hairs reflect a large amount of light. This hairiness, or **pubescence**, keeps leaves cooler by reflecting radiation, but it also reflects the visible wavelengths that are active in photosynthesis and thus it decreases carbon assimilation. Because of this problem, attempts to breed pubescence into crops to improve their water-use efficiency have been generally unsuccessful.

Osmotic Stress Induces Crassulacean Acid Metabolism in Some Plants

Crassulacean acid metabolism (CAM) is a plant adaptation in which stomata open at night and close during the day (see Chapters 8 and 9). The leaf-to-air vapor pressure difference that drives transpiration is much reduced at night, when both leaf and air are cool. As a result, the water-use efficiencies of CAM plants are among the highest measured. A CAM plant may gain 1 g of dry matter for only 125 g of water used—a ratio that is three to five times greater than the ratio for a typical C_3 plant (see Chapter 4).

CAM is very prevalent in succulent plants such as cacti. Some succulent species display facultative CAM, switching to CAM when subjected to water deficits or saline conditions (see Chapter 8). This switch in metabolism is a remarkable adaptation to stress, involving accumulation of the enzymes phosphoenolpyruvate (PEP) carboxylase (Figure 25.8), pyruvate–orthophosphate dikinase, and NADP malic enzyme, among others.

(A) Well-watered

(B) Mild water stress

(C) Severe water stress

FIGURE 25.7 Orientation of leaflets of field-grown soybean (*Glycine max*) plants in the normal, unstressed, position (A); during mild water stress (B); and during severe water stress (C). The large leaf movements induced by mild stress are quite different from wilting, which occurs during severe stress. Note that during mild stress (B), the terminal leaflet has been raised, whereas the two lateral leaflets have been lowered; each is almost vertical. (Courtesy of D. M. Oosterhuis.)

Osmotic Stress Changes Gene Expression

As noted earlier, the accumulation of compatible solutes in response to osmotic stress requires the activation of the metabolic pathways that biosynthesize these solutes. Several genes coding for enzymes associated with osmotic adjustment are turned on (up-regulated) by osmotic stress and/or salinity, and cold stress. These genes encode enzymes such as the following (Buchanan et al. 2000):

- Δ'^{1}-Pyrroline-5-carboxylate synthase, a key enzyme in the proline biosynthetic pathway
- Betaine aldehyde dehydrogenase, an enzyme involved in glycine betaine accumulation
- *myo*-Inositol 6-*O*-methyltransferase, a rate-limiting enzyme in the accumulation of the cyclic sugar alcohol called pinitol

Several other genes that encode well-known enzymes are induced by osmotic stress. The expression of glyceraldehyde-3-phosphate dehydrogenase increases during osmotic stress, perhaps to allow an increase of carbon flow into organic solutes for osmotic adjustment. Enzymes involved in lignin biosynthesis are also controlled by osmotic stress.

Reduction in the activities of key enzymes also takes place. The accumulation of the sugar alcohol mannitol in response to osmotic stress appears not to be brought about by the up-regulation of genes producing enzymes involved in mannitol biosynthesis, but rather by the down-regulation of genes associated with sucrose production and mannitol degradation. In this way mannitol accumulation is enhanced during episodes of osmotic stress.

Other genes regulated by osmotic stress encode proteins associated with membrane transport, including ATPases

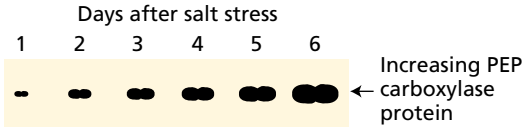


FIGURE 25.8 Increases in the content of phosphoenolpyruvate (PEP) carboxylase in ice plant, *Mesembryanthemum crystallinum*, during the salt-induced shift from C_3 metabolism to CAM. Salt stress was induced by the addition of 500 mM NaCl to the irrigation water. The PEP carboxylase protein was revealed in the gels by the use of antibodies and a stain. (After Bohnert et al. 1989.)

As discussed in Chapters 8 and 9, CAM metabolism involves many structural, physiological, and biochemical features, including changes in carboxylation and decarboxylation patterns, transport of large quantities of malate into and out of the vacuoles, and reversal of the periodicity of stomatal movements. Thus, CAM induction is a remarkable adaptation to water deficit that occurs at many levels of organization.

Table 25.2
The five groups of late embryogenesis abundant (LEA) proteins found in plants

Group (family name)[a]	Protein(s) in the group	Structural characteristics and motifs	Functional information/ proposed function
Group 1 (D-19 family)	Cotton D-19 Wheat Em (early methionine-labeled protein) Sunflower Ha ds10 Barley B19	Conformation is predominantly random coil with some predicted short α helices Charged amino acids and glycine are abundant	Contains more water of hydration than typical globular proteins Overexpression confers water deficit tolerance on yeast cells
Group 2 (D-11 family) (also referred to as dehydrins)	Maize DHN1, M3, RAB17 Cotton D-11 *Arabidopsis* pRABAT1, ERD10, ERD14 *Craterostigma* pcC 27-04, pcC 6-19 Tomato pLE4, TAS14 Barley B8, B9, B17 Rice pRAB16A Carrot pcEP40	Variable structure includes α helix–forming lysine-rich regions The consensus sequence for group 2 dehydrins is EKKGIMDKIKELPG The number of times this consensus repeats per protein varies Often contains a poly(serine) region Often contains regions of variable length rich in polar residues and either Gly or Ala., and Pro	Often localized to the cytoplasm or nucleus More acidic members of the family are associated with the plasma membrane May act to stabilize macromolecules at low water potential
Group 3 (D-7 family)	Barley HVA1 (ABA-induced) Cotton D-7 Wheat pMA2005, pMA1949 *Craterostigma* pcC3-06	Eleven amino-acid consensus sequence motif TAQAAKEKAXE is repeated in the protein Contains apparent amphipathic α helices Dimeric protein	Transgenic plants expressing HVA1 demonstrate enhanced water deficit stress tolerance D-7 is an abundant protein in cotton embryos (estimated concentration 0.25 m*M*) Each putative dimer of D-7 may bind as many as ten inorganic phosphates and their counterions
Group 4 (D-95 family)	Soybean D-95 *Craterostigma* pcC27-45	Slightly hydrophobic N-terminal region is predicted to form amphipathic α helices	In tomato, a gene encoding a similar protein is expressed in response to nematode feeding
Group 5 (D-113 family)	Tomato LE25 Sunflower Hads11 Cotton D-113	Family members share sequence homology at the conserved N terminus N-terminal region is predicted to form α helices C-terminal domain is predicted to be a random coil of variable length and sequence Ala, Gly, and Thr are abundant in the sequence	Binds to membranes and/or proteins to maintain structure during stress Possibly functions in ion sequestration to protect cytosolic metabolism When LE25 is expressed in yeast, it confers salt and freezing tolerance D-113 is abundant in cottonseeds (up to 0.3 m*M*)

[a]The protein family names are derived from the cotton seed proteins that are most similar to the family.
Source: After Bray et al. 2000.

(Niu et al. 1995) and the water channel proteins, *aquaporins* (see Chapter 3) (Maggio and Joly 1995). Several protease genes are also induced by stress, and these enzymes may degrade (remove and recycle) other proteins that are denatured by stress episodes. The protein *ubiquitin* tags proteins that are targeted for proteolytic degradation. Synthesis of the mRNA for ubiquitin increases in *Arabidopsis* upon desiccation stress. In addition, some *heat shock proteins* are

osmotically induced and may protect or renature proteins inactivated by desiccation.

The sensitivity of cell expansion to osmotic stress (see Figure 25.1) has stimulated studies of various genes that encode proteins involved in the structural composition and integrity of cell walls. Genes coding for enzymes such as *S*-adenosylmethionine synthase and peroxidases, which may be involved in lignin biosynthesis, have been shown to be controlled by stress.

A large group of genes that are regulated by osmotic stress was discovered by examination of naturally desiccating embryos during seed maturation. These genes code for so-called **LEA proteins** (named for *l*ate *e*mbryogenesis *a*bundant), and they are suspected to play a role in cellular membrane protection. Although the function of LEA proteins is not well understood (Table 25.2), they accumulate in vegetative tissues during episodes of osmotic stress. The proteins encoded by these genes are typically hydrophilic and strongly bind water. Their protective role might be associated with an ability to retain water and to prevent crystallization of important cellular proteins and other molecules during desiccation. They might also contribute to membrane stabilization.

More recently, microarray techniques have been used to examine the expression of whole genomes of some plants in response to stress. Such studies have revealed that large numbers of genes display changes in expression after plants are exposed to stress. Stress-controlled genes reflect up to 10% of the total number of rice genes examined (Kawasaki et al. 2001)

Osmotic stress typically leads to the accumulation of ABA (see Chapter 23), so it is not surprising that products of ABA-responsive genes accumulate during osmotic stresses. Studies of ABA-insensitive and ABA-deficient mutants have shown that numerous genes that are induced by osmotic stress are in fact induced by the ABA accumulated during the stress episode. However, not all genes that are up-regulated by osmotic stresses are ABA regulated. As discussed in the next section, other mechanisms for regulating gene expression of osmotic stress–regulated genes have been uncovered.

Stress-Responsive Genes Are Regulated by ABA-Dependent and ABA-Independent Processes

Gene transcription is controlled through the interaction of regulatory proteins (transcription factors) with specific regulatory sequences in the promoters of the genes they regulate (Chapter 14 on the web site discusses these processes in detail). Different genes that are induced by the same signal (desiccation or salinity, for example) are controlled by a signaling pathway leading to the activation of these specific transcription factors.

Studies of the promoters of several stress-induced genes have led to the identification of specific regulatory sequences for genes involved in different stresses. For example, the *RD29* gene contains DNA sequences that can be activated by osmotic stress, by cold, and by ABA (Yamaguchi-Shinozaki and Shinozaki 1994; Stockinger et al. 1997).

The promoters of ABA-regulated genes contain a six-nucleotide sequence element referred to as the **ABA response element** (**ABRE**), which probably binds transcriptional factors involved in ABA-regulated gene activation (see Chapter 23). The promoters of these genes, which are regulated by osmotic stress in an ABA-dependent manner, contain an alternative nine-nucleotide regulatory sequence element, the **dehydration response element** (**DRE**) which is recognized by an alternative set of proteins regulating transcription. Thus the genes that are regulated by osmotic stresses appear to be regulated either by signal transduction pathways mediated by the action of ABA (**ABA-dependent genes**), or by an **ABA-independent**, osmotic stress–responsive signal transduction pathway.

At least two signaling pathways have been implicated in the regulation of gene expression in an ABA-independent manner (Figure 25.9). Transacting *transcription factors* (called DREB1 and DREB2) that bind to the DRE elements in the promoters of osmotic stress–responsive genes are apparently activated by an ABA-independent signaling cascade. Other ABA-independent, osmotic stress–respon-

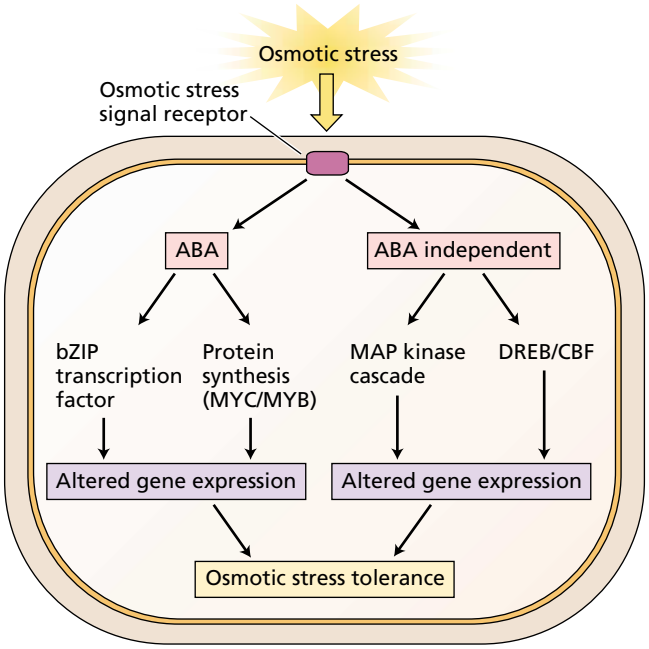


FIGURE 25.9 Signal transduction pathways for osmotic stress in plant cells. Osmotic stress is perceived by an as yet unknown receptor in the plasma membrane activating ABA-independent and an ABA-dependent signal transduction pathways. Protein synthesis participates in one of the ABA-dependent pathways involving MYC/MYB. The bZIP ABA-dependent pathway involves recognition of ABA-responsive elements in gene promoters. Two ABA-independent pathways, one involving the MAP kinase signaling cascade and the other involving DREBP/CBF-related transcription factors have also been demonstrated. (After Shinozaki and Yamaguchi-Shinozaki, 2000.)

sive genes appear to be directly controlled by the so-called MAP kinase signaling cascade of protein kinases (discussed in detail in Chapter 14 on the web site). Other changes in gene expression appear to be mediated via other mechanisms not involving DREBs.

This complexity and "cross-talk" found in signaling cascades, exemplified here by both ABA-dependent and ABA-independent pathways, is typical of eukaryotic signaling. Such complexity reflects the wealth of interaction between gene expression and the physiological processes mediating adaptation to osmotic stress.

HEAT STRESS AND HEAT SHOCK

Most tissues of higher plants are unable to survive extended exposure to temperatures above 45°C. Nongrowing cells or dehydrated tissues (e.g., seeds and pollen) can survive much higher temperatures than hydrated, vegetative, growing cells (Table 25.3). Actively growing tissues rarely survive temperatures above 45°C, but dry seeds can endure 120°C, and pollen grains of some species can endure 70°C. In general, only single-celled eukaryotes can complete their life cycle at temperatures above 50°C, and only prokaryotes can divide and grow above 60°C.

Periodic brief exposure to sublethal heat stresses often induces tolerance to otherwise lethal temperatures, a phenomenon referred to as **induced thermotolerance**. The mechanisms mediating induced thermotolerance will be discussed later in the chapter. As mentioned earlier, water and temperature stress are interrelated; shoots of most C_3 and C_4 plants with access to abundant water supply are maintained below 45°C by evaporative cooling; if water becomes limiting, evaporative cooling decreases and tissue temperatures increase. Emerging seedlings in moist soil may constitute an exception to this general rule. These seedlings may be exposed to greater heat stress than those in drier soils because wet, bare soil is typically darker and absorbs more solar radiation than drier soil.

TABLE 25.3
Heat-killing temperatures for plants

Plant	Heat-killing temperature (C°)	Time of exposure
Nicotiana rustica (wild tobacco)	49–51	10 min
Cucurbita pepo (squash)	49–51	10 min
Zea mays (corn)	49–51	10 min
Brassica napus (rape)	49–51	10 min
Citrus aurantium (sour orange)	50.5	15–30 min
Opuntia (cactus)	>65	—
Sempervivum arachnoideum (succulent)	57–61	—
Potato leaves	42.5	1 hour
Pine and spruce seedlings	54–55	5 min
Medicago seeds (alfalfa)	120	30 min
Grape (ripe fruit)	63	—
Tomato fruit	45	—
Red pine pollen	70	1 hour
Various mosses		
Hydrated	42–51	—
Dehydrated	85–110	—

Source: After Table 11.2 in Levitt 1980.

High Leaf Temperature and Water Deficit Lead to Heat Stress

Many CAM, succulent higher plants, such as *Opuntia* and *Sempervivum*, are adapted to high temperatures and can tolerate tissue temperatures of 60 to 65°C under conditions of intense solar radiation in summer (see Table 25.3). Because CAM plants keep their stomata closed during the day, they cannot cool by transpiration. Instead, they dissipate the heat from incident solar radiation by re-emission of longwave (infrared) radiation and loss of heat by conduction and convection (see Chapter 9).

On the other hand, typical, nonirrigated C_3 and C_4 plants rely on transpirational cooling to lower leaf temperature. In these plants, leaf temperature can readily rise 4 to 5°C above ambient air temperature in bright sunlight near midday, when soil water deficit causes partial stomatal closure or when high relative humidity reduces the potential for evaporative cooling. The physiological consequences of these increases in tissue temperature are discussed in the next section.

Increases in leaf temperature during the day can be pronounced in plants from arid and semiarid regions experiencing drought and high irradiance from sunshine. Heat stress is also a potential danger in greenhouses, where low air speed and high humidity decrease the rate of leaf cooling. A moderate degree of heat stress slows growth of the whole plant. Some irrigated crops, such as cotton, use transpirational cooling to dissipate heat. In irrigated cotton, enhanced transpirational cooling is associated with higher agronomic yields (see **Web Topic 25.1**).

At High Temperatures, Photosynthesis Is Inhibited before Respiration

Both photosynthesis and respiration are inhibited at high temperatures, but as temperature increases, photosynthetic rates drop before respiratory rates (Figure 25.10A and B). The temperature at which the amount of CO_2 fixed by photosynthesis, equals the amount of CO_2 released by respiration, in a given time interval is called the **temperature compensation point**.

At temperatures above the temperature compensation point, photosynthesis cannot replace the carbon used as a substrate for respiration. As a result, carbohydrate reserves decline, and fruits and vegetables lose sweetness. This imbalance between photosynthesis and respiration is one of the main reasons for the deleterious effects of high temperatures.

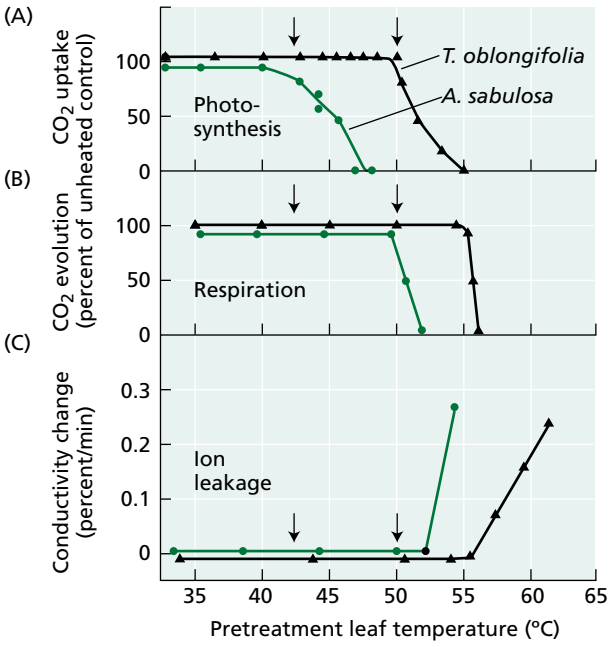


FIGURE 25.10 Response of frosted orache (*Atriplex sabulosa*) and Arizona honeysweet (*Tidestromia oblongifolia*) to heat stress. Photosynthesis (A) and respiration (B) were measured on attached leaves, and ion leakage (C) was measured in leaf slices submerged in water. At the beginning of the experiment, control rates were measured at a noninjurious 30°C. Attached leaves were then exposed to the indicated temperatures for 15 minutes and returned to the initial control conditions before the rates were recorded. Arrows indicate the temperature thresholds for inhibition of photosynthesis in each of the two species. Photosynthesis, respiration, and membrane permeability were all more sensitive to heat damage in *A. sabulosa* than in *T. oblongifolia*. In both species, however, photosynthesis was more sensitive to heat stress than either of the other two processes, and photosynthesis was completely inhibited at temperatures that were noninjurious to respiration. (From Björkman et al. 1980.)

In the same plant the temperature compensation point is usually lower for shade leaves than for sun leaves that are exposed to light (and heat). Enhanced respiration rates relative to photosynthesis at high temperatures are more detrimental in C_3 plants than in C_4 or CAM plants because the rates of both dark respiration and photorespiration are increased in C_3 plants at higher temperatures (see Chapter 8).

Plants Adapted to Cool Temperatures Acclimate Poorly to High Temperatures

The extent to which plants that are genetically adapted to a given temperature range can acclimate to a contrasting temperature range is illustrated by a comparison of the responses of two C_4 species: *Atriplex sabulosa* (frosted orache, family Chenopodiaceae) and *Tidestromia oblongifolia* (Arizona honeysweet, family Amaranthaceae).

A. sabulosa is native to the cool climate of coastal northern California, and *T. oblongifolia* is native to the very hot climate of Death Valley, California, where it grows in a temperature regime that is lethal for most plant species. When these species were grown in a controlled environment and their growth rates were recorded as a function of temperature, *T. oblongifolia* barely grew at 16°C, while *A. sabulosa* was at 75% of its maximum growth rate. By contrast, the growth rate of *A. sabulosa* began to decline between 25 and 30°C, and growth ceased at 45°C, the temperature at which *T. oblongifolia* growth showed a maximum (see Figure 25.10A) (Björkman et al. 1980). Clearly, neither species could acclimate to the temperature range of the other.

High Temperature Reduces Membrane Stability

The stability of various cellular membranes is important during high-temperature stress, just as it is during chilling and freezing. Excessive fluidity of membrane lipids at high temperatures is correlated with loss of physiological function. In oleander (*Nerium oleander*), acclimation to high temperatures is associated with a greater degree of saturation of fatty acids in membrane lipids, which makes the membranes less fluid (Raison et al. 1982).

At high temperatures there is a decrease in the strength of hydrogen bonds and electrostatic interactions between polar groups of proteins within the aqueous phase of the membrane. High temperatures thus modify membrane composition and structure and can cause leakage of ions (see Figure 25.10C). Membrane disruption also causes the inhibition of processes such as photosynthesis and respiration that depend on the activity of membrane-associated electron carriers and enzymes.

Photosynthesis is especially sensitive to high temperature (see Chapter 9). In their study of *Atriplex* and *Tidestromia*, O. Björkman and colleagues (1980) found that electron transport in photosystem II was more sensitive to high temperature in the cold-adapted *A. sabulosa* than in the heat-adapted *T. oblongifolia*. In these plants the enzymes ribulose-1,5-bisphosphate carboxylase, NADP:glyceraldehyde-3-phosphate dehydrogenase, and phosphoenolpyruvate carboxylase were less stable at high temperatures in *A. sabulosa* than in *T. oblongifolia*.

However, the temperatures at which these enzymes began to denature and lose activity were distinctly higher than the temperatures at which photosynthesis began to decline. These results suggest that early stages of heat injury to photosynthesis are more directly related to changes in membrane properties and to uncoupling of the energy transfer mechanisms in chloroplasts than to a general denaturation of proteins.

Several Adaptations Protect Leaves against Excessive Heating

In environments with intense solar radiation and high temperatures, plants avoid excessive heating of their leaves by decreasing their absorption of solar radiation. This adap-

tation is important in warm, sunny environments in which a transpiring leaf is near its upper limit of temperature tolerance. In these conditions, any further warming arising from decreased evaporation of water or increased energy absorption can damage the leaf.

Both drought resistance and heat resistance depend on the same adaptations: reflective leaf hairs and leaf waxes; leaf rolling and vertical leaf orientation; and growth of small, highly dissected leaves to minimize the boundary layer thickness and thus maximize convective and conductive heat loss (see Chapters 4 and 9). Some desert shrubs—for example, white brittlebush (*Encelia farinosa*, family Compositae)—have dimorphic leaves to avoid excessive heating: Green, nearly hairless leaves found in the winter are replaced by white, pubescent leaves in the summer.

At Higher Temperatures, Plants Produce Heat Shock Proteins

In response to sudden, 5 to 10°C rises in temperature, plants produce a unique set of proteins referred to as **heat shock proteins** (**HSPs**). Most HSPs function to help cells withstand heat stress by acting as molecular chaperones. Heat stress causes many cell proteins that function as enzymes or structural components to become unfolded or misfolded, thereby leading to loss of proper enzyme structure and activity.

Such misfolded proteins often aggregate and precipitate, creating serious problems within the cell. HSPs act as molecular chaperones and serve to attain a proper folding of misfolded, aggregated proteins and to prevent misfolding of proteins. This facilitates proper cell functioning at elevated, stressful temperatures.

Heat shock proteins were discovered in the fruit fly (*Drosophila melanogaster*) and have since been identified in other animals, and in humans, as well as in plants, fungi, and microorganisms. For example, when soybean seedlings are suddenly shifted from 25 to 40°C (just below the lethal temperature), synthesis of the set of mRNAs and proteins commonly found in the cell is suppressed, while transcription and translation of a set of 30 to 50 other proteins (HSPs) is enhanced. New mRNA transcripts for HSPs can be detected 3 to 5 minutes after heat shock (Sachs and Ho 1986).

Although plant HSPs were first identified in response to sudden changes in temperature (25 to 40°C) that rarely occur in nature, HSPs are also induced by more gradual rises in temperature that are representative of the natural environment, and they occur in plants under field conditions. Some HSPs are found in normal, unstressed cells, and some essential cellular proteins are homologous to HSPs but do not increase in response to thermal stress (Vierling 1991).

Plants and most other organisms make HSPs of different sizes in response to temperature increases (Table 25.4). The molecular masses of the HSPs range from 15 to 104 kDa (kilodaltons), and they can be grouped into five classes based on size. Different HSPs are localized to the nucleus, mitochondria, chloroplasts, endoplasmic reticulum, and cytosol. Members of the HSP60, HSP70, HSP90, and HSP100 groups act as molecular chaperones, involving ATP-dependent stabilization and folding of proteins, and the assembly of oligomeric proteins. Some HSPs assist in polypeptide transport across membranes into cellular compartments. HSP90s are associated with hormone receptors in animal cells and may be required for their activation, but there is no comparable information for plants.

Low-molecular-weight (15–30 kDa) HSPs are more abundant in higher plants than in other organisms. Whereas plants contain five to six classes of low-molecular-weight HSPs, other eukaryotes show only one class (Buchanan et al. 2000). The different classes of 15–30 kDa molecular-weight HSPs (smHSPs) in plants are distributed in the cytosol, chloroplasts, ER and mitochondria. The function of these small HSPs is not understood.

Cells that have been induced to synthesize HSPs show improved thermal tolerance and can tolerate exposure to temperatures that are otherwise lethal. Some of the HSPs are not unique to high-temperature stress. They are also induced by widely different environmental stresses or conditions, including water deficit, ABA treatment, wounding, low temperature, and salinity. Thus, cells previously

TABLE 25.4
The five classes of heat shock proteins found in plants

HSP class	Size (kDa)	Examples (Arabidopsis / prokaryotic)	Cellular location
HSP100	100–114	AtHSP101 / ClpB, ClpA/C	Cytosol, mitochondria, chloroplasts
HSP90	80–94	AtHSP90 / HtpG	Cytosol, endoplasmic reticulum
HSP70	69–71	AtHSP70 / DnaK	Cytosol/nucleus, mitochondria, chloroplasts
HSP60	57–60	AtTCP-1 / GroEL, GroES	Mitochondria, chloroplasts
smHSP	15–30	Various AtHSP22, AtHSP20, AtHSP18.2, AtHSP17.6 / IBPA/B	Cytosol, mitochondria, chloroplasts, endoplasmic reticulum

Source: After Boston et al. 1996.

exposed to one stress may gain cross-protection against another stress. Such is the case with tomato fruits, in which heat shock (48 hours at 38°C) has been observed to promote HSP accumulation and to protect cells for 21 days from chilling at 2°C.

A Transcription Factor Mediates HSP Accumulation in Response to Heat Shock

All cells seem to contain molecular chaperones that are constitutively expressed and function like HSPs. These chaperones are called **heat shock cognate proteins**. However, when cells are subjected to a stressful, but nonlethal heat episode, the synthesis of HSPs dramatically increases while the continuing translation of other proteins is dramatically lowered or ceases. This heat shock response appears to be mediated by a specific transcription factor (**HSF**) that acts on the transcription of HSP mRNAs.

In the absence of heat stress, HSF exists as monomers that are incapable of binding to DNA and directing transcription (Figure 25.11). Stress causes HSF monomers to associate into trimers that are then able to bind to specific sequence elements in DNA referred to as heat shock elements (HSEs). Once bound to the HSE, the trimeric HSF is phosphorylated and promotes the transcription of HSP mRNAs. HSP70 subsequently binds to HSF, leading to the dissociation of the HSF/HSE complex, and the HSF is subsequently recycled to the monomeric HSF form. Thus, by the action of HSF, HSPs accumulate until they become abundant enough to bind to HSF, leading to the cessation of HSP mRNA production.

HSPs Mediate Thermotolerance

Conditions that induce thermal tolerance in plants closely match those that induce the accumulation of HSPs, but that correlation alone does not prove that HSPs play an essential role in acclimation to heat stress. More conclusive experiments show that expression of an activated HSF induces constitutive synthesis of HSPs and increases the thermotolerance of *Arabidopsis*. Studies with *Arabidopsis* plants containing an antisense DNA sequence that reduces HSP70 synthesis showed that the high-temperature extreme at which the plants could survive was reduced by 2°C compared with controls, although the mutant plants grew normally at optimum temperatures (Lee and Schoeffl 1996).

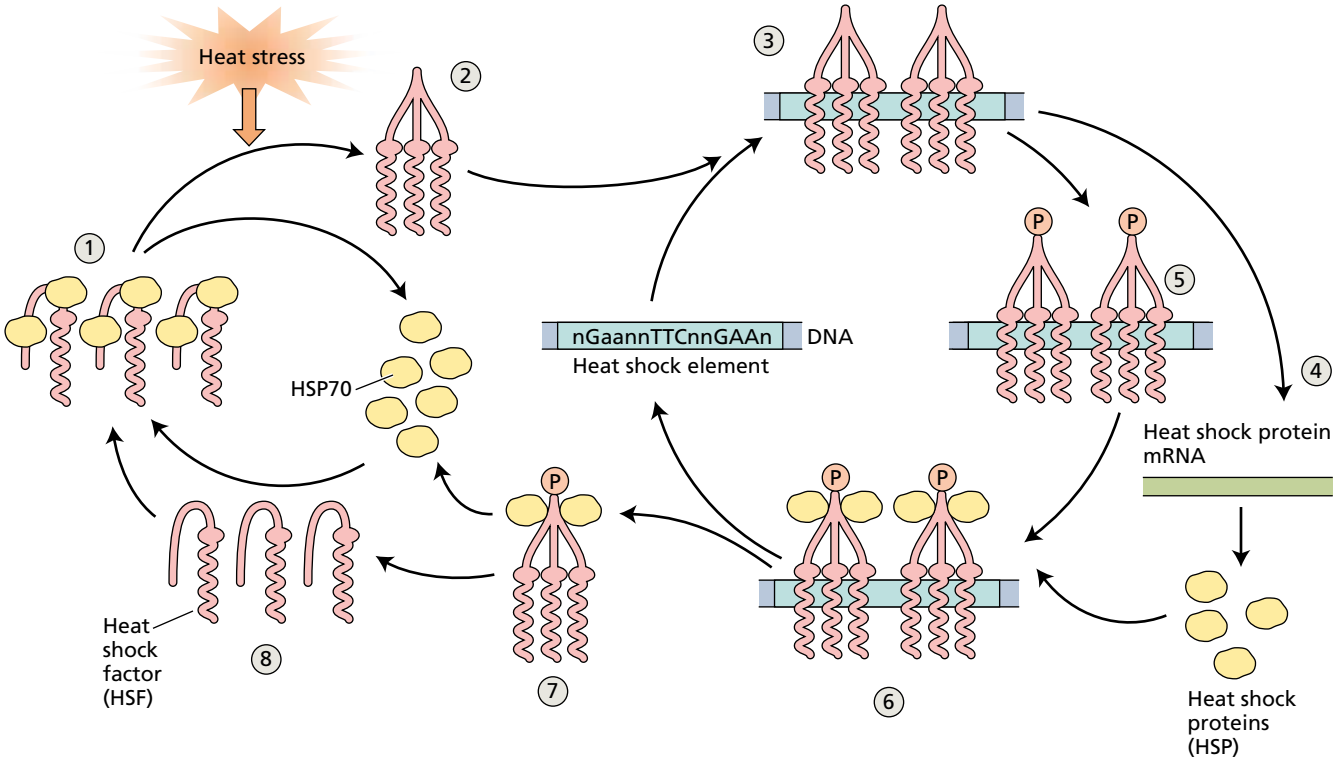


FIGURE 25.11 The heat shock factor (HSF) cycle activates the synthesis of heat shock protein mRNAs. In nonstressed cells, HSF normally exists in a monomeric state (1) associated with HSP70 proteins. Upon the onset of an episode of heat stress, HSP70 dissociates from HSF which subsequently trimerizes (2). Active trimers bind to heat shock elements (HSE) in the promoter of heat shock protein (HSP) genes (3), and activate the transcription of HSP mRNAs leading to the translation of HSPs among which are HSP70 (4). The HSF trimers associated with the HSE are phosphorylated (5) facilitating the binding of HSP70 to the phosphorylated trimers (6). The HSP70 trimer complex (7) dissociates from the HSE and disassembles and dephosphorylates into HSF monomers (8), which subsequently bind HSP reforming the resting HSP70/HSF complex. (After Bray et al. 2000.)

Presumably failure to synthesize the entire range of HSPs that are usually induced in the plant would lead to a much more dramatic loss of thermotolerance. Other studies with both *Arabidopsis* mutants (Hong and Vierling 2000) and transgenic plants (Queitsch et al. 2000) demonstrate that at least HSP101 is a critical component of both induced and constitutive thermotolerance in plants.

Adaptation to Heat Stress Is Mediated by Cytosolic Calcium

Enzymes participating in metabolic pathways can have different temperature responses, and such differential thermostability may affect specific steps in metabolism before HSPs can restore activity by their molecular chaperone capacity. Heat stress can therefore cause changes in metabolism leading to the accumulation of some metabolites and the reduction of others. Such changes can dramatically alter the function of metabolic pathways and lead to imbalances that can be difficult to correct.

In addition, heat stress can alter the rate of metabolic reactions that consume or produce protons, and it can affect the activity of proton-pumping ATPases that pump protons from the cytosol into the apoplast or vacuoles (see Chapter 6). This might lead to an acidification of the cytosol, which could cause additional metabolic perturbations during stress. Cells can have metabolic acclimation mechanisms that ameliorate these effects of heat stress on metabolism.

One of the metabolic acclimations to heat stress is the accumulation of the nonprotein amino acid γ-aminobutyric acid (GABA). During episodes of heat stress, GABA accumulates to levels six- to tenfold higher than in unstressed plants. GABA is synthesized from the amino acid L-glutamate, in a single reaction catalyzed by the enzyme glutamate decarboxylase (GAD). GAD is one of several enzymes whose activity is modulated by the calcium-activated, regulatory protein *calmodulin* (for details on the mode of action of calmodulin, see Chapter 14 on the web site).

Calcium-activated calmodulin activates GAD (Figure 25.12) and increases the biosynthesis rate of GABA (Snedden et al. 1995). In transgenic plants expressing the calcium-sensing protein aequorin, it has been shown that

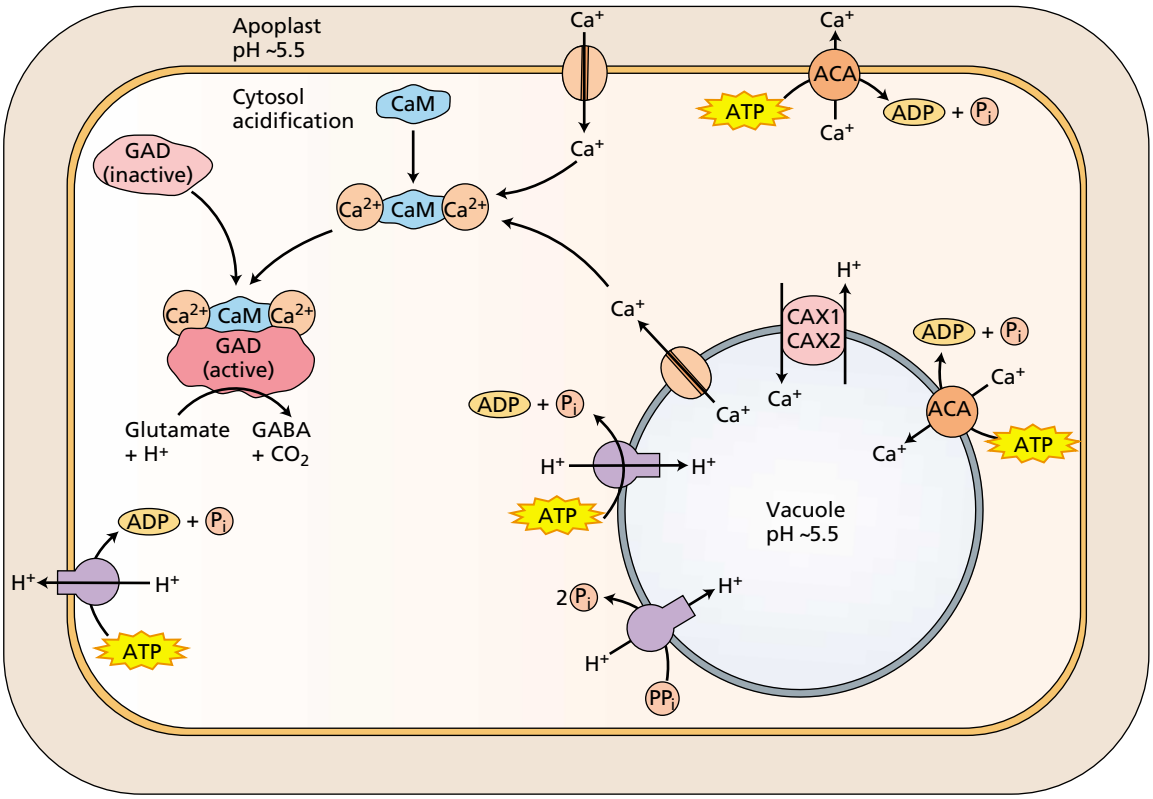


FIGURE 25.12 Heat stress causes a reduction in cytosolic pH from the normal slightly alkaline value, probably by inhibiting proton-pumping ATPases and pyrophosphatases that pump protons across the plasma membrane or into the vacuole. Additionally, heat stress effects a change in calcium homeostasis inside the cell by affecting the influx of calcium into the cytosol through either plasma membrane or vacuolar calcium channels, or by action on efflux ATPases or proton cotransporters. This increase in cytosolic calcium leads to the activation of calmodulin (CaM), which binds to glutamate decarboxylase (GAD) converting it from the inactive to the active form. Glutamate conversion to γ–aminobutyric acid (GABA) is then accomplished consuming protons in the process and mediating an increase in cytosolic pH. CAX1 and CAX2 are transport proteins, ACA: Ca^{2+} ATPase.

high-temperature stress increases cytosolic levels of calcium, and that these increases lead to the calmodulin-mediated activation of GAD and the high-temperature induced accumulation of GABA.

Although GABA is an important signaling molecule in mammalian brain tissue, there is no evidence that it functions as a signaling molecule in plants. Possible functions of GABA in heat stress resistance are under investigation.

CHILLING AND FREEZING

Chilling temperatures are too low for normal growth but not low enough for ice to form. Typically, tropical and subtropical species are susceptible to chilling injury. Among crops, maize, *Phaseolus* bean, rice, tomato, cucumber, sweet potato, and cotton are chilling sensitive. *Passiflora*, *Coleus*, and *Gloxinia* are examples of susceptible ornamentals.

When plants growing at relatively warm temperatures (25 to 35°C) are cooled to 10 to 15°C, **chilling injury** occurs: Growth is slowed, discoloration or lesions appear on leaves, and the foliage looks soggy, as if soaked in water for a long time. If roots are chilled, the plants may wilt.

Species that are generally sensitive to chilling can show appreciable variation in their response to chilling temperatures. Genetic adaptation to the colder temperatures associated with high altitude improves chilling resistance (Figure 25.13). In addition, resistance often increases if plants are first hardened (acclimated) by exposure to cool, but noninjurious, temperatures. Chilling damage thus can be minimized if exposure is slow and gradual. Sudden exposure to temperatures near 0°C, called *cold shock*, greatly increases the chances of injury.

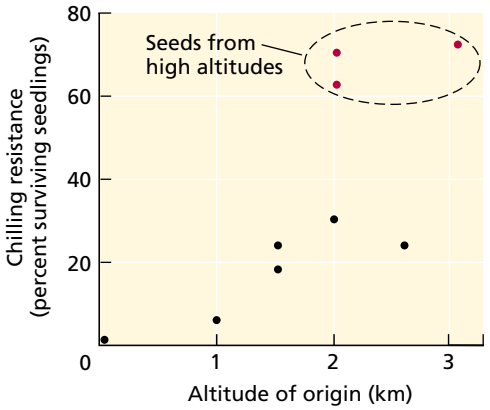


FIGURE 25.13 Survival at low temperature of seedlings of different populations of tomato collected from different altitudes in South America. Seed was collected from wild tomato (*Lycopersicon hirsutum*) and grown in the same greenhouse at 18 to 25°C. All seedlings were then chilled for 7 days at 0°C and then kept for 7 days in a warm growth room, after which the number of survivors was counted. Seedlings from seed collected from high altitudes showed greater resistance to chilling (cold shock) than those from seed collected from lower altitudes. (From Patterson et al. 1978.)

Freezing injury, on the other hand, occurs at temperatures below the freezing point of water. Full induction of tolerance to freezing, as with chilling, requires a period of acclimation at cold temperatures.

In the discussion that follows we will examine how chilling injury alters membrane properties, how ice crystals damage cells and tissues, and how ABA, gene expression, and protein synthesis mediate acclimation to freezing.

Membrane Properties Change in Response to Chilling Injury

Leaves from plants injured by chilling show inhibition of photosynthesis, slower carbohydrate translocation, lower respiration rates, inhibition of protein synthesis, and increased degradation of existing proteins. All of these responses appear to depend on a common primary mechanism involving loss of membrane function during chilling.

For instance, solutes leak from the leaves of chilling-sensitive *Passiflora maliformis* (conch apple) floated on water at 0°C, but not from those of chilling-resistant *Passiflora caerulea* (passionflower). Loss of solutes to the water reflects damage to the plasma membrane and possibly also to the tonoplast. In turn, inhibition of photosynthesis and of respiration reflects injury to chloroplast and mitochondrial membranes.

Why are membranes affected by chilling? Plant membranes consist of a lipid bilayer interspersed with proteins and sterols (see Chapters 1 and 11). The physical properties of the lipids greatly influence the activities of the integral membrane proteins, including H^+-ATPases, carriers, and channel-forming proteins that regulate the transport of ions and other solutes (see Chapter 6), as well as the transport of enzymes on which metabolism depends.

In chilling-sensitive plants, the lipids in the bilayer have a high percentage of saturated fatty acid chains, and membranes with this composition tend to solidify into a semicrystalline state at a temperature well above 0°C. Keep in mind that saturated fatty acids that have no double bonds and lipids containing *trans*-monounsaturated fatty acids solidify at higher temperatures than do membranes composed of lipids that contain unsaturated fatty acids.

As the membranes become less fluid, their protein components can no longer function normally. The result is inhibition of H^+-ATPase activity, of solute transport into and out of cells, of energy transduction (see Chapters 7 and 11), and of enzyme-dependent metabolism. In addition, chilling-sensitive leaves exposed to high photon fluxes and chilling temperatures are photoinhibited (see Chapter 7), causing acute damage to the photosynthetic machinery.

Membrane lipids from chilling-resistant plants often have a greater proportion of unsaturated fatty acids than those from chilling-sensitive plants (Table 25.5), and during acclimation to cool temperatures the activity of desaturase enzymes increases and the proportion of unsaturated lipids rises (Williams et al. 1988; Palta et al. 1993). This modification lowers the temperature at which the mem-

TABLE 25.5
Fatty acid composition of mitochondria isolated from chilling-resistant and chilling-sensitive species

	Percent weight of total fatty acid content					
	Chilling-resistant species			Chilling-sensitive species		
Major fatty acids[a]	Cauliflower bud	Turnip root	Pea shoot	Bean shoot	Sweet potato	Maize shoot
Palmitic (16:0)	21.3	19.0	12.8	24.0	24.9	28.3
Stearic (18:0)	1.9	1.1	2.9	2.2	2.6	1.6
Oleic (18:0)	7.0	12.2	3.1	3.8	0.6	4.6
Linoleic (18:2)	16.4	20.6	61.9	43.6	50.8	54.6
Linolenic (18:3)	49.4	44.9	13.2	24.3	10.6	6.8
Ratio of unsaturated to saturated fatty acids	3.2	3.9	3.8	2.8	1.7	2.1

[a] Shown in parentheses are the number of carbon atoms in the fatty acid chain and the number of double bonds.
Source: After Lyons et al. 1964.

brane lipids begin a gradual phase change from fluid to semicrystalline and allows membranes to remain fluid at lower temperatures. Thus, desaturation of fatty acids provides some protection against damage from chilling.

The importance of membrane lipids to tolerance of low temperatures has been demonstrated by work with mutant and transgenic plants in which the activity of particular enzymes led to a specific change in membrane lipid composition independent of acclimation to low temperature. For example, *Arabidopsis* was transformed with a gene from *Escherichia coli* that raised the proportion of high-melting-point (saturated) membrane lipids. This gene greatly increased the chilling sensitivity of the transformed plants.

Similarly, the *fab1* mutants of *Arabidopsis* have increased levels of saturated fatty acids, particularly 16:0 (see Table 25.5, and Tables 11.3 and 11.4). During a period of 3 to 4 weeks at chilling temperatures, photosynthesis and growth were gradually inhibited, and exposure to chilling temperature eventually destroyed the chloroplasts of this mutant. At nonchilling temperatures, the mutant grew as well as wild-type controls did (Wu et al. 1997). (For additional transformation examples, see **Web Topic 25.2**.)

Ice Crystal Formation and Protoplast Dehydration Kill Cells

The ability to tolerate freezing temperatures under natural conditions varies greatly among tissues. Seeds, other partly dehydrated tissues, and fungal spores can be kept indefinitely at temperatures near absolute zero (0 K, or –273°C), indicating that these very low temperatures are not intrinsically harmful.

Fully hydrated, vegetative cells can also retain viability if they are cooled very quickly to avoid the formation of large, slow-growing ice crystals that would puncture and destroy subcellular structures. Ice crystals that form during very rapid freezing are too small to cause mechanical damage. Conversely, rapid warming of frozen tissue is required to prevent the growth of small ice crystals into crystals of a damaging size, or to prevent loss of water vapor by sublimation, both of which take place at intermediate temperatures (–100 to –10°C).

Under natural conditions, cooling of intact, multicellular plant organs is never fast enough to limit crystal formation in fully hydrated cells to only small, harmless ice crystals. Ice usually forms first within the intercellular spaces, and in the xylem vessels, along which the ice can quickly propagate. This ice formation is not lethal to hardy plants, and the tissue recovers fully if warmed. However, when plants are exposed to freezing temperatures for an extended period, the growth of extracellular ice crystals results in the movement of liquid water from the protoplast to the extracellular ice, causing excessive dehydration (for a detailed description of this process, see **Web Topic 25.3**).

During rapid freezing, the protoplast, including the vacuole, supercools; that is, the cellular water remains liquid even at temperatures several degrees below its theoretical freezing point. Several hundred molecules are needed for an ice crystal to begin forming. The process whereby these hundreds of water molecules start to form a stable ice crystal is called **ice nucleation**, and it strongly depends on the properties of the involved surfaces. Some large polysaccharides and proteins facilitate ice crystal formation and are called ice nucleators.

Some ice nucleation proteins made by bacteria appear to facilitate ice nucleation by aligning water molecules along repeated amino acid domains within the protein. In plant cells, ice crystals begin to grow from endogenous ice nucleators, and the resulting, relatively large intracellular ice crystals cause extensive damage to the cell and are usually lethal.

Limitation of Ice Formation Contributes to Freezing Tolerance

Several specialized plant proteins may help limit the growth of ice crystals by a noncolligative mechanism—that is, an effect that does not depend on the lowering of the

freezing point of water by the presence of solutes. These *antifreeze proteins* are induced by cold temperatures, and they bind to the surfaces of ice crystals to prevent or slow further crystal growth.

In rye leaves, antifreeze proteins are localized in the epidermal cells and cells surrounding the intercellular spaces, where they can inhibit the growth of extracellular ice. Plants and animals may use similar mechanisms to limit ice crystals: A cold-inducible gene identified in *Arabidopsis* has DNA homology to a gene that encodes the antifreeze protein in fishes such as winter flounder. Antifreeze proteins are discussed in more detail later in the chapter.

Sugars and some of the cold-induced proteins are suspected to have cryoprotective (*cryo-* = "cold") effects; they stabilize proteins and membranes during dehydration induced by low temperature. In winter wheat, the greater the sucrose concentration, the greater the freezing tolerance. Sucrose predominates among the soluble sugars associated with freezing tolerance that function in a colligative fashion, but in some species raffinose, fructans, sorbitol, or mannitol serves the same function.

During cold acclimation of winter cereals, soluble sugars accumulate in the cell walls, where they may help restrict the growth of ice. A cryoprotective glycoprotein has been isolated from leaves of cold-acclimated cabbage (*Brassica oleracea*). In vitro, the protein protects thylakoids isolated from nonacclimated spinach (*Spinacia oleracea*) against damage from freezing and thawing.

Some Woody Plants Can Acclimate to Very Low Temperatures

When in a dormant state, some woody plants are extremely resistant to low temperatures. Resistance is determined in part by previous acclimation to cold, but genetics plays an important role in determining the degree of tolerance to low temperatures. Native species of *Prunus* (cherry, plum, and other pit fruits) from northern cooler climates in North America are hardier after acclimation than those from milder climates. When the species were tested together in the laboratory, those with a northern geographic distribution showed greater ability to avoid intracellular ice formation, underscoring distinct genetic differences (Burke and Stushnoff 1979).

Under natural conditions, woody species acclimate to cold in two distinct stages (Weiser 1970):

1. In the first stage, hardening is induced in the early autumn by exposure to short days and nonfreezing chilling temperatures, both of which combine to stop growth. A diffusible factor that promotes acclimation (probably ABA) moves in the phloem from leaves to overwintering stems and may be responsible for the changes. During this period, woody species also withdraw water from the xylem vessels, thereby preventing the stem from splitting in response to the expansion of water during later freezing. Cells in this first stage of acclimation can survive temperatures well below 0°C, but they are not fully hardened.
2. In the second stage, direct exposure to freezing is the stimulus; no known translocatable factor can confer the hardening resulting from exposure to freezing. When fully hardened, the cells can tolerate exposure to temperatures of –50 to –100°C.

Resistance to Freezing Temperatures Involves Supercooling and Slow Dehydration

In many species of the hardwood forests of southeastern Canada and the eastern United States, acclimation to freezing involves the suppression of ice crystal formation at temperatures far below the theoretical freezing point (see **Web Topic 25.3** for details). This *deep supercooling* is seen in species such as oak, elm, maple, beech, ash, walnut, hickory, rose, rhododendron, apple, pear, peach, and plum (Burke and Stushnoff 1979). Deep supercooling also takes place in the stem and leaf tissue of tree species such as Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*) growing in the Rocky Mountains of Colorado.

Resistance to freezing is quickly weakened once growth has resumed in the spring (Becwar et al. 1981). Stem tissues of subalpine fir, which undergo deep supercooling and remain viable to below –35°C in May, lose their ability to suppress ice formation in June and can be killed at –10°C.

Cells can supercool only to about –40°C, at which temperature ice forms spontaneously. Spontaneous ice formation sets the *low-temperature limit* at which many alpine and subarctic species that undergo deep supercooling can survive. It also explains why the altitude of the timberline in mountain ranges is at or near the –40°C minimum isotherm.

The cell protoplast suppresses ice nucleation when undergoing deep supercooling. In addition, the cell wall acts as a barrier both to the growth of ice from the intercellular spaces into the wall, and to the loss of liquid water from the protoplast to the extracellular ice, which is driven by a steep vapor pressure gradient (Wisniewski and Arora 1993).

Many flower buds (e.g., grape, blueberry, peach, azalea, and flowering dogwood) survive the winter by deep supercooling, and serious economic losses, particularly of peach, can result from the decline in freezing tolerance of the flower buds in the spring. The cells then no longer supercool, and ice crystals that form extracellularly in the bud scales draw water from the apical meristem, killing the floral apex by dehydration.

The floral buds of apple and pear, the vegetative buds of all temperate fruit trees, and the living cells in their bark do not supercool, but they resist dehydration during extracellular ice formation. Resistance to cellular dehydration is highly developed in woody species that are subject to average annual temperature minima below –40°C, particularly species found in northern Canada, Alaska, northern Europe, and Asia.

Ice formation starts at –3 to –5°C in the intercellular spaces, where the crystals continue to grow, fed by the gradual withdrawal of water from the protoplast, which remains unfrozen. Resistance to freezing temperatures depends on the capacity of the extracellular spaces to accommodate the volume of growing ice crystals and on the ability of the protoplast to withstand dehydration.

This restriction of ice crystal formation to extracellular spaces, accompanied by gradual protoplast dehydration, may explain why some woody species that are resistant to freezing are also resistant to water deficit during the growing season. For example, species of willow (*Salix*), white birch (*Betula papyrifera*), quaking aspen (*Populus tremuloides*), pin cherry (*Prunus pensylvanica*), chokecherry (*Prunus virginiana*), and lodgepole pine (*Pinus contorta*) tolerate very low temperatures by limiting the formation of ice crystals to the extracellular spaces. However, acquisition of resistance depends on slow cooling and gradual extracellular ice formation and protoplast dehydration. Sudden exposure to very cold temperatures before full acclimation causes intracellular freezing and cell death.

Some Bacteria That Live on Leaf Surfaces Increase Frost Damage

When leaves are cooled to temperatures in the –3 to –5°C range, the formation of ice crystals on the surface (frost) is accelerated by certain bacteria that naturally inhabit the leaf surface, such as *Pseudomonas syringae* and *Erwinia herbicola*, which act as ice nucleators. When artificially inoculated with cultures of these bacteria, leaves of frost-sensitive species freeze at warmer temperatures than leaves that are bacteria free (Lindow et al. 1982). The surface ice quickly spreads to the intercellular spaces within the leaf, leading to cellular dehydration.

Bacterial strains can be genetically modified so that they lose their ice-nucleating characteristics. Such strains have been used commercially in foliar sprays of valuable frost-sensitive crops like strawberry to compete with native bacterial strains and thus minimize the number of potential ice nucleation points.

ABA and Protein Synthesis Are Involved in Acclimation to Freezing

In seedlings of alfalfa (*Medicago sativa* L.), tolerance to freezing at –10°C is greatly improved by previous exposure to cold (4°C) or by treatment with exogenous ABA without exposure to cold. These treatments cause changes in the pattern of newly synthesized proteins that can be resolved on two-dimensional gels. Some of the changes are unique to the particular treatment (cold or ABA), but some of the newly synthesized proteins induced by cold appear to be the same as those induced by ABA (see Chapter 23) or by mild water deficit.

Protein synthesis is necessary for the development of freezing tolerance, and several distinct proteins accumulate during acclimation to cold, as a result of changes in gene expression (Guy 1999). Isolation of the genes for these proteins reveals that several of the proteins that are induced by low temperature share homology with the RAB/LEA/DHN (responsive to ABA, late embryo abundant, and dehydrin, respectively) protein family. As described earlier in the section on gene regulation by osmotic stress, these proteins accumulate in tissues exposed to different stresses, such as osmotic stress. Their functions are under investigation.

ABA appears to have a role in inducing freezing tolerance. Winter wheat, rye, spinach, and *Arabidopsis thaliana* are all cold-tolerant species, and when they are hardened by water shortages, their freezing tolerance also increases. This tolerance to freezing is increased at nonacclimating temperatures by mild water deficit, or at low temperatures, either of which increases endogenous ABA concentrations in leaves.

Plants develop freezing tolerance at nonacclimating temperatures when treated with exogenous ABA. Many of the genes or proteins expressed at low temperatures or under water deficit are also inducible by ABA under nonacclimating conditions. All these findings support a role of ABA in tolerance to freezing.

Mutants of *Arabidopsis* that are insensitive to ABA (*abi1*) or are ABA deficient (*aba1*) are unable to undergo low-temperature acclimation to freezing. Only in *aba1*, however, does exposure to ABA restore the ability to develop freezing tolerance (Mantyla et al. 1995). On the other hand, not all the genes induced by low temperature are ABA dependent, and it is not yet clear whether expression of ABA-induced genes is critical for the full development of freezing tolerance. For instance, research on the tolerance of rye crowns to freezing has found that the lethal temperature for 50% of the crowns (LT_{50}) is –2 to –5°C for controls grown at 25°C, –8°C for ABA-treated crowns, and –28°C after acclimation at 2°C.

Clearly exogenous ABA cannot confer the same freezing acclimation that exposure to low temperatures does. Cell cultures of bromegrass (*Bromus inermis*) show a more dramatic induction of freezing tolerance when treated with ABA: Whereas controls grown at 25°C could survive to –9°C, 7 days of exposure to ABA improved the freezing tolerance to –40°C (Gusta et al. 1996).

Typically, a minimum of several days of exposure to cool temperatures is required for freezing resistance to be induced fully. Potato requires 15 days of exposure to cold. On the other hand, when rewarmed, plants lose their freezing tolerance rapidly, and they can become susceptible to freezing once again in 24 hours.

The need for cool temperatures to induce acclimation to chilling or freezing, and the rapid loss of acclimation upon warming, explain the susceptibility of plants in the southern United States (and similar climatic zones with highly variable winters) to extremes of temperature in the winter months, when air temperature can drop from 20 to 25°C to below 0°C in a few hours.

Numerous Genes Are Induced during Cold Acclimation

Expression of certain genes and synthesis of specific proteins are common to both heat and cold stress, but some aspects of cold-inducible gene expression differ from that produced by heat stress (Thomashow 2001). Whereas during cold episodes the synthesis of "housekeeping" proteins (proteins made in the absence of stress) is not substantially down-regulated, during heat stress housekeeping-protein synthesis is essentially shut down.

On the other hand, the synthesis of several heat shock proteins that can act as molecular chaperones is up-regulated under cold stress in the same way that it is during heat stress. This suggests that protein destabilization accompanies both heat and cold stress and that mechanisms for stabilizing protein structure during both heat and cold episodes are important for survival.

Another important class of proteins whose expression is up-regulated by cold stress is the **antifreeze proteins**. Antifreeze proteins were first discovered in fishes that live in water under the polar ice caps. As discussed earlier, these proteins have the ability to inhibit ice crystal growth in a noncolligative manner, thus preventing freeze damage at intermediate freezing temperatures. Antifreeze proteins confer to aqueous solutions the property of *thermal hysteresis* (transition from liquid to solid is promoted at a lower temperature than is transition from solid to liquid), and thus they are sometimes referred to as **thermal hysteresis proteins** (**THPs**).

Several types of cold-induced, antifreeze proteins have been discovered in cold-acclimated winter-hardy monocots. When the specific genes coding for these proteins were cloned and sequenced, it was found that all antifreeze proteins belong to a class of proteins such as endochitinases and endoglucanases, which are induced upon infection of different pathogens. These proteins, called **pathogenesis-related** (**PR**) **proteins** are thought to protect plants against pathogens. It thus appears that at least in monocots, the dual role of these proteins as antifreeze and pathogenesis-related proteins might protect plant cells against both cold stress and pathogen attack.

Another group of proteins found to be associated with osmotic stress (see the discussion earlier in this chapter) are also up-regulated during cold stress. This group includes proteins involved in the synthesis of *osmolytes*, proteins for membrane stabilization, and the LEA proteins. Because the formation of extracellular ice crystals generates significant osmotic stresses inside cells, coping with freezing stress also requires the means to cope with osmotic stress.

A Transcription Factor Regulates Cold-Induced Gene Expression

More than 100 genes are up-regulated by cold stress. Because cold stress is clearly related to ABA responses and to osmotic stress, not all the genes up-regulated by cold stress necessarily need to be associated with cold tolerance, but most likely many of them are. Many cold stress–induced genes are activated by transcriptional activators called **C-repeat binding factors** (**CBF1**, **CBF2**, **CBF3**; also called DREB1b, DREB1c, and DREB1a, respectively) (Shinozaki and Yamaguchi-Shinozaki 2000).

CBF/DREB1-type transcription factors bind to **CRT/DRE elements** (C-repeat/dehydration-responsive, ABA-independent sequence elements) in gene promoter sequences, which were discussed earlier in the chapter. CBF/DREB1 is involved in the coordinate transcriptional response of numerous cold and osmotic stress–regulated genes, all of which contain the CRT/DRE elements in their promoters. CBF1/DREB1b is unique in that it is specifically induced by cold stress and not by osmotic or salinity stress, whereas the DRE-binding elements of the DREB2 type (discussed earlier in the section on osmotic stresses) are induced only by osmotic and salinity stresses and not by cold.

The expression of CBF1/DREB1b is controlled by a separate transcription factor, called ICE (*i*nducer of *C*BF *e*xpression). ICE transcription factors do not appear to be induced by cold, and it is presumed that ICE or an associated protein is posttranscriptionally activated, permitting activation of CBF1/DRE1b, but the precise signaling pathway(s) of cold perception, calcium signaling, and the activation of ICE are presently under investigation.

Transgenic plants constitutively expressing CBF1 have more cold–up-regulated gene transcripts than wild-type plants have, suggesting that numerous cold–up-regulated proteins that may be involved in cold acclimation are being produced in the absence of cold in these CBF1 transgenic plants. In addition, CBF1 tansgenic plants are more cold tolerant than control plants.

SALINITY STRESS

Under natural conditions, terrestrial higher plants encounter high concentrations of salts close to the seashore and in estuaries where seawater and freshwater mix or replace each other with the tides. Far inland, natural salt seepage from geologic marine deposits can wash into adjoining areas, rendering them unusable for agriculture. However, a much more extensive problem in agriculture is the accumulation of salts from irrigation water.

Evaporation and transpiration remove pure water (as vapor) from the soil, and this water loss concentrates solutes in the soil. When irrigation water contains a high concentration of solutes and when there is no opportunity to flush out accumulated salts to a drainage system, salts can quickly reach levels that are injurious to salt-sensitive species. It is estimated that about one-third of the irrigated land on Earth is affected by salt.

In this section we discuss how plant function is affected by water and soil salinity, and we examine the processes that assist plants in avoiding salinity stress.

TABLE 25.6
Properties of seawater and of good quality irrigation water

Property	Seawater	Irrigation water
Concentration of ions (m*M*)		
Na^+	457	<2.0
K^+	9.7	<1.0
Ca^{2+}	10	0.5–2.5
Mg^{2+}	56	0.25–1.0
Cl^-	536	<2.0
SO_4^{2-}	28	0.25–2.5
HCO_3^-	2.3	<1.5
Osmotic potential (MPa)	–2.4	–0.039
Total dissolved salts (mg L^{-1} or ppm)	32,000	500

Salt Accumulation in Soils Impairs Plant Function and Soil Structure

In discussing the effects of salts in the soil, we distinguish between high concentrations of Na^+, referred to as **sodicity**, and high concentrations of total salts, referred to as **salinity**. The two concepts are often related, but in some areas Ca^{2+}, Mg^{2+}, and $SO^2{}_4{}^-$, as well as NaCl, can contribute substantially to salinity. The high Na^+ concentration of a sodic soil can not only injure plants directly but also degrade the soil structure, decreasing porosity and water permeability. A sodic clay soil known as caliche is so hard and impermeable that dynamite is sometimes required to dig through it!

In the field, the salinity of soil water or irrigation water is measured in terms of its electrical conductivity or in terms of osmotic potential. Pure water is a very poor conductor of electric current; the conductivity of a water sample is due to the ions dissolved in it. The higher the salt concentration in water, the greater its electrical conductivity and the lower its osmotic potential (higher osmotic pressure) (Table 25.6).

The quality of irrigation water in semiarid and arid regions is often poor. In the United States the salt content of the headwaters of the Colorado River is only 50 mg L^{-1}, but about 2000 km downstream, in southern California, the salt content of the same river reaches about 900 mg L^{-1}, enough to preclude growth of some salt-sensitive crops, such as maize. Water from some wells used for irrigation in Texas may contain as much as 2000 to 3000 mg salt L^{-1}. An annual application of irrigation water totaling 1 m from such wells would add 20 to 30 tons of salts per hectare (8–12 tons per acre) to the soil. These levels of salts are damaging to all but the most resistant crops.

Salinity Depresses Growth and Photosynthesis in Sensitive Species

Plants can be divided into two broad groups on the basis of their response to high concentrations of salts. **Halophytes** are native to saline soils and complete their life cycles in that environment. **Glycophytes** (literally "sweet plants"), or nonhalophytes, are not able to resist salts to the same degree as halophytes. Usually there is a threshold concentration of salt above which glycophytes begin to show signs of growth inhibition, leaf discoloration, and loss of dry weight.

Among crops, maize, onion, citrus, pecan, lettuce, and bean are highly sensitive to salt; cotton and barley are moderately tolerant; and sugar beet and date palms are highly tolerant (Greenway and Munns 1980). Some species that are highly tolerant of salt, such as *Suaeda maritima* (a salt marsh plant) and *Atriplex nummularia* (a saltbush), show growth stimulation at Cl^- concentrations many times greater than the lethal level for sensitive species (Figure 25.14).

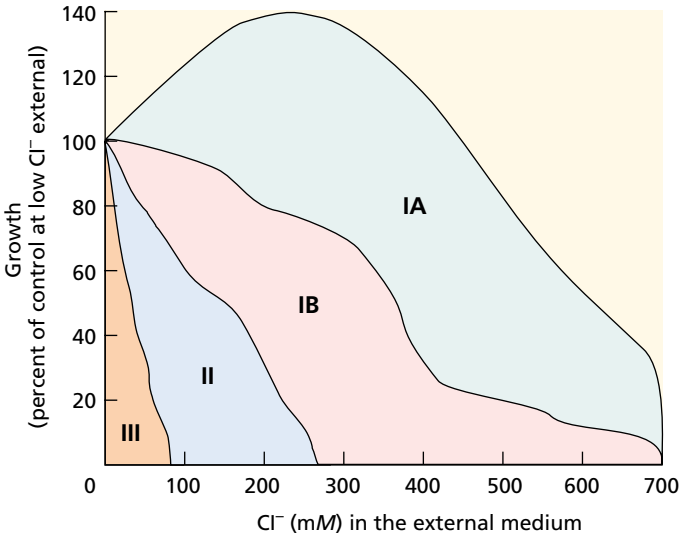
Salt Injury Involves Both Osmotic Effects and Specific Ion Effects

Dissolved solutes in the rooting zone generate a low (more negative) osmotic potential that lowers the soil water potential. The general water balance of plants is thus affected because leaves need to develop an even lower water potential to maintain a "downhill" gradient of water potential between the soil and the leaves (see Chapter 4). This effect of dissolved solutes is similar to that of a soil water deficit (as discussed earlier in this chapter), and most plants respond to excessive levels of soil salinity in the same way as described earlier for water deficit.

A major difference between the low-water-potential environments caused by salinity versus soil desiccation is the total amount of water available. During soil desiccation a finite amount of water can be obtained from the soil profile by the plant, causing ever decreasing water potentials. In most saline environments a large (essentially unlimited) amount of water at a constant, low water potential is available.

Of particular importance here is the fact that most plants can adjust osmotically when growing in saline soils. Such adjustment prevents loss of turgor (which would slow cell growth; see Figure 25.1) while generating a lower water potential, but these plants often continue to grow more slowly after this adjustment for an unknown reason that curiously is not related to insufficient turgor (Bressan et al. 1990)

In addition to the plant responses to low water potential, specific ion **toxicity effects** also occur when injurious concentrations of ions—particularly Na^+, Cl^-, or SO_4^{2-}—accumulate in cells. Under nonsaline conditions, the cytosol of higher-plant cells contains 100 to 200 m*M* K^+ and 1 to 10 m*M* Na^+, an ionic environment in which many enzymes function optimally. An abnormally high ratio of Na^+ to K^+ and high concentrations of total salts inactivate enzymes and inhibit protein synthesis. At a high concentration, Na^+ can displace Ca^{2+} from the plasma membrane of cotton root hairs, resulting in a change in plasma membrane permeability that can be detected as leakage of K^+ from the cells (Cramer et al. 1985).



Group IA (halophytes) includes sea blite (*Suaeda maritima*) and salt bush (*Atriplex nummularia*). These species show growth stimulation with Cl^- levels below 400 n*M*.

Group IB (halophytes) includes Townsend's cordgrass (*Spartina x townsendii*) and sugar beet (*Beta vulgaris*).These plants tolerate salt, but their growth is retarded.

Group II (halophytes and nonhalophytes) includes salt-tolerant halophytic grasses that lack salt glands, such as *Festuca rubra* subsp. red fescue (*littoralis*) and *Puccinellia peisonis*, and nonhalophytes, such as cotton (*Gossypium* spp.) and barley (*Hordeum vulgare*). All are inhibited by high salt concentrations. Within this group, tomato (*Lycopersicon esculentum*) is intermediate, and common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) are sensitive.

The species in **Group III (very salt-sensitive nonhalophytes)** are severely inhibited or killed by low salt concentrations. Included are many fruit trees, such as citrus, avocado, and stone fruit.

FIGURE 25.14 The growth of different species subjected to salinity relative to that of unsalinized controls. The curves dividing the regions are based on data for different species. Plants were grown for 1 to 6 months. (From Greenway and Munns 1980.)

Photosynthesis is inhibited when high concentrations of Na^+ and/or Cl^- accumulate in chloroplasts. Since photosynthetic electron transport appears relatively insensitive to salts, either carbon metabolism or photophosphorylation may be affected. Enzymes extracted from salt-tolerant species are just as sensitive to the presence of NaCl as enzymes from salt-sensitive glycophytes are. Hence the resistance of halophytes to salts is not a consequence of salt-resistant metabolism. Instead, other mechanisms come into play to avoid salt injury, as discussed in the following section.

Plants Use Different Strategies to Avoid Salt Injury

Plants minimize salt injury by excluding salt from meristems, particularly in the shoot, and from leaves that are actively expanding and photosynthesizing. In plants that are salt sensitive, resistance to moderate levels of salinity in the soil depends in part on the ability of the roots to prevent potentially harmful ions from reaching the shoots.

Recall from Chapter 4 that the Casparian strip imposes a restriction to the movements of ions into the xylem. To bypass the Casparian strips, ions need to move from the apoplast to the symplastic pathway across cell membranes. This transition offers salt-resistant plants a mechanism to partially exclude harmful ions.

Sodium ions enter roots passively (by moving down an electrochemical-potential gradient; see Chapter 6), so root cells must use energy to extrude Na^+ actively back to the outside solution. By contrast, Cl^- is excluded by negative electric potential across the cell membrane, and the low permeability of root plasma membranes to this ion. Movement of Na^+ into leaves is further minimized by absorption of Na^+ from the transpiration stream (xylem sap) during its movement from roots to shoots and leaves.

Some salt-resistant plants, such as salt cedar (*Tamarix* sp.) and salt bush (*Atriplex* sp.), do not exclude ions at the root, but instead have salt glands at the surface of the leaves. The ions are transported to these glands, where the salt crystallizes and is no longer harmful. In general, halophytes have a greater capacity than glycophytes for ion accumulation in shoot cells.

Although some plants, such as mangroves, grow in saline environments with abundant water supplies, the ability to acquire that water requires that they make osmotic adjustments to obtain water from the low-water-potential external environment. As discussed earlier in relation to water deficit, plant cells can adjust their water potential (Ψ_w) in response to osmotic stress by lowering their solute potential (Ψ_s). Two intracellular processes contribute to the decrease in Ψ_s: the accumulation of ions in the vacuole and the synthesis of compatible solutes in the cytosol.

As mentioned earlier in the chapter, compatible solutes include glycine betaine, proline, sorbitol, mannitol, pinitol, and sucrose. Specific plant families tend to use one or two of these compounds in preference to others. The amount of carbon used for the synthesis of these organic solutes can be rather large (about 10% of the plant weight). In natural vegetation this diversion of carbon to adjust water potential does not affect survival, but in agricultural crops it can reduce growth and therefore total biomass and harvestable yields.

Many halophytes exhibit a growth optimum at moderate levels of salinity, and this optimum is correlated with the capacity to accumulate ions in the vacuole, where they can contribute to the cell osmotic potential without damaging the salt-sensitive enzymes. To a lesser extent, this process also occurs in more salt-sensitive glycophytes, but the adjustment may be slower.

Besides making adjustments in water potential, plants adjusting to salinity stress undergo the other osmotic stress–related acclimations described earlier for water deficit. For example, plants subjected to salt stress can reduce leaf area and or drop leaves via leaf abscission just as during episodes of osmotic stress. In addition, changes in gene expression associated with osmotic stress are similarly associated with salinity stress. Keep in mind, however, that in addition to acclimation to a low-water-potential environment, plants experiencing salinity stress must cope with the toxicity of high ion concentrations associated with salinity stress.

Ion Exclusion Is Critical for Acclimation and Adaptation to Salinity Stress

In terms of metabolic energy, use of ions to balance tissue water potential in a saline environment clearly has a lower energy cost for the plant than use of carbohydrates or amino acids, the production of which has a significantly higher energy cost. On the other hand, high ion concentrations are toxic to many cytosolic enzymes, so ions must be accumulated in the vacuole to minimize toxic concentrations in the cytosol.

Because NaCl is the most abundant salt encountered by plants under salinity stress, transport systems that facilitate compartmentation of Na^+ into the vacuole are critical (Binzel et al. 1988). Both Ca^{2+} and K^+ affect intracellular Na^+ concentrations (Zhong and Läuchli 1994). At high concentrations of Na^+, K^+ uptake through a high-affinity K^+–Na^+ transporter, HKT1, is inhibited, and the transporter operates as an Na^+ uptake system (Figure 25.15). Calcium, on the other hand, enhances K^+/Na^+ selectivity and in so doing increases salt tolerance (Liu and Zhu 1997).

Sodium Is Transported across the Plasma Membrane and the Tonoplast

As discussed in Chapter 6, H^+ pumps in the plasma membrane and tonoplast provide the driving force (H^+ electro-

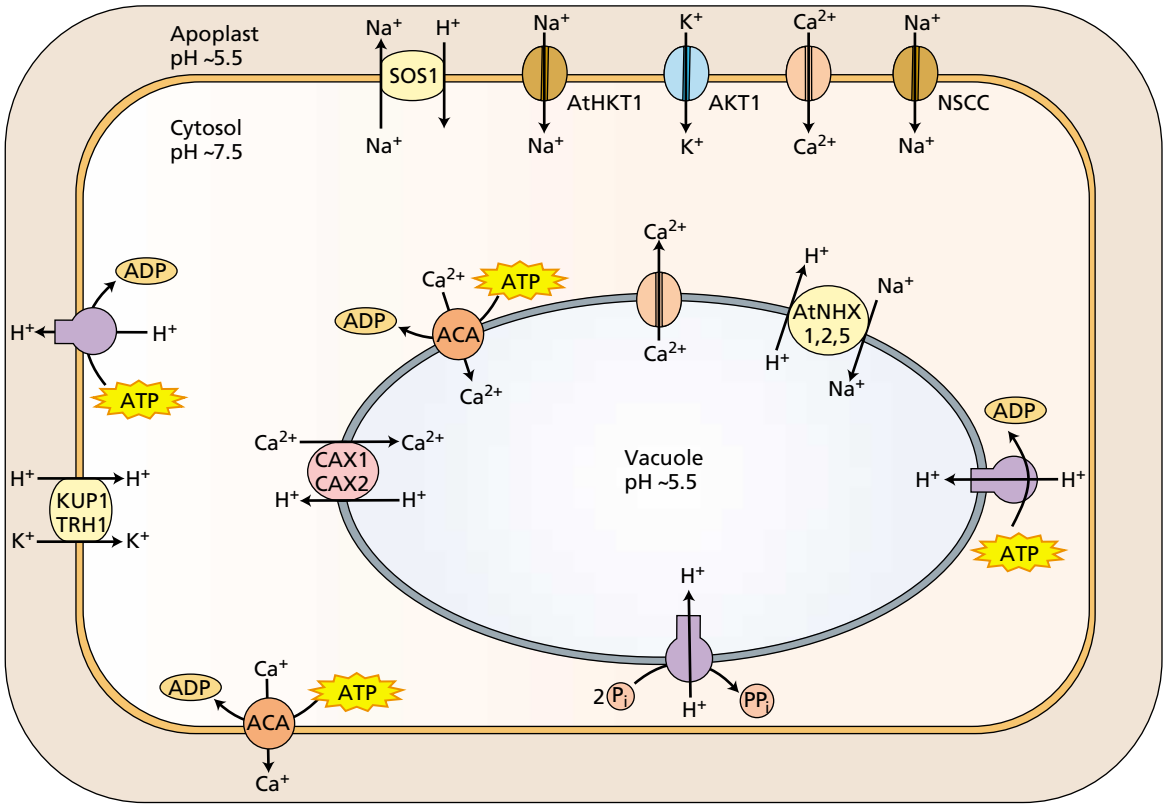


FIGURE 25.15 Membrane transport proteins mediating sodium, potassium, and calcium transport during salinity stress. SOS1, plasma membrane Na^+/H^+ antiporter; ACA, plasma/tonoplast membrane Ca^{2+}-ATPase; KUP1/TRH1, high-affinity K^+-H^+ co-transporter; atHKT1, sodium influx transporter; AKT1, K^+_{in} channel; NSCC, non selective cation channel; CAX1 or 2, Ca^{2+}/H^+ antiporter; atNHX1, 2 or 5, endomembrane Na^+/H^+ antiporter. Also indicated in the figure are proteins that have been implicated in ion homeostasis, but whose molecular identity is either not presently known or cofirmed in plants. These include plasma membrane and tonoplast calcium channel proteins, and vacuolar proton-pumping ATPases and pyrophosphatases. The membrane potential difference across the plasma membrane is typically 120 to 200 mV, negative inside; across the tonoplast 0 to 20 mV; positive inside.

chemical potential) for secondary transport of ions (see Figure 25.15). An ATPase is primarily responsible for the large ΔpH and membrane potential gradient found across the plasma membrane. A vacuolar H^+-ATPase generates a ΔpH and membrane potential across the tonoplast (Hasegawa et al. 2000).

Activity of these pumps is required for the secondary transport of excess ions associated with plant responses to salinity stress. This is indicated by findings showing that the activity of these H^+ pumps is increased by salinity, and induced gene expression may account for some of this up-regulation.

Energy-dependent transport (efflux) of Na^+ from the cytosol of plant cells across the plasma membrane is mediated by the gene product of the *SOS1* (*s*alt *o*verly *s*ensitive *1*) gene that function as a Na^+–H^+ antiporter (Figure 25.16). The SOS1 antiporter is regulated by the gene products of at least two other genes, referred to as *SOS2* and *SOS3* (Shi et al. 2000). SOS2 is a serine/threonine kinase that is apparently activated by calcium through the function of SOS3, a calcium-regulated protein phosphatase (see **Web Topic 25.4** for details on Ca^{2+} signaling and the *SOS* gene family).

Vacuolar compartmentation of Na^+ results in part from the activity of a family of Na^+–H^+ antiporters such as *Arabidopsis* AtNHX1 (see Figure 25.15). Transgenic *Arabidopsis* and tomato plants overexpressing the gene that encodes AtNHX1 exhibit enhanced salt tolerance (Apse et al. 1999; Quintero et al. 2000). (See **Web Topic 25.5** for details on molecular studies of Na^+ compartmentation.) These molecular findings are another example of the wealth of new information emerging from studies on transgenic plants, gene sequencing, and protein characterization (see **Web Topic 25.6** for details on work with transgenic plants for stress studies).

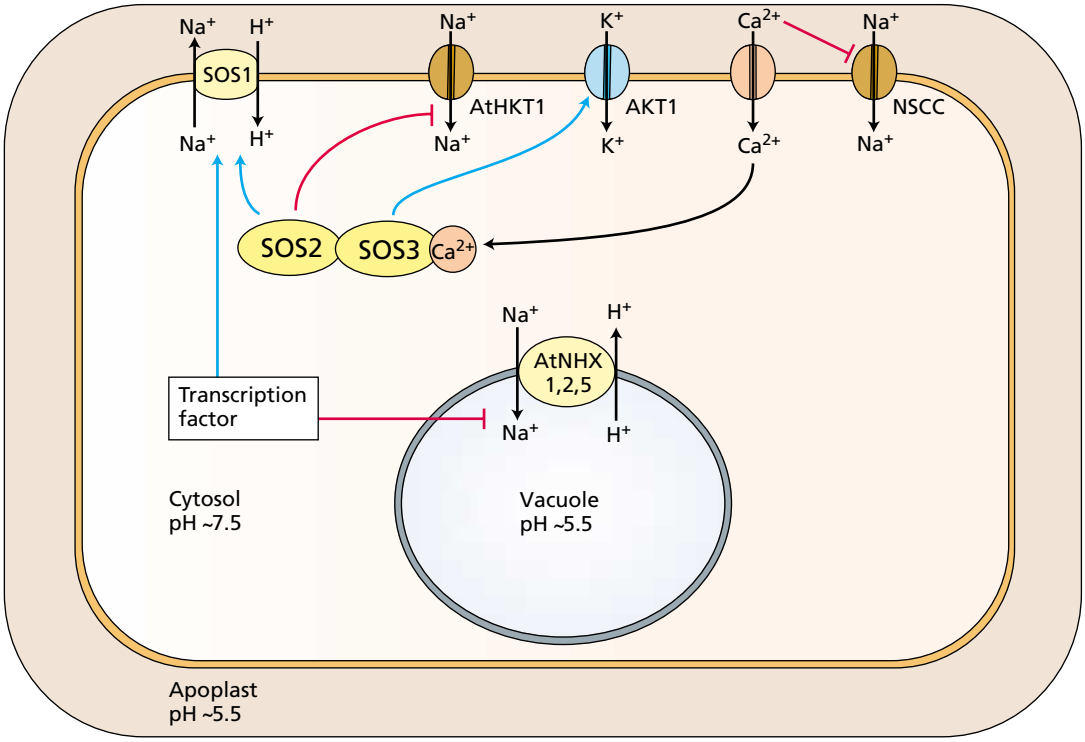


FIGURE 25.16 The regulation of ion homeostasis by the SOS signal transduction pathway, salinity stress, and calcium levels. Red arrows indicate positive regulation of the effected transport protein while blue arrows indicate negative regulation. Proteins shown in yellow are activated by salinity stress. SOS1, plasma membrane Na^+/H^+ antiporter; SOS2, serine/threonine kinase; SOS3, Ca^{2+} binding protein; HKT1, sodium influx transporter; AKT1, K^+_{in} channel; NSCC, non selective cation channel; NHX1, 2 or 5, endomembrane Na^+/H^+ antiporter; shown in orange is an undertermined calcium channel protein. Salinity stress activates a calcium channel leading to an increase in cytosolic calcium that activates the SOS cascade through SOS3. The SOS cascade must negatively regulate HKT1 which in turn secondarily regulates AKT1. At the same time, the SOS cascade increases the activity of SOS1 and AKT1. Working through an as yet undefined transcription factor the SOS cascade increases transcription of SOS1 while decreasing transcription of NHX gene(s). At low calcium NSCC can also function as an alternative sodium influx system, but this transporter is inhibited at high calcium levels. The membrane potential difference across the plasma membrane is typically 120 to 200 mV, negative inside, that of the tonoplast is 0 to 20 mV, positive inside.

OXYGEN DEFICIENCY

Roots usually obtain sufficient oxygen (O_2) for aerobic respiration (see Chapter 11) directly from the gaseous space in the soil. Gas-filled pores in well-drained, well-structured soil readily permit the diffusion of gaseous O_2 to depths of several meters. Consequently, the O_2 concentration deep in the soil is similar to that in humid air. However, soil can become flooded or waterlogged when it is poorly drained or when rain or irrigation is excessive. Water then fills the pores and blocks the diffusion of O_2 in the gaseous phase. Dissolved oxygen diffuses so slowly in stagnant water that only a few centimeters of soil near the surface remain oxygenated.

When temperatures are low and plants are dormant, oxygen depletion is very slow and the consequences are relatively harmless. However, when temperatures are higher (greater than 20°C), oxygen consumption by plant roots, and soil fauna and microorganisms, can totally deplete the oxygen from the bulk of the soil water in as little as 24 hours.

Flooding-sensitive plants are severely damaged by 24 hours of anoxia. The growth and survival of many plant species are greatly depressed under such conditions, and crop yields can be severely reduced. For example, garden pea (*Pisum sativum*) yields can be halved by 24 hours of flooding, making garden pea an example of a flooding-sensitive plant. Other plants, particularly species not adapted to grow in continually wet conditions and many crop plants, are affected by flooding in a milder way and are considered flooding-tolerant plants. **Flooding-tolerant plants** can withstand anoxia (lack of oxygen) temporarily, but not for prolonged periods of more than a few days.

On the other hand, specialized natural vegetation found in wetlands such as marshes and swamps, and crops such as rice, are well adapted to resist oxygen deficiency in the root environment. Wetland plants can resist anoxia, and they grow and survive for extended periods of up to months with their root systems in anoxic conditions. Most of these plants have special adaptations, which we will discuss here, that permit oxygen available in nearby environments to reach the tissues held in anoxic conditions. Practically all plants require oxygen when they are engaging in rapid metabolic activity, and plants can be classified according to the time they can withstand anoxic conditions in their root environment without demonstrating substantial damage.

In the following sections we discuss the damage caused by anaerobiosis to roots and shoots, how wetland vegetation copes with low oxygen tensions, and different acclimations to anoxic stress that distinguish between flooding-tolerant and flooding-susceptible species.

Anaerobic Microorganisms Are Active in Water-Saturated Soils

When soil is completely depleted of molecular O_2, the function of soil microbes becomes significant for plant life and growth. Anaerobic soil microorganisms (anaerobes) derive their energy from the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) or to nitrous oxide (N_2O) and molecular nitrogen (N_2). These gases (N_2O and N_2) are lost to the atmosphere in a process called denitrification. As conditions become more reducing, anaerobes reduce Fe^{3+} to Fe^{2+}, and because of its greater solubility, Fe^{2+} can rise to toxic concentrations when some soils are anaerobic for many weeks. Other anaerobes may reduce sulfate (SO_4^{2-}) to hydrogen sulfide (H_2S), which is a respiratory poison.

When anaerobes have an abundant supply of organic substrate, bacterial metabolites such as acetic acid and butyric acid are released into the soil water, and these acids along with reduced sulfur compounds account for the unpleasant odor of waterlogged soil. All of these substances made by microorganisms under anaerobic conditions are toxic to plants at high concentrations.

Roots Are Damaged in Anoxic Environments

Root respiration rate and metabolism are affected even before O_2 is completely depleted from the root environment. The **critical oxygen pressure** (**COP**) is the oxygen pressure at which the respiration rate is first slowed by O_2 deficiency. The COP for a maize root tip growing in a well-stirred nutrient solution at 25°C, is about 0.20 atmosphere (20 kPa, or 20% O_2 by volume), almost the concentration in ambient air. At this oxygen partial pressure (for a discussion of partial pressures, see **Web Topic 9.3**), the rate of diffusion of dissolved O_2 from the solution into the tissue and from cell to cell barely keeps pace with the rate of O_2 utilization. However, a root tip is metabolically very active, with respiration rates and ATP turnover comparable to those of mammalian tissue.

In older zones of the root, where cells are mature and fully vacuolated and the respiration rate is lower, the COP is often in the range of 0.1 to 0.05 atmosphere. When O_2 concentrations are below the COP, the center of the root becomes *anoxic* (completely lacking oxygen) or *hypoxic* (partly deficient in oxygen).

The COP is lower when respiration slows down at cooler temperatures; it also depends on how bulky the organ is and how tightly the cells are packed. Large, bulky fruits are able to remain fully aerobic because of the large intercellular spaces that readily allow gaseous diffusion. For single cells, an O_2 partial pressure as low as 0.01 atmosphere (1% O_2 in the gaseous phase) can be adequate because diffusion over short distances ensures an adequate O_2 supply to mitochondria. A very low partial pressure of O_2 at the mitochondrion is sufficient to maintain oxidative phosphorylation.

The K_m value (Michaelis–Menten constant; see Chapter 2 on the web site) for cytochrome oxidase is 0.1 to 1.0 μ*M* dissolved O_2, a tiny fraction of the concentration of dissolved O_2 in equilibrium with air (277 μ*M* at 20°C). The large difference between the COP values for an organ or tissue and

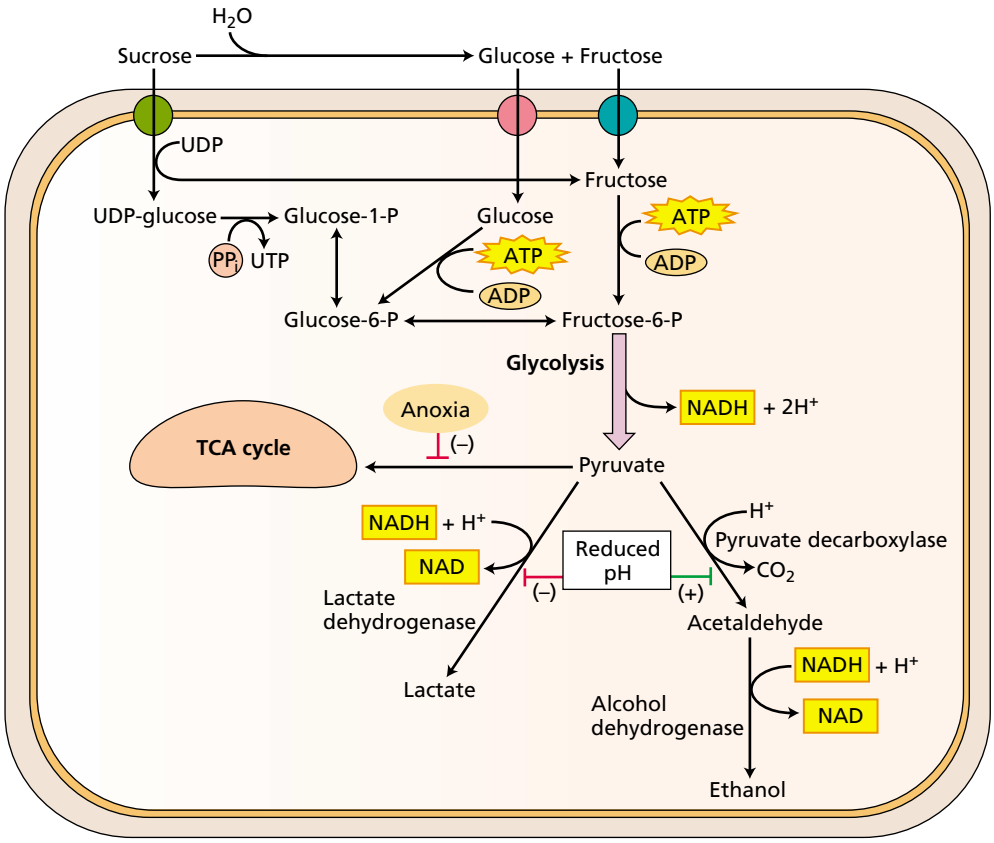


FIGURE 25.17 During episodes of anoxia, pyruvate produced by glycolysis is initially fermented to lactate. Proton production by glycolysis, and other metabolic pathways, and decreased proton translocation across the plasma membrane and tonoplast lead to a lowering of cytosolic pH. At lower pHs, lactate dehydrogenase activity is inhibited, and pyruvate decarboxylase is activated. This leads to an increase in the fermentation of ethanol and a decrease in the fermentation of lactate at lower pHs. The pathway of ethanol fermentation consumes more protons than does the pathway of lactate fermentation. This increases the cytosolic pH and enhances the ability of the plant to survive the episode of anoxia.

the O_2 requirements of mitochondria is explained by the slow diffusion of dissolved O_2 in aqueous media.

In the absence of O_2, electron transport and oxidative phosphorylation in mitochondria cease, the tricarboxylic acid cycle cannot operate, and ATP can be produced only by fermentation. Thus when the supply of O_2 is insufficient for aerobic respiration, roots first begin to ferment pyruvate (formed in glycolysis; see Chapter 11) to lactate, through the action of lactate dehydrogenase (LDH) (Figure 25.17). In the root tips of maize, lactate fermentation is transient because lowered intracellular pH quickly leads to a switch from lactate fermentation to ethanol fermentation. The shift occurs because of the different pH optima of the cytosolic enzymes involved.

At acidic pH, LDH is inhibited and pyruvate decarboxylase is activated. The net yield of ATP in fermentation is only 2 moles of ATP per mole of hexose sugar respired (compared with 36 moles of ATP per mole of hexose respired in aerobic respiration). Thus, injury to root metabolism by O_2 deficiency originates in part from a lack of ATP to drive essential metabolic processes (Drew 1997).

Nuclear magnetic resonance (NMR) spectroscopy was used to measure the intracellular pH of living maize root tips under nondestructive conditions (Roberts et al. 1992). In healthy cells, the vacuolar contents are more acidic (pH 5.8) than the cytoplasm (pH 7.4). But under conditions of extreme O_2 deficiency, protons gradually leak from the vacuole into the cytoplasm, adding to the acidity generated in the initial burst of lactic acid fermentation. These changes in pH (*cytosolic acidosis*) are associated with the onset of cell death.

Apparently, active transport of H^+ into the vacuole by tonoplast ATPases is slowed by lack of ATP, and without ATPase activity the normal pH gradient between cytosol and vacuole cannot be maintained. Cytosolic acidosis irreversibly disrupts metabolism in the cytoplasm of higher-plant cells, as it does in anoxic cells of animals. It is essentially this cytosolic acidosis that causes damage, and the timing and degree to which it is limited are the primary factors distinguishing flooding-sensitive from flooding-tolerant species.

Damaged O_2-Deficient Roots Injure Shoots

Anoxic or hypoxic roots lack sufficient energy to support physiological processes on which the shoots depend. Experiments have shown that the failure of the roots of wheat or barley to absorb nutrient ions and transport them to the xylem (and from there to the shoot) quickly leads to a shortage of ions within developing and expanding tissues. Older leaves senesce prematurely because of reallocation of phloem-mobile elements (N, P, K) to younger leaves. The lower permeability of roots to water often leads to a decrease in leaf water potential and wilting, although this decrease is temporary if stomata close, preventing further water loss by transpiration.

Hypoxia also accelerates production of the ethylene precursor **ACC** (**1-aminocyclopropane-1-carboxylic acid**) in roots (see Chapter 22). In tomato, ACC travels via the xylem sap to the shoot, where, in contact with oxygen, it is converted by ACC oxidase to ethylene. The upper (adaxial) surfaces of the leaf petioles of tomato and sunflower have ethylene-responsive cells that expand more rapidly when ethylene concentrations are high. This expansion results in epinasty, the downward growth of the leaves such that they appear to droop. Unlike wilting, epinasty does not involve loss of turgor.

In some species (e.g., pea and tomato), flooding induces stomatal closure apparently without detectable changes in leaf water potential. Oxygen shortage in roots, like water deficit or high concentrations of salts, can stimulate abscisic acid (ABA) production and movement of ABA to leaves. However, stomatal closure under these conditions can be attributed mostly to the additional production of ABA by the older, lower leaves. These leaves do wilt, and they export their ABA to the younger turgid leaves, leading to stomatal closure (Zhang and Zhang 1994).

Submerged Organs Can Acquire O_2 through Specialized Structures

In contrast to flooding-sensitive and flooding-tolerant species, wetland vegetation is well adapted to grow for extended periods in water-saturated soil. Even when shoots are partly submerged, they grow vigorously and show no signs of stress.

In some wetland species, such as the water lily (*Nymphoides peltata*), submergence traps endogenous ethylene, and the hormone stimulates cell elongation of the petiole, extending it quickly to the water surface so that the leaf is able to reach the air. Internodes of deep-water (floating) rice respond similarly to trapped ethylene, so the leaves extend above the water surface despite increases in water depth. In the case of pondweed (*Potamogeton pectinatus*), an aquatic monocot, stem elongation is insensitive to ethylene; instead elongation is promoted even under anaerobic conditions by acidification of the surrounding water caused by the accumulation of respiratory CO_2.

In most wetland plants, and in many plants that acclimate well to wet conditions, the stem and roots develop longitudinally interconnected, gas-filled channels that provide a low-resistance pathway for movement of oxygen and other gases. The gases (air) enter through stomata, or through lenticels on woody stems and roots, and travel by molecular diffusion, or by convection driven by small pressure gradients.

In many wetland plants, exemplified by rice, cells are separated by prominent, gas-filled spaces, which form a tissue called **aerenchyma**, that develop in the roots independently of environmental stimuli. In a few nonwetland plants, however, including both monocots and dicots, oxygen deficiency induces the formation of aerenchyma in the stem base and newly developing roots (Figure 25.18).

In the root tip of maize, hypoxia stimulates the activity of ACC synthase and ACC oxidase, thus causing ACC and ethylene to be produced faster. The ethylene leads to the death and disintegration of cells in the root cortex. The spaces these cells formerly occupied provide the gas-filled voids that facilitate movement of O_2.

Ethylene-signaled cell death is highly selective; cells not destined to die in the root are unaffected. A rise in cytosolic Ca^{2+} concentration is thought to be part of the ethylene signal transduction pathway leading to cell death. Chemicals that elevate cytosolic Ca^{2+} concentration promote cell death under noninducing conditions; conversely, chemicals that lower cytosolic Ca^{2+} concentration block cell death in hypoxic roots that would normally form aerenchyma. Ethylene-dependent cell death in response to hypoxia is an example of *programmed cell death*, which was discussed in Chapter 16 (Drew et al. 2000).

Some plants (or parts of them) can tolerate exposure to strictly anaerobic conditions for an extended period (weeks or months) before developing aerenchyma. These include the embryo and coleoptile of rice and of *Echinochloa crus-galli* var. *oryzicola* (rice grass), and rhizomes (underground horizontal stems) of *Schoenoplectus lacustris* (giant bulrush), *Scirpus maritimus* (salt marsh bulrush), and *Typha angustifolia* (narrow-leafed cattail). These rhizomes can survive for several months and expand their leaves in an anaerobic atmosphere.

In nature, rhizomes overwinter in anaerobic mud at the edges of lakes. In spring, once the leaves have expanded above the mud or water surface, O_2 diffuses down through the aerenchyma into the rhizome. Metabolism then switches from an anaerobic (fermentative) to an aerobic mode, and roots begin to grow using the available oxygen. Likewise, during germination of paddy (wetland) rice and of rice grass, the coleoptile breaks the water surface and becomes a diffusion pathway (a "snorkel") for O_2 to the rest of the plant. (Even though rice is a wetland species, its roots are as intolerant of anoxia as maize roots are.)

As the root extends into oxygen-deficient soil, the continuous formation of aerenchyma just behind the tip allows oxygen movement within the root to supply the apical zone. In roots of rice and other typical wetland plants, structural barriers composed of suberized and lignified

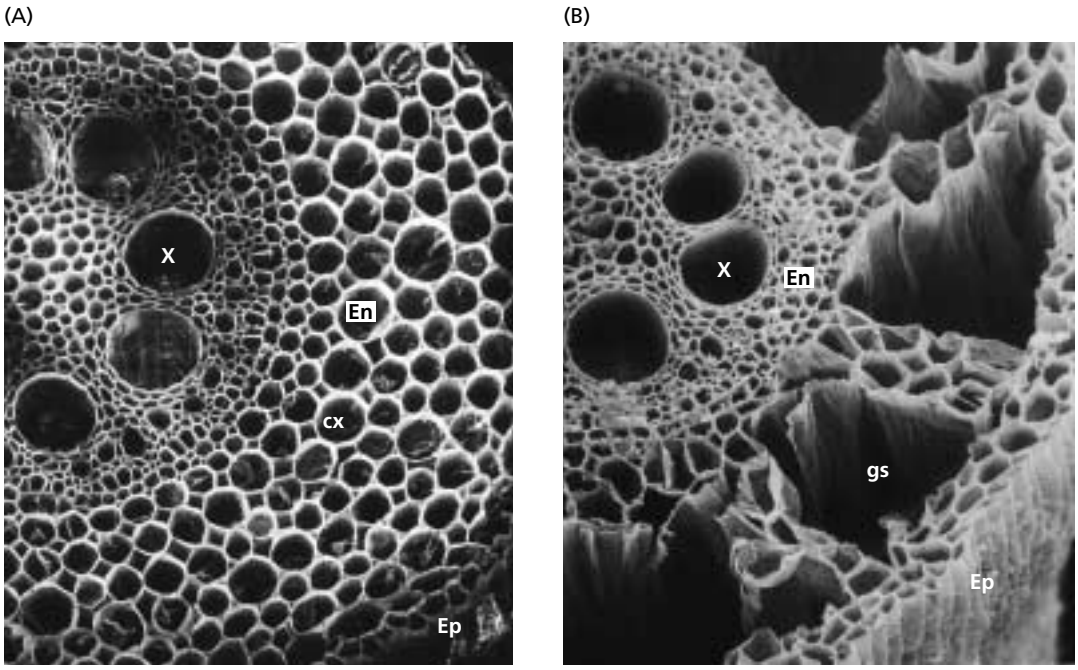


FIGURE 25.18 Scanning electron micrographs of transverse sections through roots of maize, showing changes in structure with oxygen supply. (150×) (A) Control root, supplied with air, with intact cortical cells. (B) Oxygen-deficient root growing in a nonaerated nutrient solution. Note the prominent gas-filled spaces (gs) in the cortex (cx), formed by degeneration of cells. The stele (all cells interior to the endodermis, En) and the epidermis (Ep) remain intact. X, xylem. (Courtesy of J. L. Basq and M. C. Drew.)

cells prevent O_2 diffusion outward to the soil. The O_2 thus retained supplies the apical meristem and allows growth to proceed 50 cm or more into anaerobic soil.

In contrast, roots of nonwetland species, such as maize, leak O_2, failing to conserve it to the same extent. Thus, in the root apex of these plants, internal O_2 becomes insufficient for aerobic respiration, and this lack of O_2 severely limits the depth to which such roots can extend into anaerobic soil.

Most Plant Tissues Cannot Tolerate Anaerobic Conditions

Most tissues of higher plants cannot survive prolonged anaerobic conditions. Root tips of maize, for example, remain viable for only 20 to 24 hours if they are suddenly deprived of O_2. Under anoxia, some ATP is generated slowly by fermentation, but the energy status of cells gradually declines during cytosolic acidosis. The precise combination of biochemical characteristics that allow some cells to tolerate anoxia for long periods is not fully understood. Root tips of maize and other cereals show a modest degree of acclimation if they first are made hypoxic, whereupon they can survive up to 4 days of anoxia.

Acclimation to an anaerobic condition is associated with expression of the genes that encode many of the anaerobic stress proteins (see the next section). After acclimation, the ability to carry out ethanolic fermentation under anoxia (thereby producing ATP to keep some metabolism going) is improved, and this improvement is accompanied by an ability to transport lactate out of the cytosol to the external medium, thus minimizing cytosolic acidosis (Drew 1997).

The ability of organs of wetland plants to tolerate chronic anoxia may depend on strategies similar to those just described, but they are clearly employed to greater effect: Critical features appear to be control of cytosolic pH, continued generation of ATP by glycolysis and fermentation, and sufficient storage of fuel for anaerobic respiration over extended periods. It has been suggested that synthesis of alanine, succinate, and γ-aminobutyric acid under anoxia consumes protons and minimizes cytosolic acidosis. Evidence to this effect has been found in anoxia-tolerant shoots of rice and rice grass, but not in anoxia-sensitive shoots of wheat and barley.

Organs of species that alternate between anaerobic and aerobic metabolism need to deal with the consequences of the entry of O_2 following anoxia. Highly reactive oxygen species are generated during aerobic metabolism, and they are normally detoxified by cellular defense mechanisms that involve superoxide dismutase (SOD). This enzyme converts superoxide radicals to hydrogen peroxide, which is then converted to water by peroxidase.

In anoxia-tolerant rhizomes of *Iris pseudacorus* (yellow flag), SOD activity increases 13-fold during 28 days of

anoxia. This increase is not observed in rhizomes of other *Iris* species that are not anoxia tolerant. In the tolerant species, SOD may be available to cope with the influx of O_2 that occurs when the leaves emerge into the air from water or mud, so it may assist in resisting postanoxic stress.

Acclimation to O_2 Deficit Involves Synthesis of Anaerobic Stress Proteins

When maize roots are made anoxic, protein synthesis ceases except for the continued production of about 20 polypeptides (Sachs and Ho 1986). Most of these anaerobic stress proteins have been identified as enzymes of the glycolytic and fermentation pathways.

The mechanism for sensing reduced oxygen levels under hypoxic or anoxic conditions is not completely clear. However, one of the earliest events to occur following lowering of O_2 levels is an elevation of the intracellular Ca^{2+}. Evidence suggests that this calcium signal is involved in the signal transduction of anoxia. Within minutes of the onset of anoxia, a rise in cytosolic Ca^{2+} concentration acts as a signal leading to increases in mRNA levels of alcohol dehydrogenase (ADH) and sucrose synthase in maize cells in culture.

Chemicals that block a rise in intracellular Ca^{2+} concentration also prevent the expression of the genes for ADH and sucrose synthase from being induced by anoxia, and they greatly enhance the sensitivity of maize seedlings to anoxia (Sachs et al. 1996). Further research is needed to resolve these mechanisms and to explain how intracellular Ca^{2+} concentration signals both the early survival of cells under anoxia and the induction of cell death and aerenchyma formation during prolonged hypoxia.

The accumulation of mRNAs of the anaerobic stress genes results from changes in the rate of transcription of these genes. Analysis of common sequence elements in the promoters of the ADH genes of maize and *Arabidopsis* and of the other anaerobic stress genes has led to the identification of an anaerobic stress element and a G-box element that bind *cis*-acting transcription factors leading to the transcriptional activation of these genes. However, the exact details of how oxygen deficiency is sensed, how the signal is transduced through elevations in cytosolic Ca^{2+} leading to alterations in the transcription of specific genes, remains to be determined.

Note also that there is strong evidence that some type of translational control of anaerobic stress genes is also occurring. The efficiency with which mRNAs for non-anaerobic stress–regulated genes are translated following hypoxic stress is dramatically lower than that of stress-regulated genes such as ADH.

SUMMARY

Stress is usually defined as an external factor that exerts a disadvantageous influence on the plant. Under both natural and agricultural conditions, plants are exposed to unfavorable environments that result in some degree of stress. Water deficit, heat stress and heat shock, chilling and freezing, salinity, and oxygen deficiency are major stress factors restricting plant growth such that biomass or agronomic yields at the end of the season express only a fraction of the plant's genetic potential.

The capacity of plants to cope with unfavorable environments is known as stress resistance. Plant adaptations that confer stress resistance, such as CAM metabolism, are genetically determined. Acclimation improves resistance as a result of prior exposure of a plant to stress.

Drought resistance mechanisms vary with climate and soil conditions. Indeterminate growth patterns such as that of sorghum and soybean allow these species to take advantage of late-occurring rains; plants with a determinate growth pattern, such as that of corn, lack that form of resistance to water stress. Inhibition of leaf expansion is one of the earliest responses to water stress, occurring when decreases in turgor ensuing from water deficit reduce or eliminate the driving force for cell and leaf expansion. Additional stress resistance mechanisms in response to water stress include leaf abscission, root extension into deeper, wetter soil, and stomatal closure.

Stress caused by water deficit leads to the expression of sets of genes involved in acclimation and adaptation to the stress. These genes mediate the cellular and whole-plant responses described here. The sensing and activation of signal transduction cascades mediating these changes in gene expression involve both an ABA-dependent pathway and an ABA-independent pathway.

Heat stress and heat shock are caused by high temperatures. Some CAM species can tolerate temperatures of 60 to 65°C, but most leaves are damaged above 45°C. The temperature of actively transpiring leaves is usually lower than air temperature, but water deficit curtails transpiration and causes overheating and heat stress. Heat stress inhibits photosynthesis and impairs membrane function and protein stability.

Adaptations that confer heat resistance include responses that decrease light absorption by the leaves, such as leaf rolling, and a decrease in leaf size that minimizes boundary layer resistance and increases conductive heat loss. Heat shock proteins synthesized at high temperatures act as molecular chaperones that promote stabilization and correct folding of cell proteins, and biochemical responses leading to pH and metabolic homeostasis are also associated with acclimation and adaptation to rapid rises in temperature.

Chilling and freezing stress ensue from low temperatures. Chilling injury occurs at temperatures that are too low for normal growth but are above freezing, and it is typical of species of tropical or subtropical origin exposed to temperate climates. Chilling injuries include slow growth, leaf lesions, and wilting. The primary cause of most chilling injuries is the loss of membrane properties ensuing from changes in membrane fluidity. Membrane lipids of

chilling-resistant plants often have a greater proportion of unsaturated fatty acids than those of chilling-sensitive plants.

Freezing injury is associated primarily with damage caused by ice crystals formed within cells and organs. Freezing-resistant species have mechanisms that limit the growth of ice crystals to extracellular spaces. Mechanisms that confer the resistance to freezing that is typical of woody plants include dehydration and supercooling.

Cold stress reduces water activity and leads to osmotic stress within the cells. This osmotic stress effect leads to the activation of osmotic stress–related signaling pathways, and the accumulation of proteins involved in cold acclimation. Other cold specific, non-osmotic stress–related genes are also activated. Transgenic plants overexpressing cold stress–activated signaling components demonstrate increased cold tolerance.

Salinity stress results from salt accumulation in the soil. Some halophyte species are highly tolerant to salt, but salinity depresses growth and photosynthesis in sensitive species. Salt injury ensues from a decrease in the water potential of the soil that makes soil water less available and from toxicity of specific ions accumulated at injurious concentrations. Plants avoid salt injury by exclusion of excess ions from leaves or by compartmentation of ions in vacuoles. Some of the molecular determinants of Na^+ exclusion and vacuolar partitioning have been determined, and a signaling pathway, the SOS pathway, regulating the expression of these genes involved in ion homeostasis has been established.

Oxygen deficiency is typical of flooded or waterlogged soils. Oxygen deficiency depresses growth and survival of many species. On the other hand, plants of marshes and swamps, and crops such as rice, are well adapted to resist oxygen deficiency in the root environment. Most tissues of higher plants cannot survive anaerobically, but some tissues, such as the embryo and coleoptiles from rice, can survive for weeks under anoxic conditions. The metabolic pathways for resisting anoxic damage and their regulation have been uncovered.

Web Material

Web Topics

25.1 Stomatal Conductance and Yields of Irrigated Crops
Stomatal conductance predicts yields of irrigated crops grown in hot environments.

25.2 Membrane Lipids and Low Temperatures
Lipid enzymes from mutant and transgenic plants mimic the effects of low-temperature acclimation.

25.3 Ice Formation in Higher-Plant Cells
Heat is released when ice forms in intercellular spaces.

25.4 Ca^{2+} Signaling and Activation of the Salt Overly Sensitive (SOS) Signal Pathway
Three genetically linked loci control ion homeostasis and salt tolerance.

25.5 Na^+ Transport across the Plasma Membrane and Vacuolar Compartmentation
SOS1 is an Na^+–H^+ antiporter that controls Na^+ fluxes across the plasma membrane.

25.6 Gene Transfer and Stress Tolerance
Transgenic plants are valuable tools for studying stress tolerance.

Web Essay

25.1 The Effect of Air Pollution on Plants
Polluting gases inhibit stomatal conductance, photosynthesis, and growth.

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