Abiotic Stress

31

Satish C Bhatla

The conditions which allow plants to attain maximal growth and reproductive potential, as determined by their total biomass (comprising of plant height, weight, and seed number), can be considered as the ideal growth conditions for the particular plant. Since plants grow and reproduce in complex environmental conditions composed of a multitude of abiotic and biotic factors, they are frequently exposed to stress conditions. **Stress** can be described as environmental conditions which prevent plants from attaining their full genetic potential, and, hence, it adversely affects plant growth, development, and productivity. Environmental factors which result in stress may be divided into biotic and abiotic. **Biotic** stress is imposed by direct or indirect interactions with other organisms, whereas **abiotic** stress originates from excess or deficit in the physical, chemical, and energetic conditions to which plants are exposed. Environmental conditions which have deleterious effect on plants include drought, flood, excessive soil salinity, high or low temperature, excess or insufficient light, and inadequate mineral nutrients. Phytotoxic molecules, like ozone, can also cause damage to plant tissues. Stress affects numerous plant responses ranging from changes in cellular metabolism and gene expression to modulation of growth rate and crop yield. The response of a plant is influenced by the severity, duration, and rate at which stress is imposed. A single stress or a combination of varied stress conditions can result in different stress responses. Moreover, plant responses can be elicited directly by stress or indirectly through stress-induced injury. Various factors, such as genotype, developmental stage, species, and organ or type of tissue, influence the sensitivity or resistance of plants to stress condition(s).

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31.1 Plant Responses to Abiotic Stress

31.1.1 Decline in Crop Yield

Losses due to biotic and abiotic stress conditions can result in a substantial decline in average crop productivity. Stress-tolerant crop plants, developed with the successful application of classical and biotechnological breeding techniques, can lead to an increase global food supplies, and, hence, they can be economically beneficial. Such crops can maintain yield under moderate environmental stress conditions or can enhance survival during intense or prolonged stress periods.

31.1.2 Physiological Adjustment

Stressed plants undergo flowering, fertilization, and seed set before attaining their full size, thereby resulting in smaller plants. The seeds produced may also be smaller and fewer in number due to limited leaves to provide photosynthates under stress. The specific developmental pathways adopted to maximize reproductive potential under stress conditions mainly depend on the life cycle of a plant. They are favorable for annual plants, which complete their life cycle in a single season, to adjust their developmental programs and metabolism in order to yield maximum number of viable seeds under prevailing environmental conditions. In contrast, perennial plants, which survive for over 2 years or more, tend to adjust their developmental programs and metabolism to secure requisite storage of food resources in order to survive the next season, even at the cost of seed production.

31.1.3 Resistance Mechanisms

Due to their sessile nature, plants have to adapt and develop unique mechanisms to combat varied stress conditions. Plants may deal with suboptimal environmental conditions using two general resistance mechanisms: tolerance or avoidance. **Stress tolerance** mechanisms enable the plants to withstand stress by development of resilient structures and physiological processes, whereas **stress avoidance** mechanisms prevent or minimize exposure to vulnerable stages of stress conditions by adopting life cycle strategies which limit growth to favorable periods in a varying environment (Fig. 31.1). The ability to survive varied degrees of stress may differ substantially depending upon plant species and genotype of a species. The **desert ephemerals** (short-lived weed plants growing in desert) germinate and complete their life cycles, while optimum water is available and, thus, avoids drought (Fig. 31.2). Another drought-avoidance mechanism is deep roots, which provides **phreatophytes** with improved access to groundwater and increased chances of survival during dry periods. Living organisms tend to maintain homeostasis by adjusting to stress and minimizing strain. Three modes of response to disturbance are observed in a homeostatic system. During *elastic response*, system bounces back

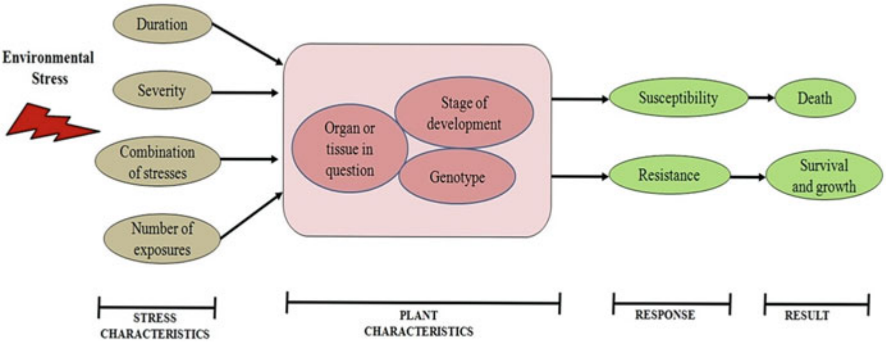


Fig. 31.1 Factors affecting stress resistance in plants

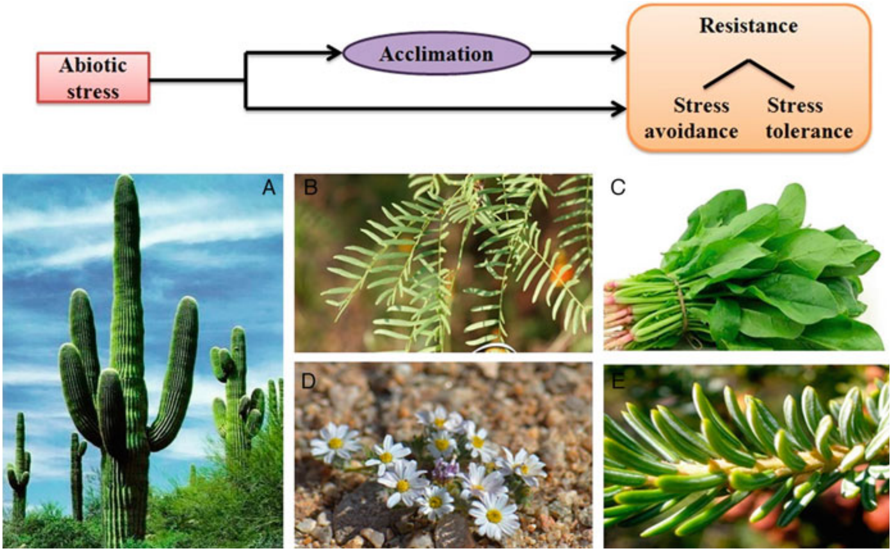


Fig. 31.2 Some examples of plant adaptations for stress tolerance. (**a**) *Cereus giganteus* (a drought-tolerant species showing succulent photosynthetic stem), (**b**) *Prosopis glandulosa* (a drought-avoiding species), (**c**) *Spinacia oleracea* (known to exhibit osmotic adjustment as acclimation mechanism), (**d**) *Monoptilon bellioides* (a desert plant which grows only in wet season), and (**e**) *Picea mariana* (a desert plant with needle-like leaves to tolerate freezing)

and regains its former state, and during *plastic response*, system is deformed and settles on a new stable configuration. A *catastrophic response* is observed when the limits of both elastic and plastic resilience are exceeded, during which the system enters into a stage of incoherence, entropy enhances, and the living system dies (Fig. 31.3). The homeostatic adjustment of an individual organism in response to fluctuating environmental factors is referred to as **acclimation**. Resistance may be conferred to an otherwise vulnerable plant by a period of acclimation before stress.

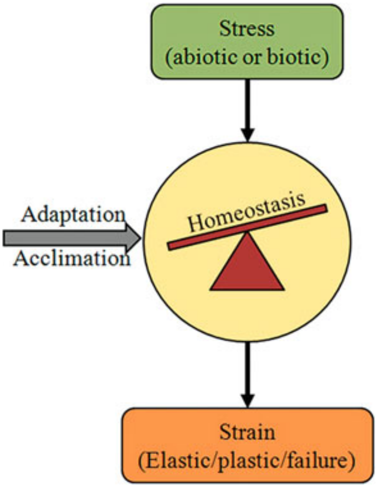


Fig. 31.3 Biotic or abiotic stress factors invoke strain in the form of elastic, plastic, or system failure responses. The extent to which homeostasis can be maintained is conditioned by the acclimatory or adaptive capacities of the organ, individual, or population

For example, in a process during gardening, *hardening off*, farmers grow plants indoors in pots under optimal conditions before moving them outdoors, so that they can be “toughened up” or “hardened” before exposure to nonlethal stress conditions. Another example of acclimatization is temperature stress, in which plants are unable to withstand freezing in summer, but some can acclimate in response to gradually declining temperatures in fall and may subsequently withstand even temperatures below −50 °C in winters. However, the physiological changes related to acclimation do not take place at genetic level and are generally reversible. Continuous or recurrent environmental stress conditions may exert selective pressure on the plants, resulting in evolution of fitness-enhancing traits. Certain morphological and physiological characteristics, such as crassulacean acid metabolism (CAM), water-storing succulent tissues, and spines, enable many **xerophytes** to tolerate stress. Irrespective of stress conditions, some constitutive, genotypically determined traits, such as light-reflective spines, deep roots, and sunken stomata, are expressed in some plants. Such evolutionary improvements, which enhance the fitness of the organism and occur over many generations and across entire populations, are termed **adaptations**. Stress resistance, based on either adaptation or acclimation, supports survival under fatal conditions, thereby maintaining crop productivity under adverse circumstances (Table 31.1).

31.1.4 Alteration in Gene Expression Patterns

Changes in the pattern of gene expression are often involved in stress-induced modifications in development and metabolism. The recognition of stress at the cellular level initiates a stress response in plants by activation of signal transduction pathways which transfer information within individual cells as well as intercellularly all over the plant. Eventually, the alterations in gene expression are amalgamated

Table 31.1 Adaptation versus acclimation in response to stress

	Adaptation	Acclimation
Level	Population	Individual
Caused by	Natural selection	Environmental conditions acting on physiological responsiveness
Reversibility	Irreversible	Reversible
Heritability	Genotypic	Generally non-heritable
Response of homeostasis to perturbation	Mostly plastic	Mostly elastic
Time scale	From generation time of the organism up to evolutionary	*Short-term* (minutes/hours) metabolic and physiological adjustments of existing components. No significant change in gene expression *Long-term* (up to weeks or months) altered patterns of gene expression and reallocation of resources, morphological change
Deployment in the life cycle	Strategic	Tactical

into a stress response by the entire plant, which modulates growth and development and can also influence its reproductive abilities. The timing and scale of response are governed by the severity and duration of the stress. Though bacterial and yeast proteins involved in initiation of signal transduction in response to stress (such as low osmotic potential) are known, similar information about stress recognition signaling molecules in plants is relatively new. Hormones, especially jasmonic acid (JA), ethylene, and abscisic acid (ABA), and second messengers, such as calcium ions, participate in the regulation of many abiotic stress responses in plants, but the intricate and intensive signaling pathways which are supposedly involved in changing the gene expression patterns are gradually being elucidated.

Enhanced or declined expression of certain genes and protein products may be exhibited in response to abiotic stress. Scientists are currently focusing on the functions of these proteins and the mechanisms involved in the regulation of their expression. Posttranscriptional regulatory mechanisms elevate levels of some protein-coding mRNAs or noncoding regulatory RNAs, increase translation, stabilize proteins, and modulate protein activity by different types of modifications. They can also influence the accumulation of gene products in addition to transcriptional activation of gene expression. Parallel or even overlapping approaches, such as activation or repression of gene expression, activation of transport activity, and production of proteins or compatible solutes, may also be adopted during plant's response to stresses. Plants are also frequently exposed to serial or concurrent abiotic stresses, such as flooding followed by drought or heat and drought. The synergistic or antagonistic interactions between varied kinds of stresses can influence the coordinated success of the activity of several genes, which enhance survival of crops in response to abiotic stress.

31.2 Oxidative Stress

31.2.1 Production of Reactive Oxygen Species (ROS)

Molecular oxygen is relatively unreactive in its ground state but can give rise to various toxic reactive forms, like free radicals, as a result of various environmental stresses. Reactive oxygen species (ROS) may result from the transfer of one, two, or three electrons to molecular oxygen (O_2) to form superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), or hydroxyl radical ($^{\bullet}OH$), respectively, and from the excitation of O_2 to form singlet oxygen ($^1O_2$). The term ROS refers not only to free radicals but also to other toxic non-radicals as well. Reactive oxygen species (ROS) are constantly produced in all aerobic organisms as by-products of various metabolic pathways (e.g., during respiration, photorespiration, and photosynthesis) localized in various cellular compartments, predominantly chloroplasts, peroxisomes, and mitochondria (Table 31.2). One-electron reduction of O_2 results in the formation of superoxide anion ($O_2^{\bullet -}$), which further gets converted into H_2O_2 upon its dismutation by the enzyme superoxide dismutase (SOD). H_2O_2, a non-radical molecule, is neutralized by glutaredoxin enzyme system [glutathione reductase, glutathione (GSH), and glutathione peroxidase] and catalase into O_2 and H_2O (Fig. 31.4). In plants, tripeptide glutathione (γ-glutamyl-L-cysteinylglycine, GSH) is one of the most common non-protein thiol metabolites. GSH serves as a major intracellular defense against ROS-induced oxidative damage. Under stress conditions, GSH concentration usually declines, and redox state becomes more oxidized, thereby causing damage to the system. So, the generation and maintenance

Table 31.2 Mechanisms of generation of various types of ROS in a plant cells

Molecule	Abbreviation	Biological source
Molecular oxygen (triplet ground state)	O_2.	Most common form of dioxygen gas
Singlet oxygen (first excited singlet state)	$^1O_2$.	Photoinhibition, PSII electron transfer
Superoxide anion	$O_2.^-$	Mitochondrial electron transfer reactions, Mehler reaction (reduction of O_2 by iron-sulfur center of PSI), photorespiration in glyoxysomes, peroxisome reactions, plasma membrane, paraquat oxidation, nitrogen fixation pathogen, reaction of O_3 and OH^- in apoplast, respiratory burst homolog (NADPH oxidase)
Hydrogen peroxide	H_2O_2	Photorespiration, β-oxidation, proton-induced decomposition of $O_2.^-$, pathogen defense
Hydroxyl radical	HO^-	Decomposition of O_3 in apoplast, pathogen defense, Fenton reaction
Perhydroxyl radical	$HO_2.^-$	Reaction of O_3 and HO^- in apoplast
Nitric oxide	NO	Nitrate reductase, nitrite reduction by the mitochondrial electron transport chain

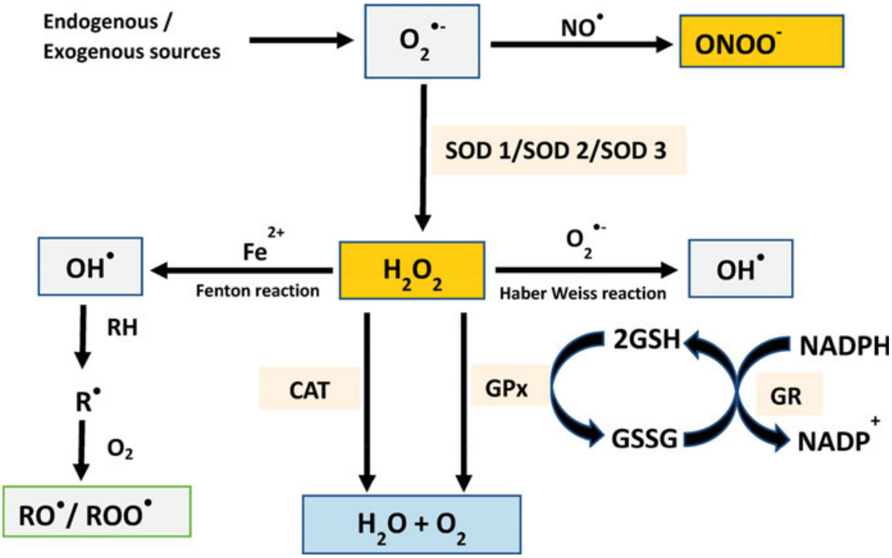


Fig. 31.4 Production and interconversion of various reactive oxygen species (ROS). *CAT* catalase, *GR* glutathione reductase, *GPX* glutathione peroxidase, *SOD* superoxide dismutase, *GSH* reduced glutathione, *GSSG* oxidized glutathione

of reduced GSH pool are crucial for the cell. Glutathione peroxidase (GPx) reduces GSH into its oxidized form (GSSG), which is again regenerated by glutathione oxidase along with the oxidation of NADPH (ascorbate-glutathione cycle). Additionally, $^{\bullet}OH$, the most reactive chemical species in the biological world, can be produced in the presence of transition metals, such as iron and copper through the Fenton reaction or by the Haber-Weiss mechanism. $O_2^{\bullet-}$ can also form peroxynitrite [$ONOO^-$, a kind of reactive nitrogen species (RNS)] upon reaction with another signaling free radical species, NO. Nitrogen-centered free radicals are termed as reactive nitrogen species (RNS). RNS include S-nitrosoglutathione (GSNO), peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), and nitrogen dioxide (NO_2).

31.2.2 Dual Role of ROS

A rapid rise in ROS concentration results in oxidative stress in the cells, known as **oxidative burst**. Rate of ROS production and cellular ROS levels both increase significantly in plants subjected to abiotic or biotic stresses. Chemically, ROS are highly reactive and biologically toxic in nature. They exhibit much shorter half-life as compared to that of oxygen. It is established that ROS play dual roles in plant cells (Fig. 31.5). At normal concentrations under unstressed conditions, they play the role of signaling mediators for different cellular responses, such as stomatal closure, root gravitropism, seed germination, plant cell death, lignin biosynthesis, osmotic stress, and hypersensitive responses, which may be activated by certain external and

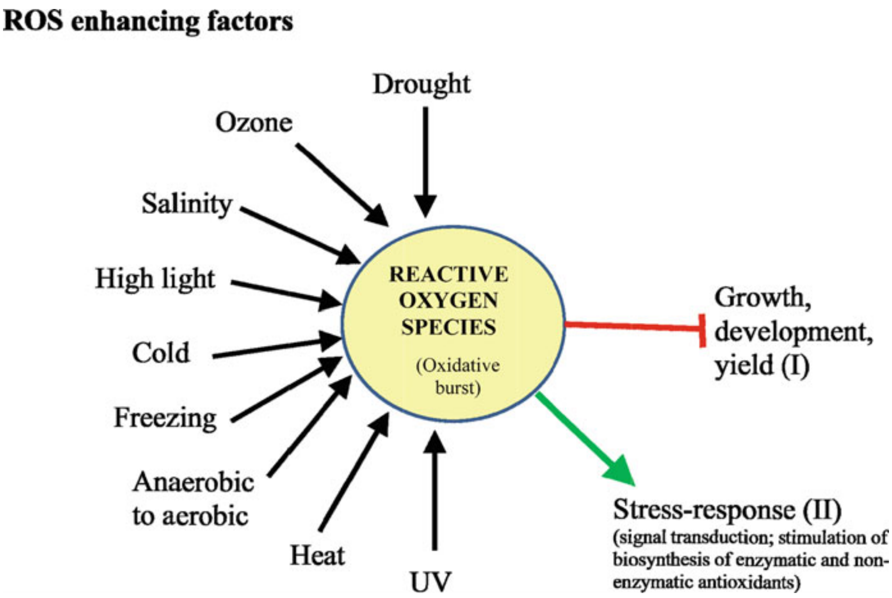


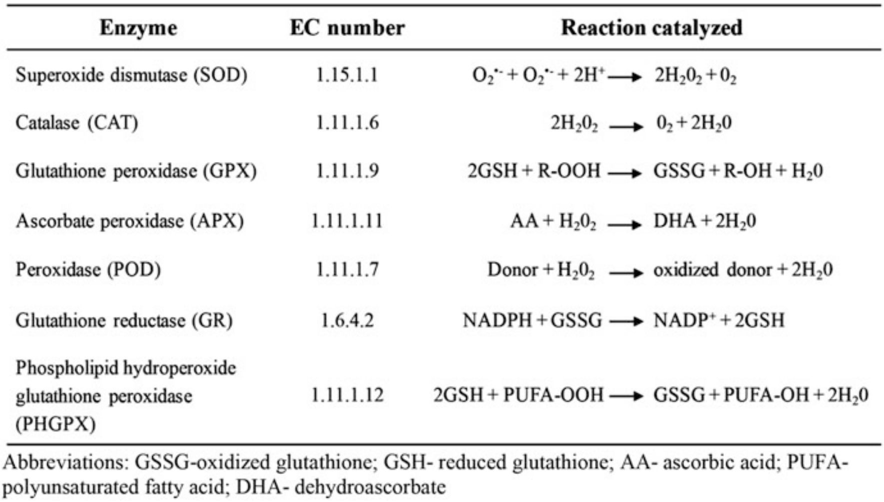
Fig. 31.5 Dual role of ROS during abiotic stress

developmental stimuli. Excess of ROS produced in plant cells under stress conditions can lead to damage to biomolecules like proteins, lipids, carbohydrates, and DNA. These, in turn, can lead to alteration in the intrinsic membrane properties such as fluidity, loss of enzyme activity, ion transport, DNA damage, inhibition of protein synthesis, etc. Under physiological steady-state conditions, ROS concentration is maintained low by different antioxidative defense mechanisms which help in scavenging ROS. Various factors may disturb the equilibrium between production of ROS and their scavenging. As a result, intracellular ROS levels may rapidly rise.

31.2.3 Cellular Antioxidative Defense System

Under normal growth conditions, plants are well-adapted to deal with the deleterious effects of ROS. However, during stress, ROS production exceeds the quenching capacity of the antioxidant protective systems. In order to overcome oxidative damage caused due to high ROS concentration and for maintaining **redox homeostasis**, plants have a well-established defense system which includes both nonenzymatic and enzymatic antioxidants. The nonenzymatic antioxidant defense system consists of vitamins (A, C, E), glutathione, ascorbate, tocopherol, carotenoids, and phenols which directly react with ROS, leading to their scavenging. The enzymatic antioxidant defense system consists of enzymes, such as peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHR),

Table 31.3 Major antioxidant enzymes involved in ROS scavenging



Enzyme	EC number	Reaction catalyzed
Superoxide dismutase (SOD)	1.15.1.1	$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \longrightarrow 2H_20_2 + 0_2$
Catalase (CAT)	1.11.1.6	$2H_20_2 \longrightarrow 0_2 + 2H_20$
Glutathione peroxidase (GPX)	1.11.1.9	$2GSH + R\text{-}OOH \longrightarrow GSSG + R\text{-}OH + H_20$
Ascorbate peroxidase (APX)	1.11.1.11	$AA + H_20_2 \longrightarrow DHA + 2H_20$
Peroxidase (POD)	1.11.1.7	$\text{Donor} + H_20_2 \longrightarrow \text{oxidized donor} + 2H_20$
Glutathione reductase (GR)	1.6.4.2	$NADPH + GSSG \longrightarrow NADP^+ + 2GSH$
Phospholipid hydroperoxide glutathione peroxidase (PHGPX)	1.11.1.12	$2GSH + PUFA\text{-}OOH \longrightarrow GSSG + PUFA\text{-}OH + 2H_20$

Abbreviations: GSSG-oxidized glutathione; GSH- reduced glutathione; AA- ascorbic acid; PUFA-polyunsaturated fatty acid; DHA- dehydroascorbate

and superoxide dismutase (SOD) which help in scavenging specific reactive oxygen species (Table 31.3) (Fig. 31.6). Various peroxidases, catalase, and the enzymes of ascorbate-glutathione cycle (APX, MDHAR, DHAR, and GR) quench peroxides through a series of coupled redox reactions (Fig. 31.7). These enzymes are nonuniformly distributed in various cellular compartments, and their isozymes are activated to varying extents upon exposure to stress. Oxidative stress can stimulate the biosynthesis of both nonenzymatic and enzymatic components of antioxidant machinery in many plants.

31.2.4 Ozone Exposure Leads to Oxidative Stress

Ozone, a normal component of stratosphere, protects Earth from hazardous UV radiation. However, since living organisms are increasingly exposed to harmful levels of ozone in the troposphere (lower atmosphere), ozone can cause damage through oxidative stress in a ROS-mediated manner. Ozone, as a pollutant, is generated in the lower atmosphere from reactions of molecular oxygen with anthropogenic hydrocarbons and oxides of sulfur (SO_x) and nitrogen (NO_x) under the effect of UV radiation. High concentration of ozone is one of the best characterized oxidative stress factors. Exposure of plants to damaging levels of ozone leads to impaired photosynthesis, reduction in growth of roots and shoots, and decrease in crop yield and leaf injury. Plants exhibit variable capacity to survive in high-ozone environments. They employ both avoidance and tolerance mechanisms to resist stress by closing stomata to exclude pollutants and by activating the antioxidant defense and varied repair mechanisms. Ozone-derived ROS, produced upon reaction

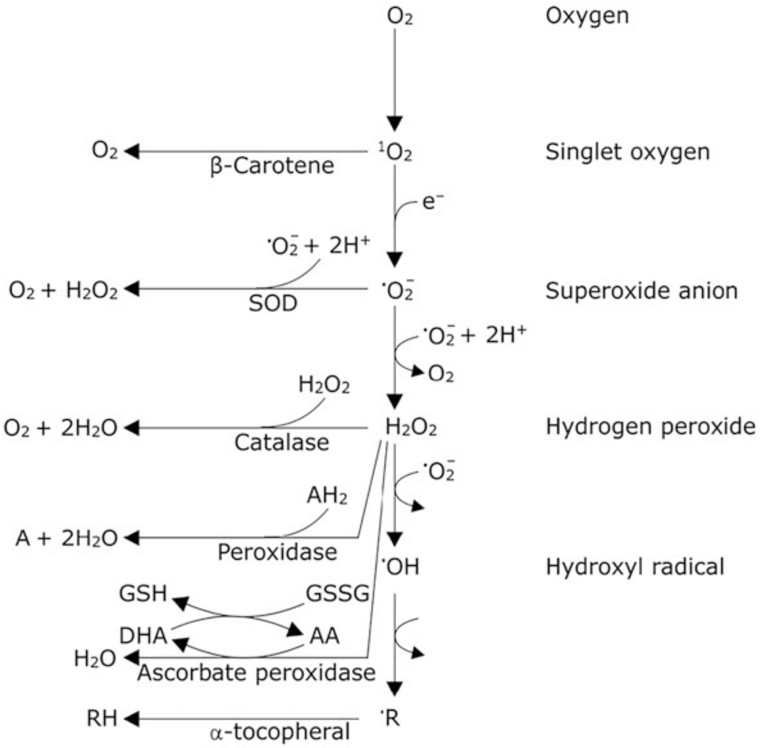


Fig. 31.6 ROS formation and action of ROS scavenging enzymes. *AA* ascorbic acid, *DHA* dehydroascorbate, *GSH* glutathione, *GSSG* oxidized glutathione, *SOD* superoxide dismutase

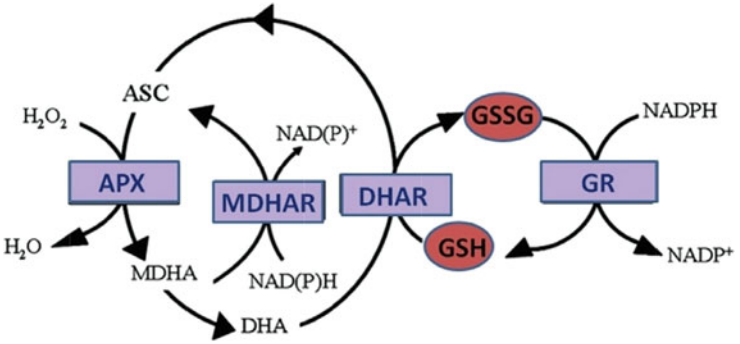


Fig. 31.7 Ascorbate-glutathione cycle (Foyer-Halliwell-Asada pathway) for quenching peroxides. *APX* ascorbate peroxidase, *MDHAR* monodehydroascorbate reductase, *DHAR* dehydroascorbate reductase, *GR* glutathione reductase, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *ASC* ascorbate, *MDHA* monodehydroascorbate, *DHA* dehydroascorbate

of ozone with ethylene and other alkenes in the apoplastic fluid, causes damage to membrane lipids, collapses membrane potential, increases Ca^{2+} uptake, inhibits H^+-pump, and enhances membrane permeability. It can target proteins, carbohydrates, and nucleic acids as well. In the atmosphere, NO can react with excess ozone, thereby reducing its (ozone's) deleterious effects on plant growth.

ROS concentration can be significantly and rapidly enhanced due to disturbed equilibrium between ROS production and scavenging under varied abiotic stress conditions. ROS levels are stimulated, and membranes and proteins are rendered dysfunctional under temperature stress, such as chilling, excessive heat, or freezing. Chilling stress increases ROS production by inactivation of cytochrome c oxidase, while heat stress impairs the respiratory electron transport chain in mitochondria resulting in peroxidation of membrane lipids due to oxidative stress. In chloroplasts, ROS levels are enhanced due to damage to the photosynthetic machinery in response to freezing and chilling stresses. Varied stress conditions, such as drought, salinity, deoxygenation following flooding, and osmotic stress also result in increase in ROS and hence ROS-associated injury.

31.3 Salt Stress

One of the major widespread environmental stresses is caused by soil salinity. It can limit growth and development of plants due to excess chloride and sodium ions in soil, particularly in arid and semiarid regions. **Salinity** refers to an excessive accumulation of salts in soil solution. Soil salinity is a major threat to global food security. Up to 22% of the world's irrigated land, which produces one-third of the world's food, is salt affected. Global warming leads to more variations in atmospheric temperature, rise in temperature, and erratic rainfall leading to increase in salt concentration in top soil due to drying of soil, and a rise in sea levels results in salt water entering the rivers where water that is being used for irrigation is from. Terrestrial plants are rarely salt-tolerant due to their evolution from freshwater algae. The functions of Na^+ in marine ancestry are fulfilled by K^+ and H^+ in all terrestrial plants. When the concentrations of the sulfates or chlorides of Na, Mg, and Ca in the soil are enough to produce an electrical conductivity of at least 4 $dS.m^{-1}$ in the soil extract and a sodium absorption ratio [SAR; based on $Na/(Ca^{2+} + Mg^{2+}]$ of less than 13, it is referred to as **saline soil**. Saline soils contain high levels of NaCl together with some fraction of $CaSO_4$, $MgSO_4$, and Na_2SO_4, while soils containing high Na_2CO_3 and high SAR due to low calcium and magnesium concentrations are called **sodic soils** (Table 31.4).

Table 31.4 Types and properties of salt-containing soils

Soil type	Constituent salts	pH range
Saline soils	$CaSO_4$, $MgSO_4$, NaCl, Na_2SO_4	pH 6–7
Saline-sodic soils	NaCl, $CaSO_4$, $MgSO_4$	pH < 8.5
Sodic soils	High NaCl/Na_2SO_4 (most deleterious for plants)	pH 8.5–12

Fig. 31.8 Some examples of halophytes. (**a**) *Atriplex nummularia*, (**b**) *Disphyma australe*, (**c**) *Suaeda maritima*

Plants develop mechanisms for salt exclusion or salt tolerance to deal with deleterious effects of salt stress. They are classified as halophytes or glycophytes, depending upon their capacity to survive on substrate containing high salt concentration. **Halophytes** (salt-tolerant plants) can grow at considerably high concentrations of salt, for example, *Atriplex nummularia*, *Disphyma australe*, and *Suaeda maritima* (Fig. 31.8). Depending upon the salt sensitivity, varied types of responses have been categorized in terrestrial plants: *Type I response*, plants which require some salt for adequate growth but are inhibited at seawater salinities, e.g., saltbush; *Type II response*, plants which exhibit significant decline in growth at EC of 4 $dS.m^{-1}$, such as rice and barley; and *Type III response*, salt-sensitive plant species which are unable to grow in saline soils, e.g., chickpea (Fig. 31.9). Salt-tolerant plants (halophytes) comprise of 0.25% of known angiosperms and include around 350 species mainly from Caryophyllales, Alismatales, Malpighiales, Poales, and Lamiales. Usually terrestrial plants are unable to tolerate salt stress and are referred as **glycophytes** (sweet plants). Most of the world's staple food crops are salt-sensitive, and some crops exhibit tolerance to mild salinity. Glycophytes and halophytes have strong differences in terms of their evolutionary and adaptational backgrounds. At low concentrations of around 1 mm Na^+ in the soil, some crops show Na^+-induced enhancement in growth, and also small amounts of sodium ions are essential for species with C_4 and CAM photosynthetic pathways. Thus, for these plants, sodium is a *beneficial element* (Table 31.5).

31.3.1 Disruption of Ionic Homeostasis due to Salt Stress

Salinity imposes stress both at cellular and whole plant levels. NaCl is an osmotically active compound and hence predominantly affects plant growth through *osmotic stress*. It reduces the water potential of soil, thereby restricting water uptake, and hence it leads to water deficiency. Osmotic stress so developed changes cell volume and results in loss of turgor pressure. Moreover, salinity causes *ionic stress* due to excessive uptake of Na^+ and Cl^-, leading to alterations in ion distribution

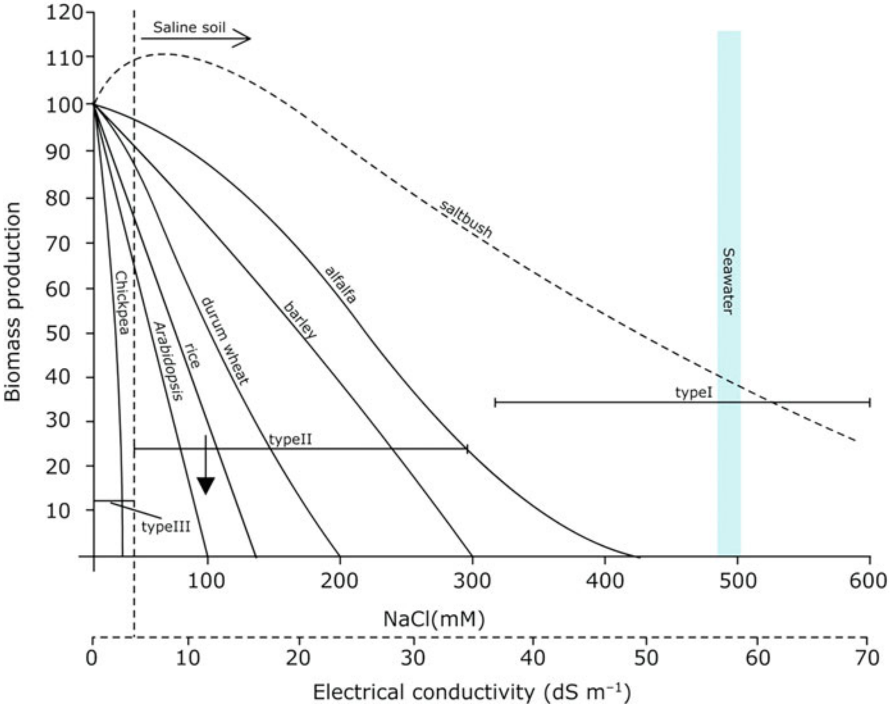


Fig. 31.9 Salt-tolerance limits of selected plants in terms of biomass production with reference to electrical conductivity of the growth medium

Table 31.5 Sodium as an essential element for plant development

Plant	Effect
Anabaena cylindrica Lemm.	Growth enhancement
C4 plants	Aids in metabolism by regeneration of
Cynodon dactylon	Phosphenol pyruvate and its uptake in
Amaranthus tricolor	Chloroplasts
Portulaca grandiflora	
Panicum miliaceum	

pattern (cytotoxicity) and aberrations in associated biochemical pathways. Potassium transporters take part in the maintenance of high concentration of K^+ in the plant cells and because of similarity of Na^+ to K^+ in terms of their ionic radii and ion hydration energies, these two ions compete for uptake via transporters under saline conditions. Plant growth is considerably reduced due to high Na^+:K^+ ratio, ultimately leading to toxicity (Fig. 31.10). High concentrations of sodium (Na^+) and chloride (Cl^-) ions in soil result in high salinity and, hence, cause both hyperionic and hyperosmotic conditions. A range of deleterious effects are caused by salinity stress. These include inhibition of photosynthesis rate, chlorophyll content, damage

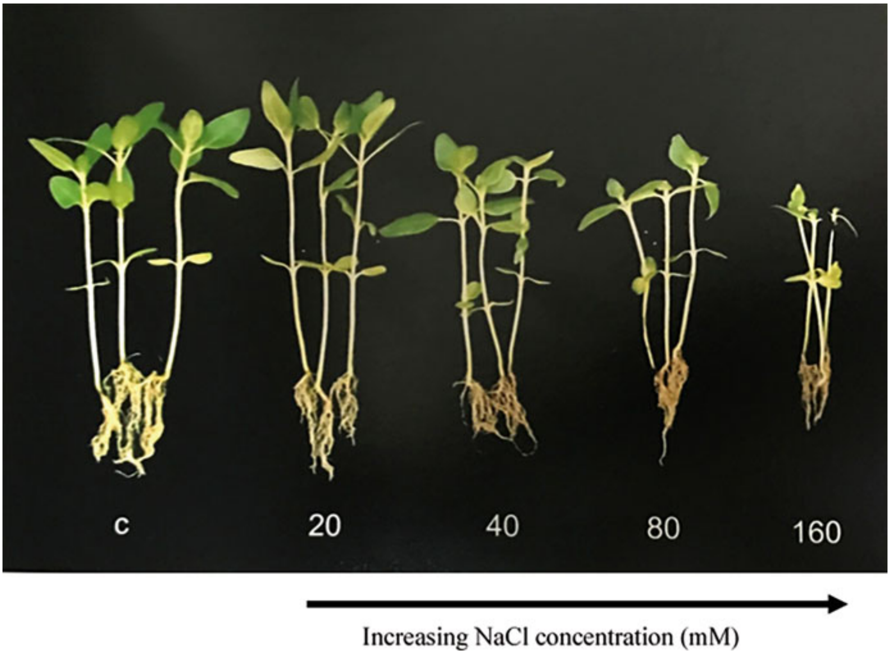


Fig. 31.10 Effect of NaCl salinity levels on the growth of entire sunflower plants grown in sand culture (25 days old) and irrigated with Hoagland nutrient solution supplemented with 20, 40, 80, and 160 mm of NaCl. C represents control plants (treated with only Hoagland nutrient medium)

to plasma membrane permeability, alteration in nutrient uptake, accumulation of toxic ions, osmotic and oxidative stress, and other metabolic disturbances in a number of plants.

31.3.2 Sodium Entry Through Symplastic and Apoplastic Pathways

Low cytosolic concentrations (1–5 mm) of sodium ions in plant cells lead to strong electrochemical driving force which facilitates inward entry of Na^+ through passive transport systems. Sodium influx into the root cells and its sequestration or long-distance transport are highly regulated. Entry of Na^+ into the epidermis of the root cells is followed by its apoplastic and symplastic movement to the cortical zone, endodermis, and xylem parenchyma cells (Fig. 31.11a). Development of Casparian strips is initiated in the differentiating zone of root endodermis, thereby restricting toxic solutes to enter the stelar cells. The suberized Casparian strip rarely acts as a complete barrier to apoplastic movement of Na^+. Such apoplastic bypass plays a crucial role in the entry of Na^+ in many species. Elongation zone of the roots is highly susceptible to Na^+ influx due to higher absorption capability in this region. In the plant roots, nonselective cation channels (NSCCs) are primarily responsible for Na^+ influx across the plasma membrane. Na^+/H^+ exchangers (NHXs) carried out the

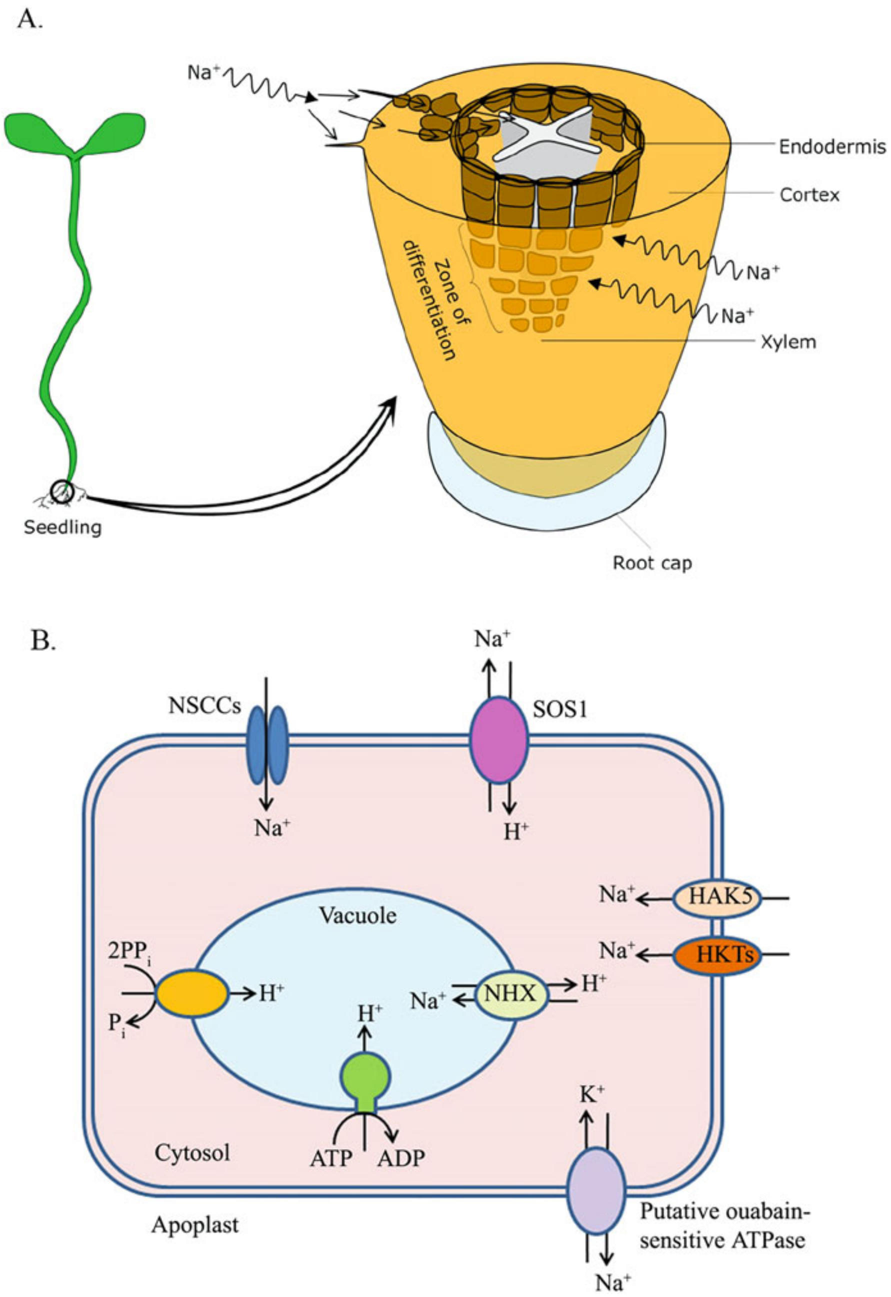


Fig. 31.11 (**a**) Anatomical features of root apex and subapical region highlighting the zone for Na^+ uptake. (**b**) Mechanisms of Na^+ influx and efflux in plant cells

compartmentalization of Na^+ into vacuoles. Na^+ efflux from the roots is mediated by the salt overly sensitive 1 (SOS1) protein catalyzed activity of Na^+/H^+ antiporters. In animals, ouabain (OU)-sensitive Na^+, K^+-ATPase (a P-type ATPase) mediates sodium efflux. The evolution of P-type ATPases in higher plants revealed the possibility of an OU-sensitive sodium efflux mechanisms similar to that present in animal cells (Fig. 31.11b).

31.3.3 Salt Stress-Induced Signal Transduction Events

Plant responses upon salt exposure include extensive changes in gene expression and numerous physiological changes. Such responses to salt stress involve initiation of ABA-dependent and ABA-independent signaling pathways. The perception of salt stress involves signaling pathway regulating ion homeostasis. This pathway has been characterized with the help of analysis of *Arabidopsis* mutants, which are overly sensitive to salt (**salt overly sensitive**; ***sos*** **mutants**). Intracellular calcium concentration is enhanced in response to water deficiency induced by salt stress. **SOS3 protein** (**a calcium-binding protein**) undergoes a conformational change upon binding with calcium, facilitating its further association with a **serine/threonine protein kinase, SOS2**. This binding relieves the auto-inhibitory regulatory domain of SOS2 and hence activates its kinase activity, which plays an important role in adaptation to high salinity (Fig. 31.12). Thereafter, a plasma membrane **Na^+/H^+ antiporters and SOS1**, which participates in the exclusion of Na^+ from the cell, is induced through phosphorylation by SOS3-SOS2 complex. Hence, SOS1 activation results in reduced cytosolic Na^+ under saline conditions. SOS3-SOS2 complex can also activate tonoplast-localized Na^+/H^+ antiporter to enhance vacuolar sequestration of sodium ions and also plasma membrane-localized low-affinity Na^+ transporter to facilitate Na^+ export. SOS pathway is the first line of defense against salt

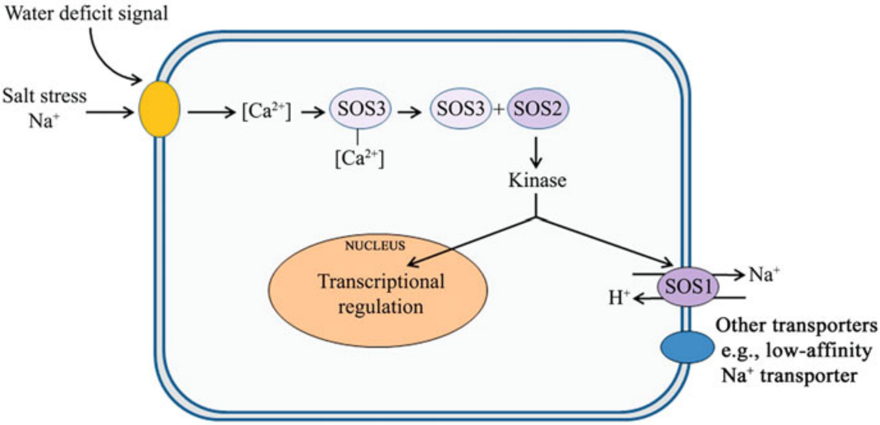


Fig. 31.12 Signal transduction pathway showing response to salt stress in *Arabidopsis* roots

stress in terrestrial plants. However, since Na^+ efflux from the plant cells is not sufficient to survive under high sodium stress because of its limited capacity, varied tolerance and avoidance mechanisms have evolved as a prominent part of response to salt stress.

31.3.4 Salt Stress Tolerance Mechanisms

Salt stress signaling involves a series of physiological events which coordinate to maintain ion homoeostasis. Salt stress-induced signaling events can be classified as early and late responses. Early responsive events are associated with rapid fluctuations in the levels of cytosolic and organellar [Ca^{2+}] and its perception by calcium-dependent proteins. Late responsive events involve long-distance transport of Na^+ from root to the aerial organs and modulation of antioxidant machinery to prevent reactive oxygen species (ROS)-mediated oxidative damage to plant cells. Salt tolerance in plants depends upon the following factors: 1. accumulation and sequestration of Na^+ and Cl^-; 2. maintenance of K^+ and Ca^{2+} pools as essential macronutrients in the presence of high Na^+ levels; 3. regulation of long-distance Na^+ transport and its accumulation in different aerial organs following transpiration; 4. accumulation of suitable osmolytes to prevent desiccation; and 5. modulation of efficient antioxidant machinery.

31.3.5 Accumulation and Sequestration of Na^+ and Cl^-

Under low external water potential during salt stress, water uptake is accomplished through a regulated uptake of Na^+ along with other counterbalancing ions, such as Cl^-, into the vacuoles. This ion uptake leads to water uptake in the cells. In the shoots of halophytes, cellular osmolarity is 2–3 times higher than the osmolarity of soil solution, but the cytoplasmic concentration of ions, including Na^+ and Cl^-, is maintained at nontoxic levels. This is attained by the action of tonoplast-localized Na^+ and Cl^- importers, which sequester Na^+ and Cl^- in the vacuoles (Fig. 31.13). The enhanced osmolarity in the vacuole is counterbalanced by accumulation of organic solutes in the cytoplasm. Apart from balancing of osmolarity, the organic solutes also function as osmoprotectant stabilizers of membranes, proteins, and free-radical scavengers. In halophytes, the uptake of Na^+ across the plasma membrane takes place by varied mechanisms. Usually Na^+ and Cl^- uptake occurs through ion channels, but uptake by plasma membrane invaginations (pinocytosis) can also supplement ion uptake. Low-affinity K^+ transporters are another mode of Na^+ entry. The driving force for the active sequestration of Na^+ into the vacuoles by Na^+-H^+ antiporter is generated by tonoplast-localized H^+-ATPase and H^+-pyrophosphatases (Fig. 31.11b). This may be followed by passive Cl^- entry through a specific tonoplastic uniport channel into the vacuoles. Na^+-H^+ antiporter may be expressed constitutively in some halophytes, and it can be induced rapidly in some salt-tolerant glycophytes under high external sodium stress. In glycophytes cells of

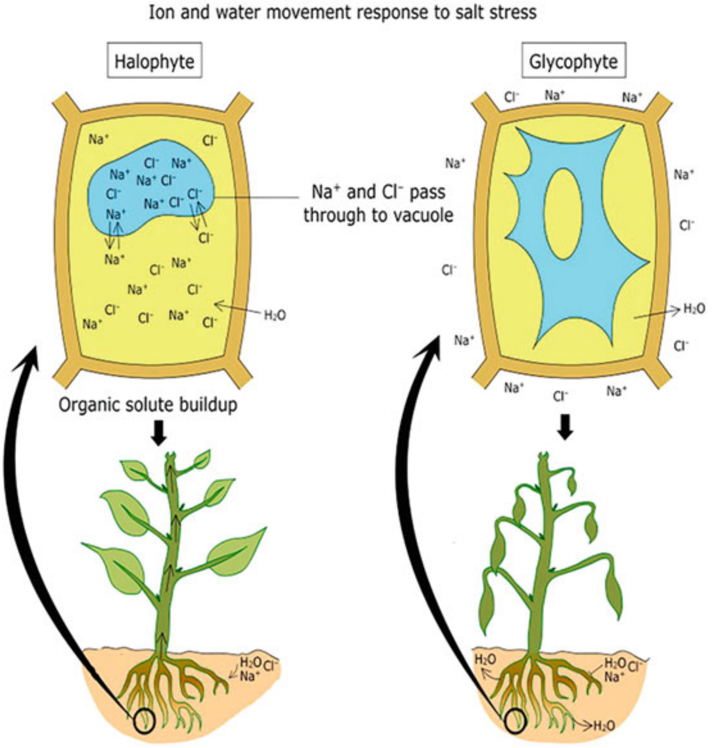


Fig. 31.13 Ion movement and consequent water movement in plants. Diagrams depict responses to salt stress in a halophyte and a salt-sensitive glycophyte

roots respond to high Na^+ concentration in soil by loss of cytoplasmic water leading to plasmolysis.

31.3.6 Expulsion of Salt Ions

With the help of Na^+-H^+ antiporter and H^+ ATPase localized on the plasma membrane, halophytes are able to expel Na^+ and Cl^- ions from the cytoplasm. When there are sudden salt shocks instead of long-term adjustment to high salt, expulsion of salt ions is used as a tolerance mechanism. This mechanism operates in an opposite manner to the mechanisms operating during salt sequestration in the vacuoles. This is so because cytoplasmic proteins and enzymes in halophytic plants are not tolerant to very high salt stress levels as the cytoplasm does not generally consists of high anion or cation concentrations.

31.3.7 Accumulation of Organic Solutes

In halophytes and salt-tolerant glycophytes, another distinctive feature is the accumulation of organic solutes in the cytoplasm in response to accumulation of salt ions in vacuoles. Accumulation of such nontoxic, “compatible” osmolytes provides osmotic balance and also protects the contents of cytoplasm. These osmoprotective compounds are synthesized under drought stress as well and are mainly categorized into amino acids, polyols/sugars, and quaternary ammonium (“onium”) compounds. The most commonly produced quaternary ammonium compound is **glycine betaine**. Glycine betaine, derived from serine via a choline intermediate, is a ubiquitous compound in all plant species, whether present in trace amounts or accumulated under abiotic stress. Apart from its role as an osmoprotective compound, it also has cryoprotective effects as it leads to a decline in osmotic potential without causing disturbance to macromolecule-solvent interactions. Other betaines, such as β-alanine in *Limonium* sp. and β-dimethylsulfoniopropionate (DMSP) in *Wedelia biflora*, also accumulate in some plant species under salt stress. Accumulation of “onium” compounds is not a typical response to salt stress in all halophytic species. Some plant species, such as ice plant (*Mesembryanthemum crystallinum*), accumulate methylated inositols, namely, ononitol and pinitol (polyols), under low temperature and salt stress. Also, adjustment of gene expression to enhance encoding of water channels, aquaporins, is another mechanism to facilitate uptake of water in halophytes.

31.3.8 Morphological Adaptations to Salt Stress

Certain morphological features in some plants help their survival under high external salt stress. Since soil water potential in saline soils is as low as that of desert soils, stress responses of many halophytes are xeromorphic in nature, so as to help the plant to survive under osmotic and ionic challenges imposed by salinity. Numerous halophytes are succulents. Their thick and fleshy leaves facilitate storage of water. Under water-deficit conditions, water stored in tissue can maintain plant cells (especially in photosynthetic cells) in hydrated state to support carbon fixation. Majority of the leaf tissue contains large and thin-walled cells to store water in the central region in halophytic succulents, such as sea blite (*Suaeda maritima*) and prickly saltwort (*Salsola kali*). The photosynthetic mesophyll cells are localized around the periphery of this water-storing tissue. In certain plants like silver goosefoot (*Oblone portulacoides*), salt bladders (modification of leaf hairs) are formed from supplementation of the central water reserve in the leaf with swollen modified epidermal hairs (Fig. 31.14a). The underlying photosynthetic cells extract water from the bladder-like surface cells and the central aqueous tissue under high salinity conditions resulting in contraction of bladder cells. Salt bladder cells regenerate their water reservoir when the availability of water increases. In some plants, such as asparagus, succulence is developed only under salt stress. Extreme halophytes, such as *Salicornia* species, are stem succulents.

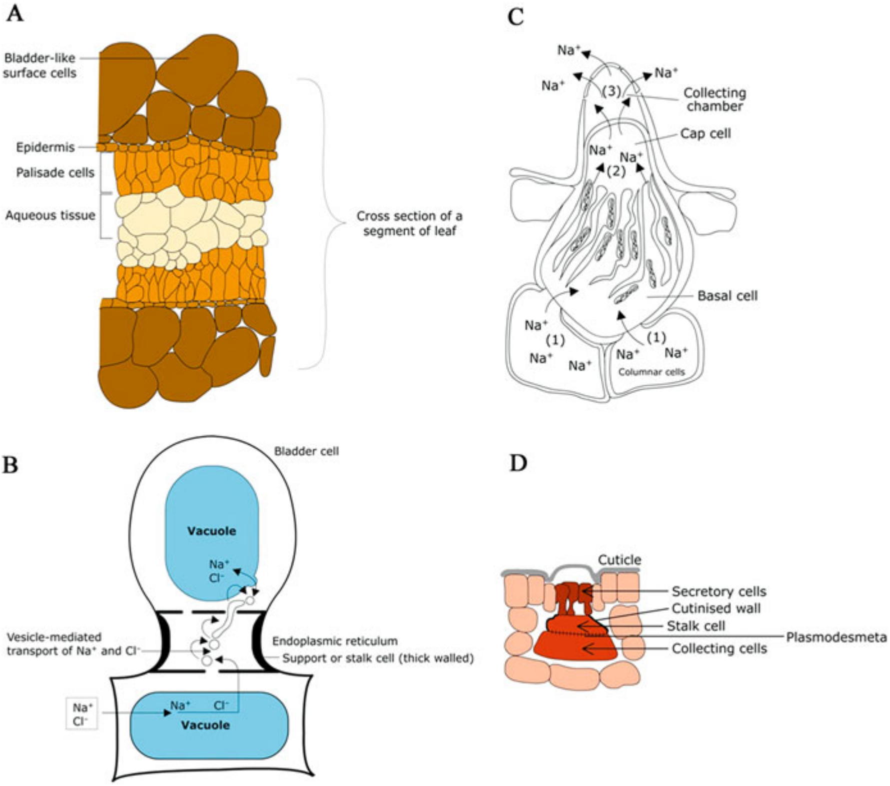


Fig. 31.14 (**a**) Cross section of a part of the leaf of silver goosefoot (*Obione portulacoides*)—a halophyte, showing bladder-like cells on the leaf surface. (**b**) Salt gland of the salt brush (*Atriplex lentiformis*). Membrane transport followed by vesicular transport facilitates the influx of Na$^+$ and Cl$^-$ into the vacuole of the bladder cell. (**c**) The bicellular salt glands of the Poaceae. (1) NaCl is transported into the salt gland from cells with large vacuoles that are often found in columns beneath it and that probably help to conduct salt toward the gland. (2) NaCl solution is transported into the cap cell under pressure, probably via active processes in the mitochondria-rich basal cell. (3) Salt solution is squeezed from a collecting chamber through pores in the cuticle onto the leaf surface, where it can either crystallize or be washed away. Bicellular salt glands have been found in halophytic grasses in the genera *Aeluropus*, *Chloris*, *Distichlis*, *Cynodon*, *Spartina*, *Sporobolus*, and *Zoysia.* (**d**) Longitudinal section of the multicellular salt gland of mangrove (*Avicennia officinalis*)

Xeromorphic halophytes have extensive vacuolated parenchyma in leaves to assist in water storage and intracellular compartmentalization of NaCl. Even the stomatal aperture and density are low in halophytes relative to glycophytes. Stomata in halophytes are often localized in crypts below the leaf surface. They may also be surrounded by trichomes or papillae to prevent high transpiration rates. Thick cuticles and trichomes facilitate in the reflection of light from the surface. Under high saline environments, specialized secretory adaptations termed as salt glands, may assist in secretion of salt in halophytes. Salt secretion can take place via two mechanisms: secretion into vacuoles of the gland cells and direct external secretion through pores. Salt glands are not universally present in halophytes but are common in few mangrove plants, such as black mangrove (*Avicennia germinans*). There are various kinds of salt glands. Multicellular hairs consisting of 1–3 cells form salt glands in *Atriplex* species. Such salt glands comprise of a terminal cell, which acts as a bladder cell with a large vacuole for active salt transportation, and one to two thick-walled and cutinized support cells, which assist in maintenance of directional flow of salt and water. Na^+ ions accumulate in the vacuole of bladder cell via vesicles traversing from the vacuole of basal epidermal cells through the support cells (Fig. 31.14b). In halophytic NaCl-excreting grasses, simple bicellular salt glands are present. They consist of a large basal cell surmounted by a cap cell. A porous cuticular dome, called collecting chamber, is formed by detachment of the cuticle from the cell wall (Fig. 31.14c). The salt glands in black mangrove consist of 2–4 basal collecting cells with a stalk cell surrounded by radially arranged secretory cells. The gland is covered with cuticles containing numerous pores (Fig. 31.14d). The stalk cell is heavily cutinized on side walls and is linked to basal cells via numerous plasmodesmata. Salt is transported to the secretory cells via apoplastic pathway in the collecting cells and further symplastic pathways in the stalk cell. Cutinized walls of the stalk cell prevent the back flow and apoplastic flow of salt solutions. NaCl is actively secreted into the space beneath the cuticle from the secretory cells, and it is further squeezed out through the cuticular pores. The secretory cells show cytoskeletal and mitochondrial specialization that helps to actively excrete salt into a subcuticular cavity from which it seeps out onto the leaf. The collecting cells are not linked via plasmodesmata to the mesophyll cells but collect salt excreted into the apoplast by them. **Hydathodes** are formed during early leaf development and assist in removal of excess water which may be detrimental for the growth of young leaves in highly humid environment. They may be adapted to exclude excess salts as well in some halophytes. The rate of Na^+ secretion and density of salt glands is strongly related to salinity tolerance in halophytes containing salt glands.

31.3.9 Physiological Adaptations to Salt Stress

Stomatal responses under salt stress are regulated in numerous halophytic species, thereby influencing transpiration rate. Na^+ ions stimulate stomatal closure in halophytes and promote stomatal opening in glycophytes. Stomatal aperture movements are controlled by comparable sets of plasma membrane-localized ion channels, namely, K^+ inward- and outward-rectifying channels in glycophytes and halophytes. In guard cells of halophytes, continuous salt exposure leads to stomatal closure through inhibition of K^+ inward-rectifying channel. The opposite response with respect to stomatal aperture during salt stress in glycophytes and halophytes is probably due to signaling pathways involving cytosolic Ca^{2+} which hinders K^+ inward-rectifying channel in halophytes.

31.4 Water Deficit

The deficiency of the basic necessity of life, water, is one of the most widespread repercussions of environmental stresses in plants. Salinity, drought, and low temperature result in shortage of water either through deficit of available water in environment or through decline in the external water potential, thereby preventing the entry of water into plant cells. The two most crucial physiological activities for crop yield, growth and photosynthesis, are adversely affected by water deficit. The global loss in possible agricultural yield due to water-deficit stress is considered to be more than the losses caused by other stresses together. The effects of these abiotic stresses are linked in terms of signaling mechanisms and metabolic and biochemical responses elicited. Deficiency of cellular water hinders plant growth and can cause membrane damage, alteration in solute concentration, protein denaturation, and loss of turgor. To prevent these detrimental changes and to restrict water loss via transpiration, plants exhibit certain physiological and cellular adaptations which are discussed below.

31.4.1 Avoidance of Water Stress

Some plants maintain a high water potential in the tissues to avoid water stress by reducing the loss of water to minimal level under conditions of acute water deficit. Many desert plants, like cacti, are included in the category of water stress avoiders. Desert ephemerals which emerge for a short time in the spring and die back to underground parts thereby live for most of their life span as dormant seeds. They are considered as supreme water stress avoiders. Usually these plants germinate following rainstorms and complete their life cycle very quickly while the water supply lasts. In climates with frequent dry season like Mediterranean, annuals synchronize their growth period annually with the water availability period, and perennials can store water in an underground organ during rainy season for successive growth. Water stress avoidance is crucial for plants especially in arid habitats. A xerophyte may develop water-conserving mechanism for defense against xeric habitat, but during extreme conditions of water deficit, plants need to develop tolerance. For example, resurrection plants usually avoid water stress, but desiccation-tolerant flowering plants are also observed.

31.4.2 Developmental Adaptations

Numerous flowering plants undergo a stage of low water content in the form of mature seeds (water content of 5–20%). Some seeds have been observed to survive drying conditions of water content below 1%. But all seeds are not highly resistant to water deficit. Thus, seeds of many species belonging to humid tropics are dehydration-sensitive. The resistance against desiccation of seeds is slowly lost during germination, and hence flowering plants are unable to endure water deficit for the rest of their life cycle. The **resurrection plants**, which can tolerate extreme desiccation and revive quickly under wet conditions in mature state, are an exception, for example, *Talbotia elegans*, *Borya nitida*, *Myrothamnus flabellifolia*, etc. Under severe drought conditions, the water potential values decline up to −160 MPa in the leaves of such plants, whereas the ψ values vary between −3 and −22 MPa for leaves of most mesophytic species. A regular seasonal fluctuation in water stress tolerance is evident in the perennials of arctic and temperate climates, it being highest during winter months. Plants are exposed to water deficit in winter season due to freezing of soil. Consequently the water uptake capacity of root is reduced even if the soil contains aqueous water at a high ψ. The stomatal closure to maintain low transpiration rate results in reduced carbon fixation and hence limits plant growth. In numerous plants adapted to saline or water-deficit stress, a specialized metabolism, crassulacean acid metabolism (CAM), is found. CAM pathway is explained in detail in Photosynthesis chapter.

31.4.3 Specialized Xeromorphic Features

Xerophytes are the plants which grow in arid environments. Usually major xerophytic vegetation constitutes drought-tolerant perennials which have specialized morphology categorizing them into succulents or non-succulent perennials. **Succulents** store water to tolerate water stress conditions. Water storage can take place in the stem (e.g., in cacti and spurges), in leaves (e.g., *Aloe*, *Agave*), or in roots (e.g., *Fouquieria* spp.) (Fig. 31.15). Enlarged vacuoles in the parenchymatous cells are the sites of succulence. Moreover, succulent plants exhibit crassulacean acid metabolism, low transpiration rate, and thick cuticles to prevent water loss. Shallow root systems are found in certain cacti in order to assist them to tap the transitory surface moisture of the soil. This is unlike other xerophytes which have extensive root systems to tap deep water reserves. Some species are drought deciduous as well,

Fig. 31.15 Succulent plants. (**a**) The ponytail palm (*Nolina recurvata*), (**b**) living stones (*Lithops gesincoe*), and (**c**) the candelabra tree (*Euphorbia candelabrum*)

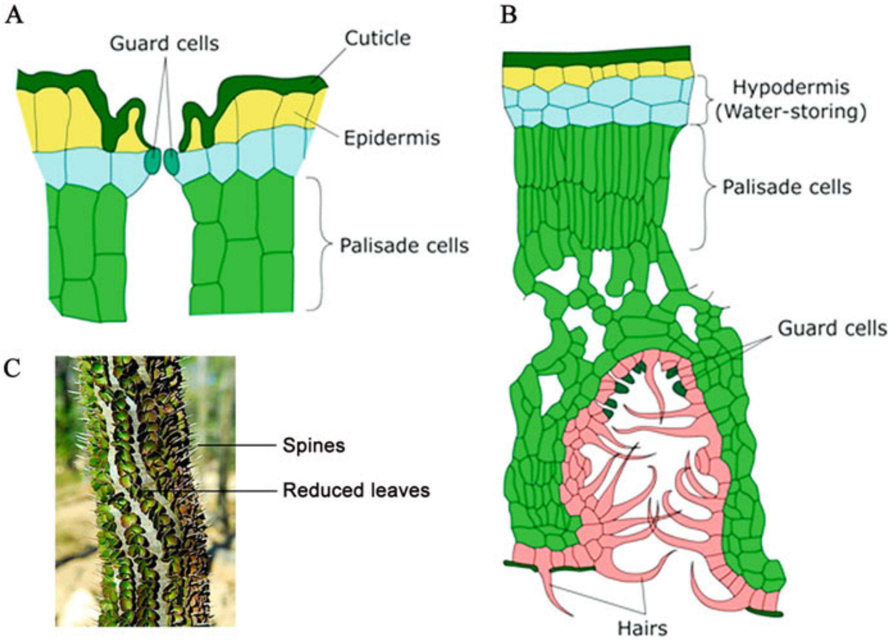


Fig. 31.16 Morphological adaptations to drought. (**a**) Thick cuticle and sunken stomata observed in the section of leaf of Agave (*Agave americana*). (**b**) Thick cuticles and stomata confined to pits observed in the leaf of oleander (*Nerium oleander*). (**c**) Small leaves on a spiny tree (*Alluaudia procera*)

i.e., the rootlets abscise under water stress conditions and then regenerate quickly after rain. Numerous distinctive morphological adaptations, such as sunken stomata in grooves or pits, profuse production of trichomes (hairs), reduced leaf area, and thick cuticles, are shown by non-succulent perennials (Fig. 31.16). The presence of thick cuticle prevents water loss and also protects leaves from any damage or breakage due to wilting. Some cuticles have shiny surface to reduce the heat load of leaves by reflection of light. The copious production of trichomes assists in trapping a layer of air near to the leaf surface, and it also helps in reflection of light, so as to reduce leaf temperature. Reduced leaf area in non-succulent perennials assists in limitation of water loss from the leaf surface, such as asparagus. Some plants do not even possess leaves, and photosynthesis in such plants is restricted to petiole or stem. To prevent wilting, sclerenchymatous cells are reinforced in the tissues so as to prevent shrinkage. Another common xerophytic feature in plants is the presence of spines. Some drought deciduous plants can even lose their leaves under dry conditions. For example, *Salvia mellifera* loses 90% of leaves under water deficit. The retained leaves are generally smaller than those lost and rotate in such a manner that their white undersides are exposed so that they can reflect light to maintain low plant temperature. Certain other plants such as Kentucky bluegrass (*Poa pratensis*) retain their leaves under dry conditions, but the orientation or shape

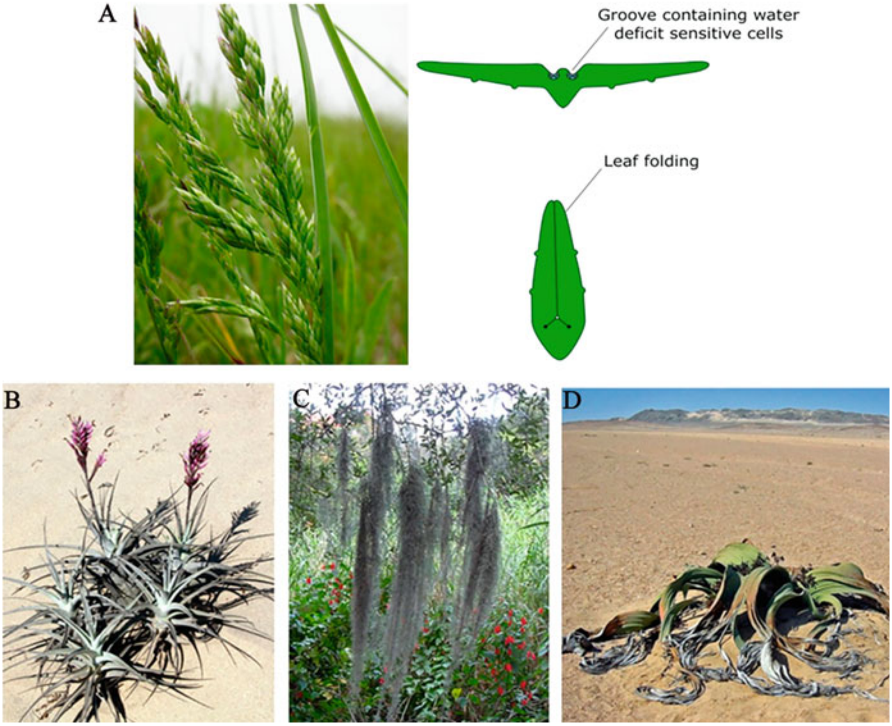


Fig. 31.17 (**a**) Folding of leaves of Kentucky bluegrass (*Poa pratensis*) in response to water deficit. The leaves fold when the cells in the grooves lose turgidity. (**b** and **c**) represent plants without roots, and these plants can obtain water from mist. (**b**) *Tillandsia purpurea*, positioned on sand or (**c**) epiphyte Spanish moss, *Tillandsia usneoides.* (**d**) *Welwitschia mirabilis*, growing in the desert

of leaves is modified. Under water deficit, large cells with enhanced water content (present along the longitudinal furrows of the leaves) lose turgidity leading to collapsing of furrows and folding of leaves (Fig. 31.17a). Some grasses undergo "aestivation" under dry conditions, i.e., their shallow root system completely dries out. The leaves die but do not undergo abscission so that the dead leaves protect the young buds at the soil surface. An epiphyte, *Tillandsia usneoides* (Spanish moss), uses dense, specialized nail-shaped trichomes to absorb water from the aerial sources (Fig. 31.17b, c).

Apart from perennials, an abundant flora of annual plants is present in dry regions with periods of rainfall. After several imbibitions during rain, seeds of annual plants germinate during favorable temperatures. Generally, the root system of annuals is shallow so as to absorb the surface moisture. Some plants, such as *Welwitschia mirabilis*, survive drought periods as subterranean perennating organs rather than as roots (Fig. 31.17d). Several annuals complete their life cycle by germination in the cool, wetter climates and organizing their vegetative leaves into a rosette. A warmer and humid microenvironment is created in a rosette as compared to 0–10 °C aboveground air temperature in winters, and as the temperature increases in spring, the stem undergoes elongation and produces cauline leaves. The cauline leaves and green stem perform photosynthesis, and the rosette leaves die.

31.4.4 Implications of Water-Deficit Stress

Water deficit affects almost all processes in plants due to the ubiquitous involvement of water in all cellular structures and processes. It is very difficult to differentiate between primary consequences of water deficit and secondary effects due to primary damage and to follow a series of events following primary perception to effects. The consequences of desiccation are time-dependent, i.e., damage increases with time. The extremity of water deficiency determines the physiological effects of desiccation stress. As soon as water content of the cells declines below the saturation level, growth begins to decrease due to extreme sensitivity of growth rate to water stress. This sensitivity can be due to decrease in cell turgor as the expansion growth is dependent on the yielding of cell wall under turgor pressure. The moderate level of water deficiency leads to rapid decline in the rate of photosynthesis in mesophytes. The decrease in photosynthetic rate coincides with the stomatal closure both in xerophytes and mesophytes. Under severe water stress, the reaction center of photosystem II degenerates, and the proportion of chloroplast proteins may decline as well. The decrease in photosynthetic activity may result in shortage of nutrients, thereby hindering plant growth. The excess of light energy absorbed, due to repressed fixation of CO_2, can lead to light-driven accumulation of reactive oxygen species (ROS). This may further impair photosynthetic potential of the plants due to ROS-mediated oxidative damage to the membranes. Depending upon concentration of the substrates, rates of metabolic reactions can be significantly affected as low cellular water content results in high solute concentrations. Extreme water deficit can result in an overall metabolic disturbance in the cell, thereby resulting in cell death. Moreover, drying out of cell affects structural organization at all levels from macromolecular structure to microscopically visible damage. With severe dehydration, the volume of protoplast contracts, resulting in tension and adherence to cell wall. Such physical stresses can result in tearing of the plasmalemma. The repetition of desiccation and rehydration causes cellular damage and even death at times. Protein denaturation enhances due to withdrawal of the hydration water during water stress. Also, hydrophilic heads of the lipid region of the membranes are held together by water of hydration, and water deficit can lead to semisolid state of lipids and hence loss of membrane integrity.

31.4.5 Water-Deficit Tolerance

Long-term exposure to conditions with low water can trigger the development of xeromorphic characters. Certain pressure sensor proteins on the plasma membrane, **osmosensors**, are involved in the primary perception of water deficit. In

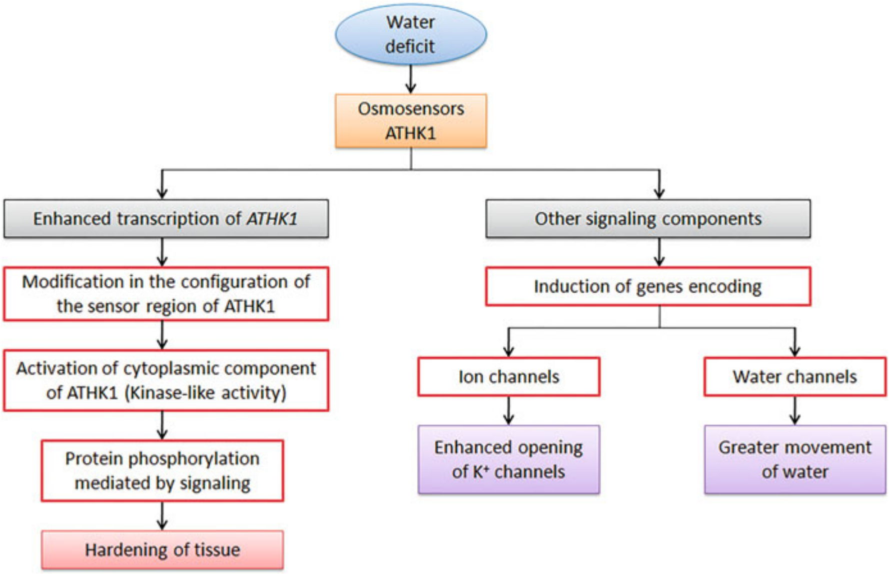


Fig. 31.18 Mechanisms of water-deficit perception and signal transduction in plant cells

Arabidopsis, one such osmosensor, *ATHK1*, has been characterized. The transcription of *ATHK1* is enhanced under water stress, and presumably pressure changes modify the configuration of the sensor region leading to activation of cytoplasmic component (Fig. 31.18). The cytoplasmic component acts as a kinase to stimulate hardening via protein phosphorylation-mediated signal transduction chain. A significant change in the gene activity and protein synthesis is also evident under desiccation stress. Genes encoding ion channels and water channels (aquaporins) are also induced in response to water deficit. Plasma membrane-localized potassium channels permit enhanced Na^+ uptake to assist in osmotic adjustment upon induction by stress conditions. The expression of genes expressing aquaporins is elevated to facilitate the water movement into and out of cells.

31.4.5.1 Osmotic Adjustment and Accumulation of Solutes

Water movement across soil-plant-atmosphere continuum is possible only if water potential (ψ) decreases along that path. A biochemical mechanism which helps in plants acclimatization to dry or saline soil is osmotic adjustment. Several drought-tolerant plant cells accumulate solutes to regulate their solute potential and, hence, lower ψ during transient or extended periods of water stress. This capacity is called **osmotic adjustment** (Fig. 31.19). This net increase in solute content is independent of the change in volume of the cell due to water loss. The decline in ψ is usually restricted to about 1.2–1.8 MPa. Two processes are involved in osmotic adjustment, one takes place in the vacuole and the other in the cytosol. In order to increase the solute concentration in the root cells, ions can be taken up from the soil or can be transported from other plant organs to the root. Such a response is commonly

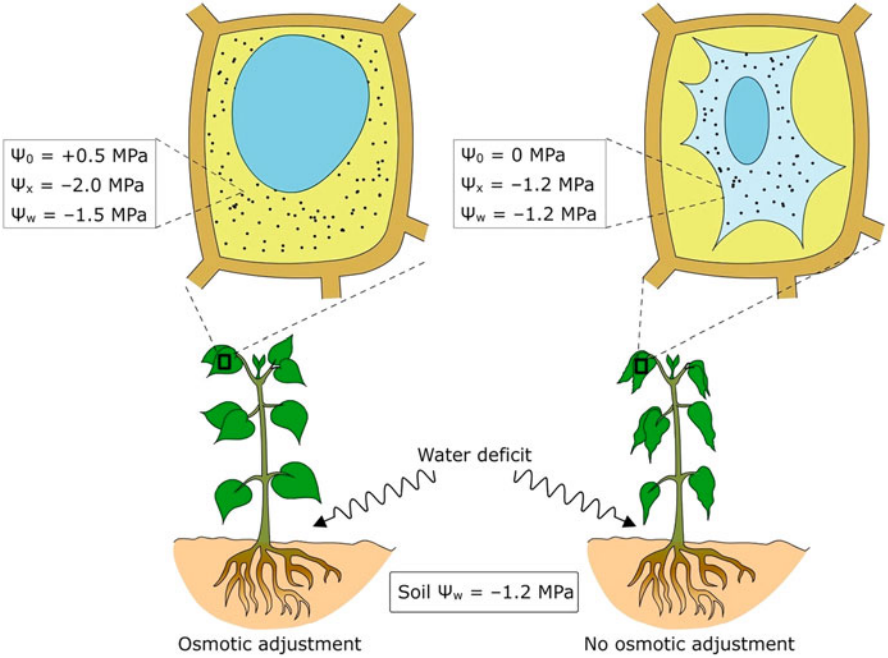


Fig. 31.19 Positive turgor pressure in a cell caused by solute accumulation leads to osmotic adjustment. Ψ drops due to solute accumulation, thereby enhancing water flow into the cell. In cells that fail to adjust osmotically, solutes are concentrated passively, but turgor is lost

observed in plants growing in saline soils, which are rich in ions, such as potassium and calcium. These ions are readily available to the plant, but their uptake must be electrically balanced either by the synthesis and vacuolar accumulation of organic acids, such as malate or citrate, or by uptake of inorganic ions, such as Cl^-. Due to ion uptake, a potential problem is faced by plants, i.e., accumulation of high concentrations of elements like sodium and chloride, which can have a detrimental effect on cell metabolism. Hence, accumulation of ions is majorly limited to vacuoles so as to keep ions out of contact with cytosolic enzymes. Accompanying accumulation of high concentration of ions in the vacuole, certain solutes called **compatible solutes or osmolytes** accumulate in the cytosol in order to maintain the water potential equilibrium between the two compartments. Compatible solutes or osmolytes are osmotically active organic compounds in the cell which, even at high concentrations, do not interfere with the functions of enzymes or damage the membrane (and hence termed “compatible”). They consist of sugar alcohols, like sorbitol, quaternary ammonium compounds like glycine betaine, and amino acids like proline (Fig. 31.20). Some compatible solutes like proline may act as **osmoprotectant** as well. Osmoprotective effects include stabilization of protein and membrane structure, scavenging of free radicals and hence protecting against oxidative damage, and providing cellular source of carbon and nitrogen to the cell

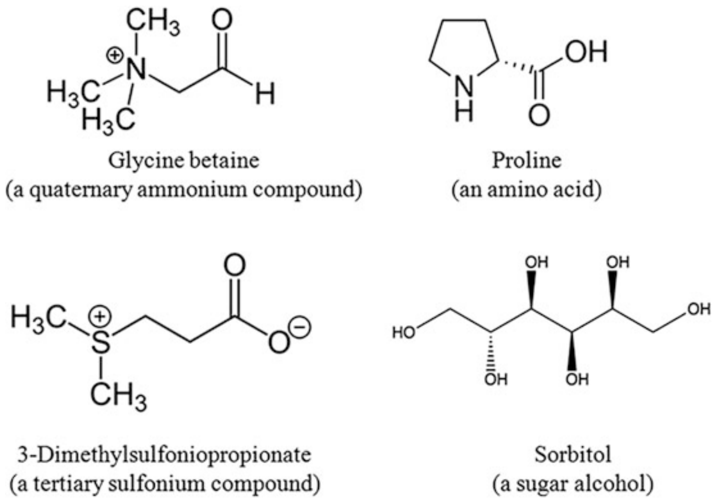

Glycine betaine
(a quaternary ammonium compound)
Proline
(an amino acid)
3-Dimethylsulfoniopropionate
(a tertiary sulfonium compound)
Sorbitol
(a sugar alcohol)

Fig. 31.20 Molecules often serving as compatible solutes in plant cells. These are amino acids, sugar alcohols, quaternary ammonium compounds, and tertiary sulfonium compounds. All these compounds are small and have no net charge

when conditions normalize. The synthesis of compatible solutes requires energy, and hence their production in plants reduces crop yield. Some plants upregulate the genes involved in encoding proline transporters to enhance the proline levels.

31.4.5.2 LEA Proteins

In addition to solutes, late embryogenesis abundant (LEA) proteins also accumulate in plant cells exposed to water stress. LEA proteins are hydrophilic globular proteins. They were initially characterized to accumulate in seeds during the process of maturation and desiccation. These proteins retain water and assist in stabilization and protection of proteins by acting as chaperones under water-deficit conditions. Chaperone action is crucial under water stress conditions to ensure correct folding of proteins, thereby preventing denaturation/aggregation (Fig. 31.21). In order to enhance tolerance against stress, salt or drought-sensitive plants can be transformed with genes encoding enzymes which are crucial for the production of osmoprotectants. Such plants can then be assayed for the increased accumulation of compatible osmolytes and ability to adjust osmotically under stress conditions.

31.5 High Temperature Stress

Temperatures over 45 °C are lethal for most plants, and temperatures over 30 °C are usually stressful. The high temperature tolerance range varies for different kinds of plant species, such as some Mediterranean species can survive at 48–55 °C, sub-tropical woody plants at 50–60 °C, and tropical trees at 45–55 °C (Fig. 31.22a, b).

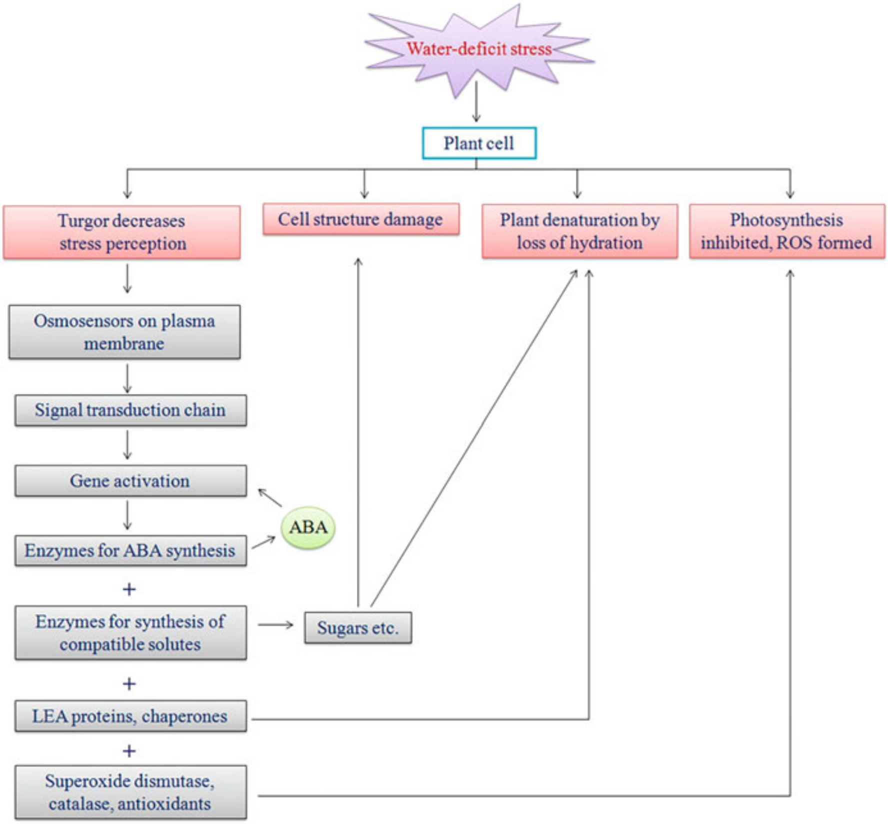


Fig. 31.21 Main events accompanying the development of water stress resistance. The perception of stress initiates signal transduction chain leading to the synthesis of proteins. These include enzymes for synthesis of ABA, which can then activate other genes. The new proteins or their products counteract (upward arrows) the deleterious effects of water stress noted at the top of the diagram. *ROS* reactive oxygen species, *ABA* abscisic acid

Generally, an ambient temperature over 30 °C is stressful for plants even if they can survive in hot climates. If concurrent water shortage leads to stomatal closure, leaf temperature can rise up to 5–10 °C higher than the air temperature. Internal temperature of about 40–50 °C may be attained in fleshy leaves, while the ambient temperature is at 20–30 °C. Desiccated cells and tissues, such as pollen grains and seeds, have a much higher tolerance against high temperature as compared to higher plants. At times, soil temperature can rise quite high above the air temperature, leading to death of seedlings at soil level due to overheating.

Thermal death point of a plant is the heat-killing temperature depending upon the time of exposure. Higher temperatures can be endured if the exposure time is short, such as observed in the cells of *Tradescantia discolor* which are killed within 7 min upon exposure to 60 °C, in 4 h at 50 °C and in 22 h at 40 °C. Generally thermal

Fig. 31.22 Heat tolerant plants. (**a**) Purple coneflower (*Rudbeckia purpurea*), a heat-tolerant perennial. (**b**) *Cynoglossum magellense*, a member of the borage family. Its silvery-blue foliage reflects light and reduces the heat load of the leaves. (**c**, **d**) Leaves of wild olive (*Olea europaea)* are covered by hairs which give the foliage a silvery appearance. The hairs reflect light and reduce heat load

death point of hydrated organs of temperate plants lies between 45 and 55 °C upon exposure for some hours. Aquatic and shade plants have thermal death points of about 40 °C, and desert plants, like cacti, are able to survive over 60 °C. Usually high temperature stress is accompanied with periods of water shortage. Hence plants adapted to hot environments can survive both water and heat stress. Net carbon gain is sensitive to heat stress as the rate of photosynthetic carbon fixation elevates less quickly than the rate of photorespiration during high ambient temperatures. This results in reduced net carbon gain by plants. The compensation point is attained when carbon assimilation by photosynthesis is equivalent to the carbon loss via photorespiration. Plant growth is slowed down or ceased as the net carbon gain declines with elevated temperature toward or beyond the compensation point. Photosynthesis is also sensitive to heat stress. The photosynthetic protein complexes are dispersed between both the non-stacked and the stacked regions of thylakoid membranes. At high temperatures, organized structure of thylakoid membranes is damaged, thereby hindering effective electron transfer, detaching reaction centers from antennae pigments, and uncoupling photophosphorylation. Reactive oxygen species (ROS) production is also enhanced under high temperature stress.

31.5.1 Thermal Injury

A catastrophic collapse of cellular organization at high temperatures owing primarily to denaturation of proteins can result in death within a few seconds or minutes. At less extreme temperatures, a complex phenomenon of slower heat injury takes place when development of damage occurs over a period of few hours or some days. Protein denaturation occurs at a slower rate resulting in various disturbances in cell organization, including metabolic abnormalities and membrane organization. Thermal motion of molecules is increased causing enhanced membrane fluidity and extreme fluidity, which are detrimental for cell.

31.5.2 Developmental Adaptations to Heat Stress

31.5.2.1 Heat Avoidance

Plants living in hot climates usually exhibit developmental adaptations which are advantageous in water-deficit conditions as well. These adaptations help plants to maintain temperature below the ambient temperature. High transpiration rate helps in dissipating heat as water evaporates and hence leads to cooling of leaves and protects against heat stress-induced injury. This mechanism is, however, observed in tropical plants which have ample water supply, and it is not suitable for plants in dry conditions undergoing high temperature stress. Orientation of leaves at steep angles to the incident light can help in reducing the temperature of leaves by 3–5 °C. This can prevent overheating of leaves. Such an adaptation is commonly found in plants adapted to hot and dry summers. The reflectance of incident light, by pale-colored leaf surface and also by profuse production of trichomes (epidermal hairs which reflect incident light), can lead to reduction in heat load of leaves (Fig. 31.22c, d). In hot, dry climates, some plants possess thick corky bark to reduce absorption of heat and also to provide insulation to the phloem and cambium from water loss.

31.5.2.2 Heat Hardening and Heat Tolerance (Thermotolerance)

Tolerance to heat stress is majorly attributed to properties of cellular proteins and membrane lipids. A drastic change occurs in protein synthesis when a plant is exposed to a temperature above threshold. Although the biosynthesis of certain proteins declines in both prokaryotic and eukaryotic organisms in response to high temperature stress, a new set of proteins known as **heat shock proteins (HSPs)** are synthesized as a generalized physiological response (Fig. 31.23). The synthesis of HSPs is induced by higher than normal but sublethal temperatures. They are also produced upon gradual rise in temperature and result in hardening. HSPs provide

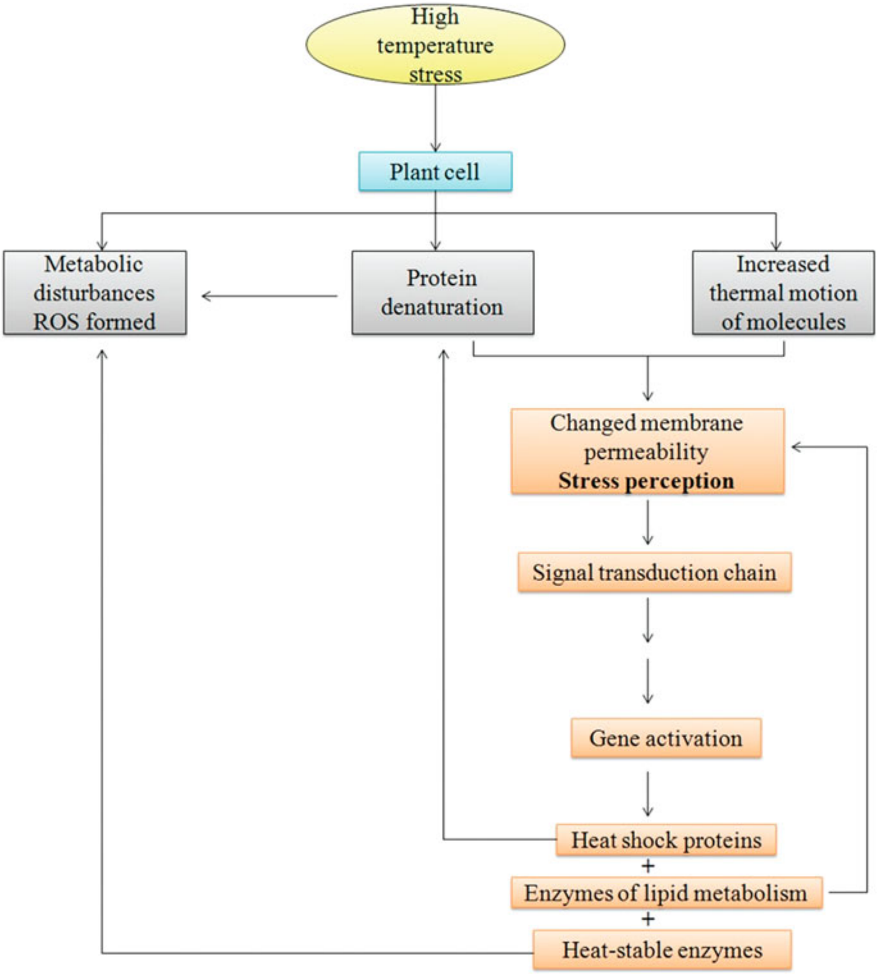


Fig. 31.23 Mechanisms of high temperature stress tolerance and heat hardening of plant cells. Signal perception includes sensing of denatured proteins in the cytosol and effect on membranes. Upward arrows indicate the counteraction of deleterious effects of the high temperature by gene activation products (hardening process)

protection to organisms from acute damage, assist in acclimation by allowing metabolic and cellular activities to resume, and also increase the temperature tolerance range for survival. Depending upon the optimal growth temperatures for different plant species, heat shock response is invoked at specific temperature, for example, at 35 °C for temperate species (e.g., rye grass) and 45 °C for tropical cereals (e.g., millet and sorghum). Depending on molecular weight, the heat shock proteins from all type or organisms fall in five major groups: HSP100, HSP90, HSP70, HSP60, and small HSPs. All HSPs play the role of molecular chaperones by recognizing and binding to proteins which are in an unstable or inactive state. High temperature stress results in denaturation of proteins, i.e., loss of the three-dimensional structure essential for proper activity. HSPs prevent the manufacturing of inactive, misfolded proteins and assist in stabilizing and controlling the cycles of protein refolding. Many plants, which are previously exposed to smaller temperature increase, have been shown to survive otherwise lethally high temperatures. This thermotolerance is acquired in plants as a result of production of HSPs in response to pre-treatment. Interestingly, biosynthesis of HSPs is evoked by other abiotic stresses as well, such as drought and cold. Thermotolerance acquired subsequent to heat stress also provides cross protection against other environmental stresses due to stabilization of proteins by HSPs.

Molecular chaperons play a crucial role in both stressed and non-stressed cells by preventing misfolding of newly synthesized proteins. In all kinds of organisms, chaperones related to HSP90, HSP70 and HSP60 are functional at normal temperatures. The hydrolysis of ATP to ADP is essential for chaperone activity, and hence molecular chaperones usually consist of domains which bind to adenine nucleotides (ATP and ADP). In addition to ATPase domains, HSP70s contain an adjacent peptide-binding domain and also interact with co-chaperones, which are proteins assisting in the activity of HSPs to maintain target proteins in their native conformation. Although genes encoding the members of all five categories of HSPs are present in a wide variety of organisms, the role of specific HSP families in the heat shock responses or in acquired thermotolerance varies among species. The synthesis of HSPs in response to high temperature stress is controlled by regulation of HSP gene transcription, and the regulatory mechanism is similar in all eukaryotes.

The synthesis of other cellular proteins resumes after a certain period, as the production of HSPs is a transient phenomenon. Upon return to below threshold temperature, the synthesis of HSPs ceases leading to loss of thermotolerance. Each time the plant faces high temperature stress, new synthesis of HSPs is required. Under high temperature stress, fluidity of membrane is enhanced due to increased thermal motion of the molecules. Due to excessive fluid membrane, the precise spatial distribution of membrane proteins is disrupted. Saturated membrane lipids have been observed to confer more heat tolerance to plants due to high melting point which thereby contributes to greater rigidity of membranes.

31.6 Low Temperature Stress

31.6.1 Freezing Versus Chilling Stress

Exposure to suboptimal temperatures can lead to either chilling or freezing stress in plants depending upon the intermolecular arrangement of phospholipids in the cell membranes and physical state of water. Low temperature initially exhibits thermodynamic effects on rates of chemical reactions, and longer exposure results in more complex physiological changes. Chilling stress occurs in the temperature range of 0–15 °C, when water is in the liquid state, and freezing stress occurs when the ambient temperature leads to ice formation. Both these stresses have direct as well as indirect effects on plant growth, development, and metabolism. Cellular injury occurs due to both the stresses, but freezing stress has more severe indirect effects due to the formation of ice crystals in the intracellular spaces which further cause osmotic dehydration in the cell.

31.6.2 Low Temperature and Water Deficit

Apart from tropical plants, low temperature causes a major problem only when the freezing is induced. Due to lower solute content in the extracellular fluid in comparison to cells, freezing leads to ice formation in extracellular compartments. Ice formation results in reduced water potential outside the cell, thereby enhancing the movement of unfrozen water outside the cells, down the gradient. More than 90% of the osmotically active water moves into the intracellular spaces at −10 °C resulting in water deficit. Hence, many of the physiological adaptations in plants which make them survive low temperature stress are similar to adaptations during salt stress or drought.

31.6.3 Plant Species of Warm Climates

Temperate plants are usually able to survive chilling temperatures. However, plant species adapted to warm climates are damaged by low, non-freezing temperatures and hence are **chilling-sensitive**. The chilling-sensitive species include crops originated in tropical climates, such as tomato, soybean, cucumber, and maize. Low temperature limits productivity in these crops primarily by reducing reaction rates for carbon fixation.

31.6.4 Low Temperature Sensing Systems in Plants

Changes in temperature must be perceived by plants to undergo requisite adaptations to low temperature. *Synechocystis* PCC6803, a cyanobacterium, possesses a histidine kinase Hik33 which acts as a putative low temperature sensor. Changes in membrane fluidity lead to signal-mediated activation of Hik33, followed by autophosphorylation and transfer of phosphate group to Rre26, the response regulator. Hik33 is involved in the regulation of expression of numerous cold-regulated genes (Fig. 31.24). No Hik33 orthologs have been discovered in higher plants yet, but two-component systems consisting of a combination of His and Rre play a role in some phytohormone responses. The involvement of such two-component systems in sensing of low temperature in plants is yet to be deciphered. There are many possible candidates involved in sensing of low temperature in plants, but these have not been confirmed. Alteration in membrane fluidity is considered to participate in the sensing of temperature drop. In alfalfa cells, changes in membrane fluidity, due to benzyl

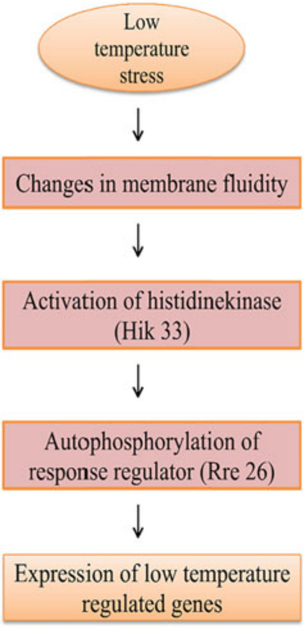


Fig. 31.24 Signal transduction events associated with low temperature stress in a cyanobacterium (*Synechocystis* sp. PCC 6803). Similar events may be involved in higher plants as well

alcohol treatment as a membrane stabilizer and dimethyl sulfoxide as a membrane fluidizer, have been shown to modulate both cold stress tolerance and cold-induced gene expression. Calcium influx and cytoskeleton organization have been observed to be affected as well under such conditions. Hence, alterations in membrane fluidity may play the role of cold stress sensor and trigger a chain of signal transduction events.

31.6.5 Membrane Destabilization as a Response to Chilling and Freezing

31.6.5.1 Chilling Stress

At optimal temperatures, membrane lipids exist in a liquid crystalline (fluid) state to ensure normal cellular functions. The lipids which have high melting temperatures undergo solidification to form a gel phase as the temperature declines and thus form a phase separation within the membrane (Fig. 31.25). The gel phase of membrane makes them leaky or even dysfunctional and hence intracellular solutes and water are lost. The reactions associated with membrane, such as enzyme-mediated processes, receptor functions, and carrier-mediated transport, are also disabled. One of the major effects of low temperature stress is impairing of electron transport chain, thereby limiting photosynthesis. Photoenergy reception is not altered by chilling stress, and, hence, exposure of chloroplasts to excess excitation energy leads to

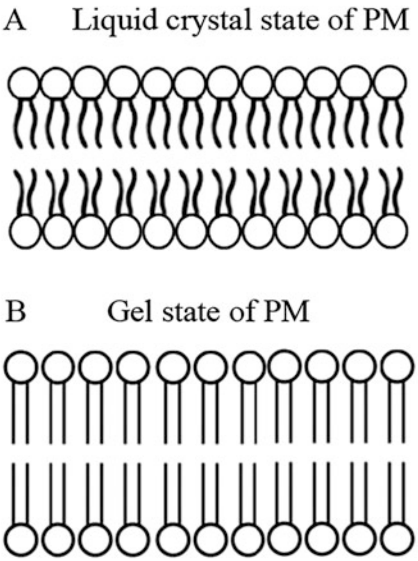


Fig. 31.25 (**a**) Diagrammatic cross-section of a phospholipid bilayer in a fluid (liquid crystal) state. The hydrophobic tails of the lipids point toward the middle of the bilayer, but the arrangement is not very regular and it changes from moment to moment owing to thermal motion of the molecules. (**b**) Bilayer in a gel state, as at low temperature. The tails are now regularly arranged and the structure is much more rigid, due to negligible thermal motion

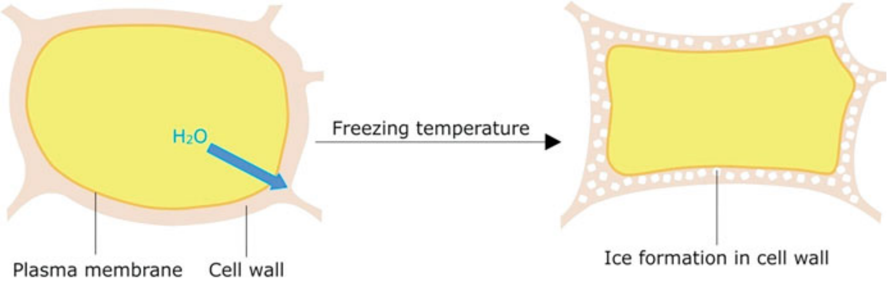


Fig. 31.26 Effect of freezing temperatures in plants leading to cellular water deficit, as water travels down its potential gradient, crossing the plasma membrane into the cell wall and intercellular spaces. Slow rate of freezing prevents formation of ice crystals in the cytoplasm, cell dehydrates, and freezing occurs in the apoplast

photoreduction of oxygen molecules producing ROS. The oxidative stress further imposes damage on membrane proteins and lipids.

31.6.5.2 Freezing Stress

The water-deficit stress occurs due to the effect of freezing on cellular water. Ice has a relatively lower chemical potential than the unfrozen water. Also the extracellular ice has a lower vapor pressure than the cytoplasm- or vacuole-localized water. During ice formation in extracellular compartments, the cellular water moves across the plasma membrane toward the extracellular ice, down the water potential gradient (Fig. 31.26). In addition to distortion of cellular shape due to extracellular ice formation, this results in enhanced intracellular solute concentration and decreased cell volume. Further decline in temperature causes an increase in such types of stress conditions resulting in enhanced cellular injury. The majority of injury due to freezing stress takes place at the plasma membrane, and the subsequent damage involves membrane destabilization as a result of dehydration of plant cells. The cellular dehydration causes alterations in membrane interactions and structures leading to close apposition of plasma membrane with the membranes of organelles, such as the chloroplasts, further resulting in membrane destabilization. Varied forms of injuries can occur in plants due to membrane destabilization, such as loss of osmotic responsiveness and expansion-induced lysis. Repetitive freeze-thaw cycles also impose osmotic and mechanical stress which contributes to membrane destabilization. Increase in solute concentration in the cytoplasm and other intracellular compartments due to osmotic dehydration causes inactivation of membrane-associated enzyme and transporter activities. The disassociation of membrane proteins can also occur due to changes in hydrophobic and electrostatic interactions as a result of direct interaction of solutes with the membrane. Enhanced solute concentration can electrostatically interact with charged head groups of lipid bilayer leading to alteration in lipid phase behavior. Extracellular ice formation can cause mechanical stress-mediated membrane destabilization by directly damaging the plasma membrane and hence leading to deformation of the cell.

31.6.6 Adaptive Mechanisms for Low Temperature Stress

31.6.6.1 Cold Acclimation and Change in Metabolite Profile

Prior exposure to low but non-freezing temperatures confers the ability to survive low temperature (below freezing) in plants, and this process is known as **cold acclimation**. For example, in winter rye, the temperature at which 50% of plants die is decreased from −6 °C in non-acclimated plants to −21 °C in acclimated plants and in *Arabidopsis* from −3 °C to −10 °C, when acclimated by 2 days exposure to 4 °C before freezing. Cold tolerance is an accumulative process which can be reversed, restarted, or even stopped. For example, in cereals, the cold acclimation begins at the threshold temperature of 10 °C, with an optimum of 3 °C. The rate of cold acclimation is enhanced as the temperature falls below 10 °C. Cold acclimation may occur in different parts of a plant independently. Post-cold acclimation, the cold hardiness in plants, is retained as long as the temperatures remain below freezing. Also when temperature increases above 10 °C, cold hardiness is rapidly lost. So, even cold-tolerant species are damaged during summer frosts. In non-acclimated plants, primary damage due to exposure to low temperatures is caused by membrane damage. Membrane damage takes place due to loss of osmotic responsiveness, phase transition (an abrupt change in the physical properties of membrane, such as permeability and fluidity), and expansion-induced lysis (takes place during thawing). A series of physiological events take place during acclimation, which assist in protection of cellular membranes from freezing damage. During cold acclimation, the proportion of phospholipids (phosphatidylserine and phosphatidylcholine) is enhanced, while that of cerebrosides (lipids composed of ceramide and a sugar residue) is declined in the plasma membrane. This alteration in the proportion of lipids leads to a decline in tendency for membrane lipids to undergo fusion and rearrange to form pores and decrease in incidence of expansion-induced lysis. These changes have an additive effect, thereby diminishing the probability of freezing-induced membrane damage and cell death. During cold acclimation, expression of certain proteins is induced to stabilize the membranes by bringing down the incidence of phase transition. These proteins probably serve as nucleating sites for such changes. The levels of sugar-synthesizing enzymes are also raised to enhance membrane stability. To protect against water-deficit stress, novel hydrophilic protein and LEA proteins are also induced to reduce the probability of protein denaturation during cold acclimation. Protein denaturation resulting in functional loss is also reduced by the induction of chaperonins during cold acclimation.

In conjunction with freezing stress in many plants species, various compatible solutes, such as glycine betaine, proline, and simple sugars (glucose, raffinose, stachyose, fructose and sucrose), accumulate in the cells. The molecular functions of these compatible solutes during cold stress acclimation are similar to those in water-deficit stress, leading to osmotic and dehydration stress. Though sugars contribute toward enhanced freezing tolerance post-cold acclimation by protecting membranes, they alone are not sufficient for cold acclimation in plants.

31.6.6.2 Changes in Gene Expression

The alterations induced by cold acclimation are mediated by changes in the gene expression at low temperature. A group of genes, called **cold regulated genes** (COR), are induced during cold acclimation, and many of COR genes are induced during water-deficit stress as well. Each COR gene may contribute to freezing tolerance. COR genes involved in anthocyanin biosynthesis may also play an indirect role in cold acclimation. Though the functions of proteins encoded by COR genes are not yet deciphered, they are predicted to be highly hydrophilic in nature. The action of COR proteins on electrolyte leakage suggests their possible role in stabilizing plasma membrane during freezing stress, similar to LEA proteins. Overexpression of a spinach-derived novel hydrophilic COR proteins in transgenic tobacco conferred enhanced freezing tolerance by reducing cellular damage. Certain protein called as **antifreeze proteins (AFPs)** is also produced during cold acclimation. AFPs are secreted into the extracellular space/apoplast and prevent the nucleation of ice crystals or re-formation of ice crystals post a freeze-thaw cycle. Though accumulation of antifreeze proteins alone cannot determine the low temperature limit for plants, there is correlation between the accumulation of antifreeze proteins and cold tolerance. Interestingly, **molecular chaperones (chaperonins)** like HSP90 and HSP70.12 are also encoded by some COR genes to prevent protein denaturation during freezing. Proteins involved in low temperature signaling, like calmodulin-related proteins, certain transcription factors, and MAP kinase (MAPK) and MAP kinase kinase kinase (MAP3K), are also encoded by some COR genes. In *Arabidopsis*, three transcription factors have been deciphered which induce COR gene expression (Fig. 31.27).

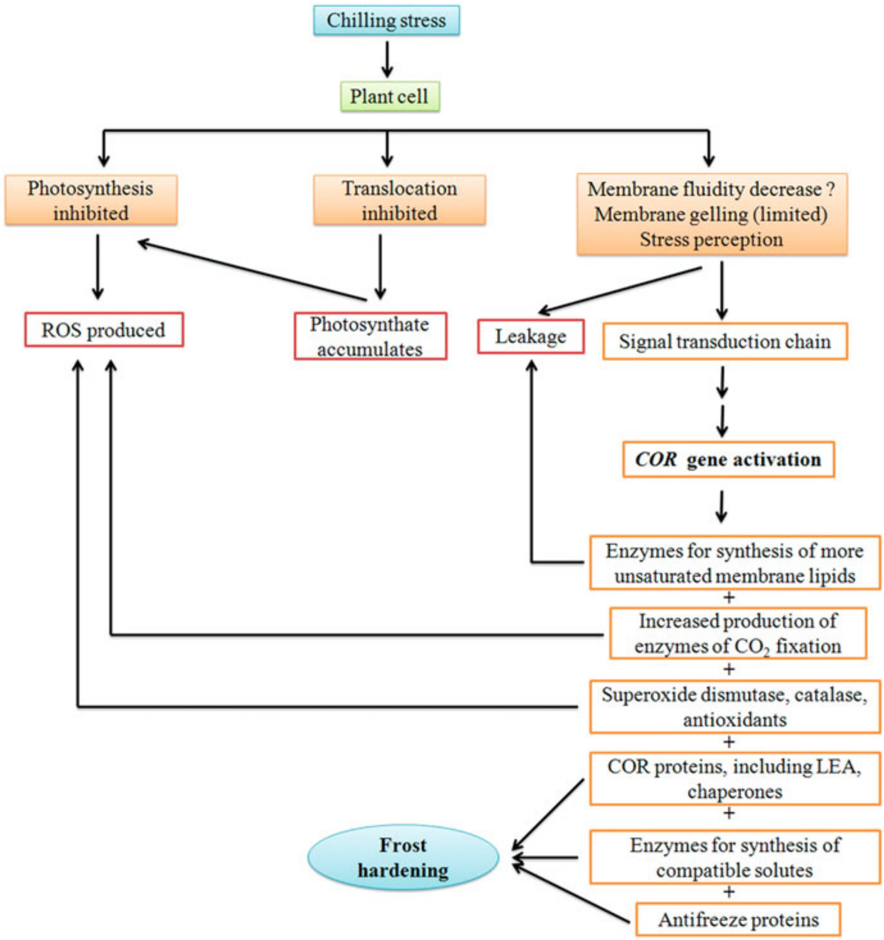


Fig. 31.27 Mechanisms of chilling stress and frost hardening of plant cells. Stress perception leads to a signal transduction chain leading to gene activation. New proteins with upward arrows are active in counteracting deleterious effects of chilling. Others (listed at the bottom) are involved in frost hardening. *COR* gene products and compatible solutes can also act in chilling tolerance. *ROS* reactive oxygen species

31.6.6.3 Low Temperature Signaling

The series of signals involved in low temperature stress are not yet fully characterized. During low temperature, a rapid increase in the concentration of cytosolic free calcium is evident, primarily due to influx from extracellular sources. This abrupt rise in cytosolic calcium is essential for induction of COR genes and hence freezing tolerance. Protein phosphorylation is induced by increase in cytosolic calcium which further induces cold acclimation response. A MAPK cascade may also be involved in signaling pathway during low temperature acclimation as some MAP kinases are induced during cold temperature. Abscisic acid level also plays a role in enhancing response to low temperature in some species. Both ABA-dependent and ABA-independent signaling pathways take place during low temperature stress.

31.6.6.4 Vernalization and Cold Acclimation

Vernalization involves exposure of seeds to a period of low temperature to accelerate flowering and reproduction. To fulfill this requirement for vernalization, a few plant species are adapted for specific periods of growth at low temperature. Vernalization ensures that the temperate plants reach the stage of flowering at appropriate time as flowering is specifically sensitive to damage caused by low temperature. Vernalization is essential for flowering in winter wheat, whereas spring wheat does not have any such requirement and will flower irrespective of growth conditions. Vernalization and many cold acclimation traits, such as antifreeze protein accumulation, sugar accumulation, and flowering time, map to the same chromosomal region on chromosome 5A in wheat indicating that the two are functionally related.

31.7 Flooding (Anaerobic) Stress

Plants, like most eukaryotes, are **obligate aerobes**, i.e., they cannot live without oxygen. Excess of soil water (flooding) leads to environmental stress for land plants. Wetlands account for about 6% of the world's terrestrial habitats. Rice, one of the important crops of tropical and subtropical regions, is predominantly cultivated on flood, anaerobic soils. Waterlogging commonly occurs in winter in northern latitudes, and, in tundra, low oxygen levels may be created by ice crusts by prevention of diffusion of oxygen. Even in warmer climates, lack of oxygen in soil can occur due to higher rates of respiration by soil microflora. Bogs and swamps are known to exhibit permanent waterlogging. In plants growing in permanently flooded conditions, such as black mangroves (*Avicennia* sp.), negatively geotropic roots (pneumatophores) rise above the water level to allow passage of oxygen from the atmosphere to the roots down its concentration gradient via large air spaces. Oxygen supply to the root cells is affected by a number of factors such as soil temperature, water content, porosity, root density, and the presence of aerobic microorganisms and algae. Oxygen concentration in the root tissue may also be affected by the thickness and depth of root, volume of intercellular spaces, and metabolic activity. Plant or cellular oxygen status can be defined as normoxic (normal oxygen levels), hypoxic (reduced oxygen levels), or anoxic (no oxygen). Oxygen concentration in well-drained, porous soils is nearly equal to atmospheric concentration of 20.6%. Because of the available high concentration of oxygen in the atmosphere and its very rapid diffusion in the air, the atmosphere has a high oxygen-supplying capacity. Since oxygen diffuses slowly in water, so in saturated soil, the microbial demand for oxygen is generally much greater than the rate at which it can diffuse from the atmosphere. This results in depletion of oxygen in the soil for the plant roots. Submergence or flooding lowers O_2 supply all the more. Such a situation presents a significant challenge to the terrestrial plants. Soil aggregates with a

Table 31.6 Some examples of wetland, flood-tolerant and flood-sensitive plants

Wetland plants	Flood-tolerant plants	Flood-sensitive plants
Barnyard grass (*Echinochloa phyllopogon*)	*Arabidopsis thaliana*	Pea (*Pisum sativum*)
Coral tree (*Erythrina caffra*)	Barnyard grass (*Echinochloa crus-pavonis*)	Soybean (*Glycine max*)
Common reed (*Phragmites australis*)	Barley (*Hordeum vulgare*)	Tomato (*Lycopersicon esculentum*)
Marsh dock (*Rumex palustris*)	Corn (*Zea mays*)	
Rice (*Oryza sativa*)	Potato (*Solanum tuberosum*)	
Rice grass (*Echinochloa crus-galli*)		
Sweet flag (*Acorus calamus*)		

diameter greater than 30 mm can have waterlogged centers because microbial demand for O_2 is greater than the rate of penetration of oxygen into the aqueous phase around soil particles. Hypoxia (oxygen limitation) starts when gaseous oxygen level in soil falls below 50 mmol/m^3. Flooding/submergence lowers soil oxygen level by 60–95% within a day although the rate of depletion is influenced by soil type and temperature. The diffusion coefficient of oxygen in air is about 1000 times greater than in water. Oxygen is displaced from soil due to its low solubility and low rate of diffusion in water, and its loss is further accelerated due to oxygen consumption by soil microbes.

31.7.1 Flooding Sensitivity of Plants

Plants exhibit variations in their ability to tolerate flooding. Depending on their sensitivity to flooding, plants can be classified as wetland, flood tolerant, or flood sensitive (Table 31.6).

31.7.1.1 Wetland Plants

As a consequence of various morphological, anatomical, and physiological features, wetland plants survive in waterlogged soil and in partial submergence. Such plants exhibit one or more of the following features (Fig. 31.28):

1. Elongation of stem or leaf petioles toward water surface.
2. Thinning of leaves to facilitate underwater photosynthesis.
3. Thick root hypodermis to reduce loss of oxygen to the anaerobic soil.
4. Aerenchyma formation to facilitate oxygen transport from aerial structures to submerged roots.
5. Adventitious roots formation, which develop aerenchyma to facilitate O_2 transport from aerial plant parts to the O_2-deprived tissue. Adventitious roots also develop in non-wetland species in the aerial parts under submerged conditions.
6. Pneumatophores.

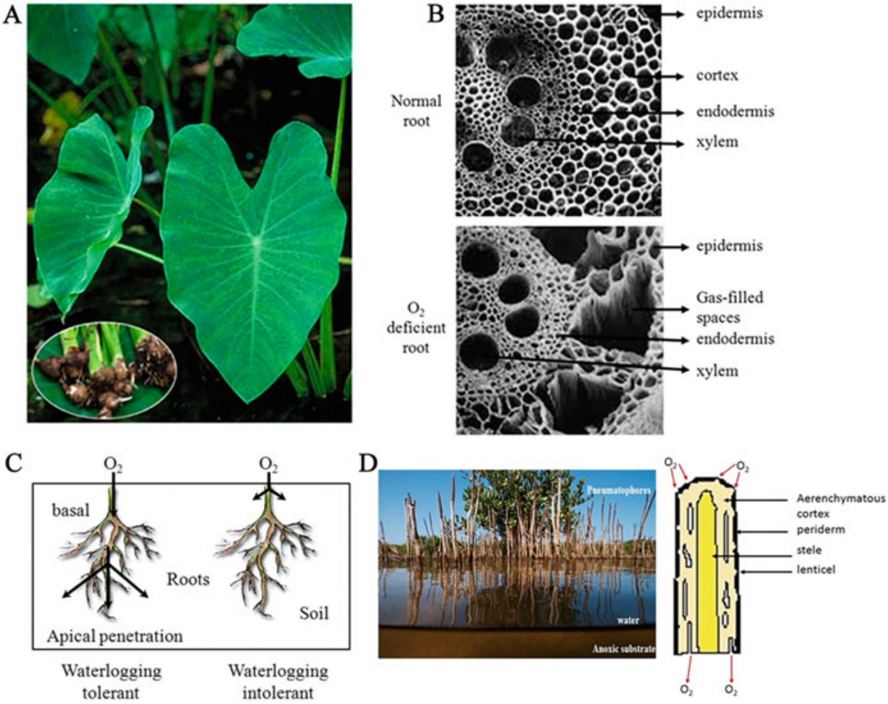


Fig. 31.28 Adaptive features in plants in response to flooding. (**a**) *Colocasia esculenta* showing large cordate leaves (elephant ear) and swollen stem base known as corm. (**b**) Scanning electron micrographs of a normal and oxygen-deficient root. (**c**) Penetration of oxygen in the roots during waterlogging. (**d**) Pneumatophores with aerenchyma for gaseous exchange

7. Lenticels for gaseous exchange, e.g., in willow and alder trees.
8. Shallow roots.

31.7.1.2 Flood-Tolerant Plants

Flood-tolerant plants exhibit only temporary anoxic conditions under flooding. Some plants/tissues can tolerate anaerobic conditions in flooded soil for few weeks before developing aerenchyma. These include the embryo and coleoptile of rice and rhizomes of giant bulrush (*Schoenoplectus lacustris*) and *Typha angustifolia*. These rhizomes expand their leaves under anaerobic conditions for a long time. In the spring season, once leaves have expanded above the surface of water, oxygen diffuses down through aerenchyma into the rhizome. Metabolism then switches from anaerobic (fermentative) to aerobic mode. Similarly, in paddy fields, the coleoptile from germinating rice breaks through water surface, thereby creating a diffusion route for O_2 into the submerged roots. Although rice is a wetland species, its roots (as that of maize) are intolerant of anoxia. With the continued extension of roots in oxygen-deficient soil, continuity of aerenchyma allows oxygen movement up to the apical region. Additionally, suberized and lignified cell walls prevent oxygen diffusion outward to the soil. These adaptations allow root growth of rice plants in anaerobic soil. Roots of non-wetland species, such as maize, exhibit leakage of oxygen, causing insufficient oxygen for aerobic respiration. This results in reduced extension growth of roots of such plants as maize.

31.7.1.3 Flood-Sensitive Plants

These get injured in response to anoxia because of cytoplasmic acidification. The cells of root meristem of flooded sensitive plants exhibit reduced rate of protein synthesis, inhibition of cell division, degradation of mitochondria, cell death, and disruption of ion transport, in response to flooding. Such plants do not develop root aerenchyma and do not generally survive for more than 24 h. Most plant species are tolerant to short-term hypoxia. Pre-treatment or acclimation of plants to hypoxia enhances tolerance to sustained hypoxia but not anoxia (lack of O_2). The stem and roots of most wetland plants develop longitudinally interconnected, gas-filled channels which provide low-resistance pathway for movement of oxygen or other gases. The chemistry of flooded soil varies with its composition and microbial interactions. In an alkaline soil, the pH of soil tends to decrease, whereas in as acidic soil, the pH increases. Sulfide is one of the most important plant toxins produced in many flooded soils as a result of reduction of SO_4^{-2} by the action of bacteria such as *Desulfovibrio* sp. This can further lead to the production of physiologically active and toxic H_2S. Additionally, there is an increase in the metabolic activity of **methanogens** under anoxic conditions, leading to enhanced production of methane.

31.7.2 Methane Emissions from Wetlands

Wetlands are often characterized by high organic matter and low redox potential. Such a region is very favorable for the multiplication of methanogenic microorganisms in soil. Almost one-third of methane released into the atmosphere globally comes from wetlands, and up to two-third of this is from rice fields alone. Landfill sites are another source of atmospheric methane. Methane produced in waterlogged soil exhibits a slow diffusion into the atmosphere because of high diffusive resistance. But plants growing in wetlands facilitate this process of methane release into the atmosphere because of rapid diffusion of methane as an uncharged molecule into and up through the plant to the atmosphere. Methanotrophic bacteria also use methane as a terminal electron acceptor, producing some toxic carbon compounds, both in anaerobic and aerobic conditions. The rate of methane release from wetlands into the atmosphere is, thus, determined by the combined metabolic activities of methanogenic and methanotrophic microbes in soil (Fig. 31.29). A decrease in the organic content of soil and control of the activity of aerobic methanotrophs are some of the management systems developed for rice fields to decrease methane emissions and manage the production of reduced organic compounds by methanotrophs. Methanotrophic bacteria from wetlands also

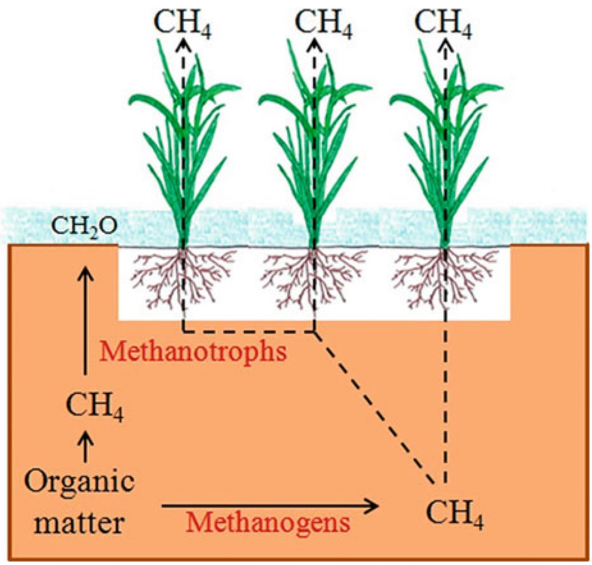


Fig. 31.29 Methane in anoxic soils: Methane is produced from organic matter by anaerobic methanogens in waterlogged anoxic soils which further leads to the production of formaldehyde by methanotrophs at anoxic-oxic boundaries

contribute to bioremediations due to their ability to oxidize a range of small carbon compounds and some organic pollutants.

31.7.3 Adaptations of Wetland Plants

Mangroves are known to inhabit environments which are flooded regularly. In order to provide support against tidal flow, mangrove trees develop secondary growth so as to develop prop roots. This inhibits oxygen influx to the cortex. Oxygen influx in mangroves is enhanced by the development of negatively geotropic pneumatophores which grow vertically upward above the ground. Pneumatophores have well-developed aerenchyma as long and continuous columns in the straightened cortex of the root system. Pneumatophores also provide structural support to the tree because of well-developed phelloderm that includes a layer of sclerified cells. The bark-like phellem provides barrier to the entry of oxygen. Extensive lenticels present on the surface of pneumatophores at the distal end facilitate entry of oxygen into the cortex from the atmosphere. Thus, pneumatophores both provide structural support and facilitate gaseous exchange in wetlands. Other roots (lateral roots, stilt roots, feeding roots) present in mangrove trees also have abundance of lenticels to allow influx of O_2 to aerenchyma. Pneumatophores are also known to develop colonies of cyanobacteria for nitrogen fixation.

31.7.3.1 Changes in Root Anatomy

Porosity Plants exhibit an internal oxygen gradient if roots are growing in an anoxic environment in waterlogged soil in contrast with shoots exposed to oxygen-rich environment. Internal resistance to diffusion of oxygen down its gradient is high from shoot to root in majority of flooding intolerant plants. In flood-tolerant plants, this resistance to diffusion rapidly decreases. Plants exhibit this variation in the extent of diffusion of oxygen by altering the porosity of cells in roots. Thus, in wheat roots not adapted to wet soil, porosity between the cells just few centimeters from the root tip, is as low as 1%, thereby leading to high resistance to oxygen diffusion. In case of rice roots which are adapted to wet soil, the cells in subtropical region of roots (elongation zone) are loosely packed, and their porosity increases up to 10%, upon flooding. Porosities of root cells as high as 40–50% have been observed in some wetland species. Such plants also exhibit significant changes in the otherwise complex anatomy of root-shoot complex so as to increase porosity for long-distance flow of oxygen from shoot to the root under flooding.

Aerenchyma The porous tissue of waterlogging-tolerant plants is referred to as **aerenchyma**. It mostly constitutes the loosely packed cortical cells between the endodermis and epidermis of plant roots, and several **lacunae** extend longitudinally for a significant distance within the root. In some species, these lacunae can be continuous between root and the shoot. Aerenchyma can develop as a result of cell death and dissolution (lysigeny), separation of cells without collapse (schizogeny), or a combination of lysigeny and schizogeny (schizolysigeny). In most wetland plants, aerenchyma formation is a part of normal development and is initiated in young plants before flooding, and it gets further proliferated in response to flooding. Most of the aerenchyma, formed in the roots of plants in response to waterlogging, is the result of death and dissolution of targeted cortical cells by the process of lysigeny. Lysigeny is different from programmed cell death occurring in animal cells since it (lysigeny) is without phagocytic activity or removal of debris via a circulatory system. Instead, lysigeny involves digestion of cellulosic cell walls. Lysigenous aerenchyma normally occurs in the zone of cell elongation in roots and does not occur where lateral emerge. It is constitutive in the roots of rice and can be induced in response to waterlogging in flood-tolerant species (e.g., maize). During constitutive aerenchyma formation, lysis is evident in oxic conditions. Soil compaction (leading to decreased aeration) and nutrient deficiency are some other factors which can promote lysigenous aerenchyma formation in plant roots irrespective of waterlogging. Aerenchyma enhances the porosity of roots from the shoot to the roots.

Hypodermis A number of wetland plant roots have hypodermis—a layer of dense, hexagonally placed cells beneath the epidermis. In rice, hypodermis also contains sclerified fibers. Hypoxia-tolerant plant roots also exhibit deposition of suberin. Hypodermis helps to reduce the efflux of oxygen and influx of toxins as a result of suberization. It also provides structural support for root with aerenchyma in the cortex.

Stele Roots adapted to hypoxia also show much reduced stele. Thus, for example, in comparison with wheat roots in which stele occupies about 15% of cross-sectional area near the root tip. In the hypoxia-adapted rice roots, stele occupies nearly 5% of cross-sectional area to minimize oxygen demand at the center of the root. The root anatomy of waterlogging-tolerant plants is thus well-adapted to maximize oxygen supply.

Lenticels and Lateral Roots In the stem of some plants, gaseous flow is facilitated by lenticels. Some flood-tolerant woody plants are particularly rich in lenticels above the soil surface. They are used for oxygen supply to aerenchyma. In mangroves, the secondary growth in adventitious roots not only provides mechanical support; they are also rich in lenticels and aerenchyma to facilitate flow of oxygen. Lateral roots penetrate the hypodermis and provide a route for oxygen efflux. Laterals on adventitious roots can also significantly oxygenate the rhizosphere.

Epinasty and Hyponasty In some plants which are not adapted to flooding, waterlogging can lead to epinastic growth (Fig. 31.30). Nastic growth refers to growth response to a nondirectional stimulus, such as flooding. During **epinasty**, there is excess growth of the leaves on the upper (adaxial) surface than the lower (abaxial) surface, leading to downward curvature of leaves. Epinasty was first reported in tomato and is thought to be a characteristic of Solanaceae plants. Epinasty is generally not evident in flood-tolerant plants, indicating that their physiological adaptations help them avoid it. With increasing intensity or duration of flooding, many hypoxia-tolerant species exhibit upward growth of leaves (away from water), a phenomenon referred as **hyponasty**. This is caused by excess growth

Fig. 31.30 Epinasty in plants under waterlogged soils

of leaves on the lower surface, directing leaves up to the water surface. The epinastic strategy may be adopted by plants during flooding by which downward directed leaves act as sails in wind. Such a bending of leaves facilitates faster removal of water vapors just below them, thereby facilitating faster aeration of top soil layer. Epinasty may also enhance transpiration rate. Both epinasty and hyponasty are triggered by enhanced ethylene available to the leaves. Flooded roots make ACC, the precursor to ethylene. ACC is transported from roots to the leaves along the xylem stream, where it gets converted into ethylene in the presence of oxygen. Depending on the environmental conditions, the leaf tissue can then respond differently to the available excess amount of ethylene. It is now believed that epinasty occurs through three different biochemical routes: (1) release of ethylene under root anoxic conditions; (2) high levels of salicylic acid during permanent flooding which keep stomata open, causing epinasty; and (3) by ABA during desiccation, when water circulation is still needed but transpiration is negligible.

31.7.3.2 Primary Metabolism

Under normal conditions, plants oxidize 1 mole of hexose sugar through glycolysis, citric acid cycle, and oxidative phosphorylation to produce 30–36 ATP. Under anoxic conditions, ATP is produced only by glycolysis (2–4 moles) and noncyclic mode of citric acid cycle (1 mole) per pyruvate metabolized. Under hypoxic conditions, the partial pressure of O_2 limits ATP production by oxidative phosphorylation. In waterlogging-sensitive plants, onset of hypoxia decreases flow of electrons to O_2, but glycolysis still occurs, using ADP and NAD^+ to produce some ATP and NADH. Without replenishment of NAD^+, glycolysis is inhibited, and metabolic activity ceases completely. Plants can replenish NAD^+ for a short time during hypoxia, using lactate dehydrogenase and NADH-dependent fermentation enzyme that converts pyruvate to lactic acid. Lactate dehydrogenase has limited capacity to replenish NAD^+ since its activity is inhibited by acidity created by lactic acid formation in the cytosol. Thus, plants use fermentation pathways to delay the effects of hypoxia.

31.7.3.3 Aquaporin Gating

Another early response to excess water is the modulation of gating of PIP_2 (plasma membrane intrinsic protein 2) aquaporin by cytosolic acidosis. This leads to a decrease in water uptake in response to flooding. In water-sufficient plants, PIP_2 are phosphorylated and open, while under hypoxia and in drought, dephosphorylation, protonation, and bound calcium close aquaporins.

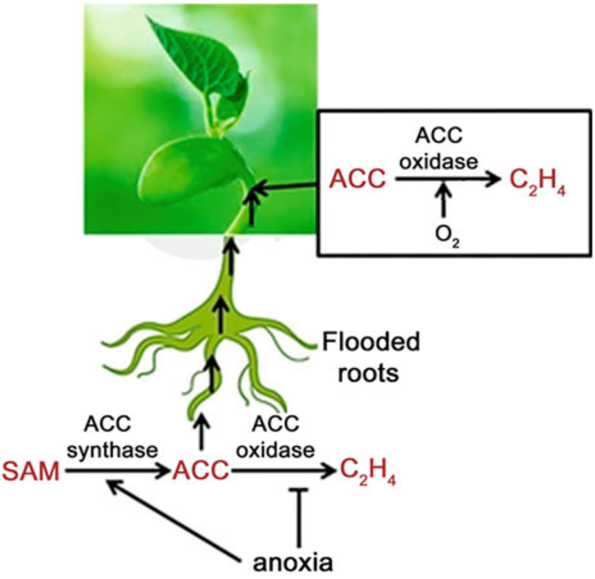
31.7.3.4 Ion Toxicity

Many plants which are non-adapted to waterlogging exhibit ion toxicity caused by a reduction in their ability to select ions during uptake to the stele. When the water leading to flooding is saline, plants also lose their ability to discriminate Na^+ from K^+, hence leading to disturbance in K/Na ratio in the tissue.

31.7.4 Ethylene Action in Anaerobic Stress

The ethylene-mediated pathways are important for physiological responses as well as for morphological and anatomical adaptations to hypoxia. Ethylene is constitutively synthesized from methionine in plant cells. It triggers lysigenous aerenchyma production. Anaerobic stress in plant roots caused by flooding leads to enhanced ethylene levels in plants by one or more of the following reasons: (1) ethylene does not diffuse away from roots; (2) anoxic conditions stimulate expression of genes for ethylene biosynthesis; and (3) anoxic conditions promote enhanced ethylene synthesis. Oxygen is required for the activity of ACC oxidase which is one of the enzymes (the other being ACC synthase) required for ethylene biosynthesis. Under hypoxic conditions, however, ethylene levels increase due to ACC oxidase activity (Fig. 31.31). During flooding, temporal and spatial differences in ethylene concentration, ethylene receptors, and their activity are likely to help explain the different responses of tissues to ethylene, particularly in flood-tolerant plants. Figure 31.32 highlights the likely sequence of biochemical events triggered by ethylene in response to waterlogging, which leads to lysigenous formation of aerenchyma. Hypoxia brings about a rapid increase in cytoplasmic calcium concentration and altered expression of many genes for calcium signaling molecules, namely, calcineurin and CaM-like proteins, in hypoxic roots. An important target of increased Ca^{2+} levels is an NADPH oxidase involved in cell lysis through production of H_2O_2. Genes encoding ROS scavengers are also downregulated in roots producing aerenchyma lysigenously. This indicates mitogen-activated protein kinase (MAPK) cascades, leading to cell death and dissolution, including chromatin

Fig. 31.31 Response of aerial tissues to flood stress



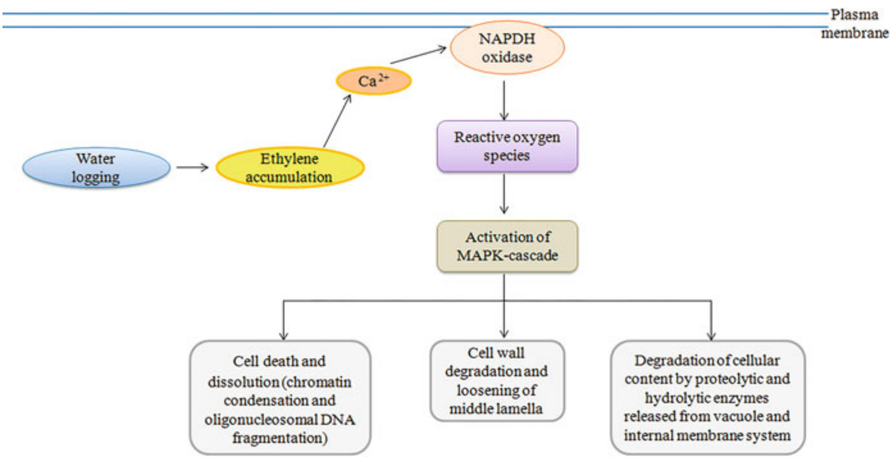


Fig. 31.32 Process of lysigeny in plant cells

dissolution, hydrolysis and proteolysis of cell contents by enzymes released from the vacuole and ER, and loosening of middle lamella and degradation of cell wall.

31.8 Signaling Pathways in Response to Abiotic Stress Conditions

During signaling, diverse signals reflecting external conditions are perceived by receptors, which further transduce them. Receptors, in many cells, are generally membrane-bound proteins to which molecular entities from environment can bind. In plant cells, fluctuating environmental conditions can be detected by changing concentration or state of molecules and/or ions. Signals indicating environmental changes are transduced in cells by receptors often through change in conformation and further alteration in activity of G-proteins, ion transporters, enzymes, or kinases. Heteromeric G-proteins, bound to receptors, are uncoupled upon binding of the ligand and further transduce the information to specific signaling pathways. Conformational changes in the receptors disturb the delicate ionic balance in plant cells and trigger rapid flow of ions down the electrochemical gradient, thereby transducing the external signal information. Signal responses are also initiated by kinases linked with other receptors. For appropriate response against stress, which often involves amplification cascades, transduced signal information has to be relayed to other parts of the cell. These complex cascades involved in transmittance and amplification of signal information from the receptors can involve varied cellular entities/molecules, such as Ca^{2+}, reactive oxygen species (ROS), reactive nitrogen species (RNS), inositol lipids, and cyclic nucleotides. The amplification of signals through these signaling networks can be limited to specific parts of a cell. This confinement can be achieved by the presence of 14-3-3 proteins which provide scaffolding.

31.8.1 Rapid Signaling Stress Sensors

Various signaling molecules and transcription factors are known to play crucial roles in the maintenance of cellular homeostasis under stress conditions. Several possible sensors involved in early stress-sensing mechanisms have been identified (Fig. 31.33). A multitude of signal transduction pathways, involving calcium, reactive oxygen species (ROS), protein kinases, protein phosphatases, transcriptional regulators, and plant hormones, are transduced by rapid stress sensors as a downstream signal. These specific stress-responsive signals further lead to activation or suppression of different networks, which either allow continued growth and reproduction under stress or enable the survival of plants until the return of favorable conditions. Varied transcription factors can be phosphorylated or dephosphorylated by the activities of protein kinases and phosphatases, sensitized by the elevated levels of calcium and ROS during early stage of stress response. During abiotic stress, changes in the redox status of the cell can also be directly sensed by specific transcriptional regulators, thereby activating or inhibiting some transcription factors. Upon exposure to multiple stresses, an intensive crosstalk may take place between the protein kinases or phosphatases and hormones of individual stress response pathways in plant cells. For example, several abiotic stress responses are regulated by mitogen-activated protein kinases (MAPKs) in *Arabidopsis*. MAPK modules, constituting of MAP3K/MAP2K/MAPK cascades, regulate responses under varied abiotic stresses, such as salinity and osmotic stress, drought stress, oxidative stress, and temperature stress. Similar upstream signaling intermediates like ROS, phosphatidic acid, and calcium are involved in signaling response to the

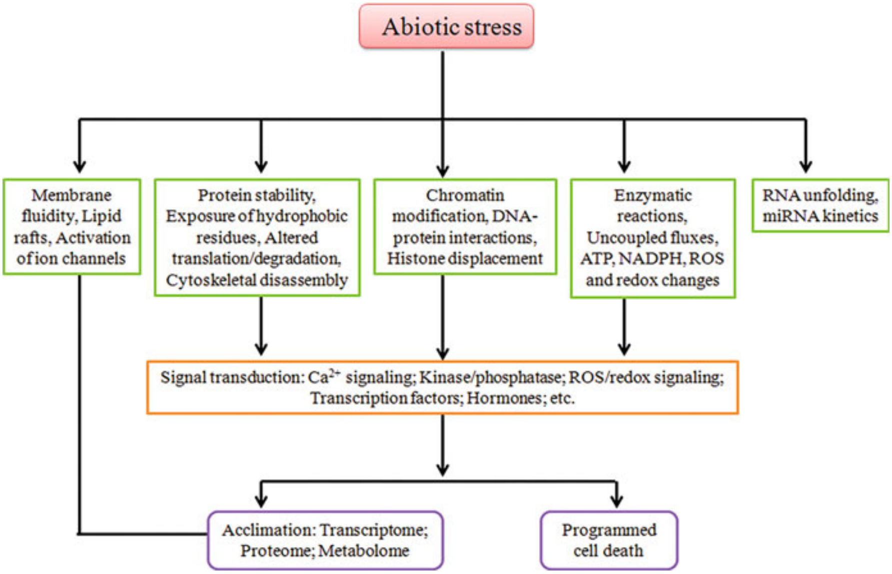


Fig. 31.33 Early events associated with abiotic stress-sensing in plants

abovementioned four stresses, and hence production of these signaling intermediates during one of the stress response signaling mechanisms can also affect the responses of the other three stresses.

31.8.2 Involvement of Transcriptional Regulatory Networks (Regulons) During Stress Acclimation

Proteins which result in the activation or suppression of expression of various genes by binding to specific DNA sequences are termed as **transcriptional regulators or transcriptional factors**. A specific transcription factor can affect expression of hundreds of different genes simultaneously by binding to their promoters. A transcription factor also has the capacity to activate or suppress the expression of another transcription factor by binding to the promoter of the gene encoding the respective transcription factor. Hence, a cascade of transcriptional regulation of gene expression can take place. With the activation and suppression of some genes, a gene network can be created in response to a specific abiotic stress by combination of varied transcription factors. These transcriptional regulatory networks generated in response to abiotic stress are known as **stress-response regulons**. These regulons provide the advantage of simultaneous activation of stress-responsive pathways and suppression of other nonessential or even harmful pathways and hence are beneficial in regulating response of plants to a specific abiotic stress. For example, genes encoding photosynthetic antenna proteins might be suppressed, while other genes encoding antioxidant machinery might be activated under high light conditions.

31.8.3 Acquisition of Systemic Acquired Acclimation (SAA)

Acclimation can be acquired in specific plant parts not exposed to abiotic stress, by the transport of the signals generated in the plant part subjected to stress. This phenomenon is called **systemic acquired acclimation (SAA)**. In response to varied abiotic stresses, such as cold, salinity, high light intensity, and heat, a rapid SAA mediated by a self-propagating wave of ROS production has been demonstrated. This self-propagated ROS wave is dependent on a specific NADPH oxidase called **respiratory burst oxidase homolog D (RBOHD)**, localized in the plasma membrane.

31.8.4 Role of Epigenetic Mechanisms and Small RNAs in Stress-Response (Long-Term Stress Adaptive Mechanisms)

Apart from reversible acclimation processes, including signaling cascades and changes in gene expression, in response to abiotic stress, potential long-term adaptation can be provided by epigenetic changes. Certain alterations in chromatin are mitotically and meiotically heritable, and these epigenetic changes in response to stress might have evolutionary implications. The processes, such as stable or heritable DNA methylation and histone modifications, can be associated with certain abiotic stresses. Small RNAs, including microRNAs (miRNAs) and endogenous short-interfering RNAs (siRNAs), play important role in some plant responses against environmental stressors. MicroRNAs and siRNAs can result in posttranscriptional gene silencing through degradation of mRNA in the cytosol, mediated via RISC (RNA-induced silencing complex). siRNA can also result in suppression of gene expression by changes in chromatin properties in the nuclei through a ribonuclease complex, RNA-induced transcriptional silencing (RITS). Small RNAs have also been speculated to play a role in suppression of protein translation under stress conditions. They have been demonstrated to regulate gene expression during various abiotic stresses, such as nutrient deficiency, salinity, cold, dehydration, and oxidative stresses.

31.8.5 Regulation of Abiotic Stress Responses by Hormonal Interactions

A vast range of essential stress-responses involving adaptations are mediated by hormones in plants. One of the most rapid responses to abiotic stress in plants is the biosynthesis of abscisic acid (ABA). ABA biosynthesis or redistribution plays an effective role in closure of the stomata. Under water-deficit stress conditions, ABA accumulation in stressed leaves results in reduced water loss via transpiration. ABA synthesis has also been shown to be induced by cold stress, and exogenously applied ABA enhances cold tolerance in plants. Cytokinins also play crucial role in acclimation to different abiotic stresses. ABA and cytokinins are known to have opposite effects with respect to stomatal opening, transpiration and photosynthesis. ABA levels are enhanced, and cytokinin levels are declined during drought stress conditions. Though ABA is essential for closure of stomata to prevent excessive water loss, drought stress can lead to inhibition of photosynthesis and also premature leaf senescence. The effects of drought stress seem to be ameliorated by cytokinins by protecting the biochemical processes related to photosynthesis and delaying senescence. Gibberellic acid, salicylic acid, brassinosteroids, auxin, jasmonic acid, and ethylene are also known to play significant roles in stress responses in plants. Auxins play an essential role in acclimation to drought conditions in plants, and gibberellic acid and brassinosteroids can link growth regulation to responses against abiotic stresses. The comprehensive overlapping among various genes regulated by hormones elucidates the existence of a multiplex network involving crosstalk among varied hormonal pathways. The capacity of plants to acclimate to different abiotic stress conditions is greatly influenced by the coordination and mutual regulation of hormone biosynthetic pathways and also by synergistic or antagonistic nature of hormone action. Figure 31.34 depicts an interaction among ethylene, ABA, and GA in response to flooding, leading to rapid extension growth of rice seedlings.

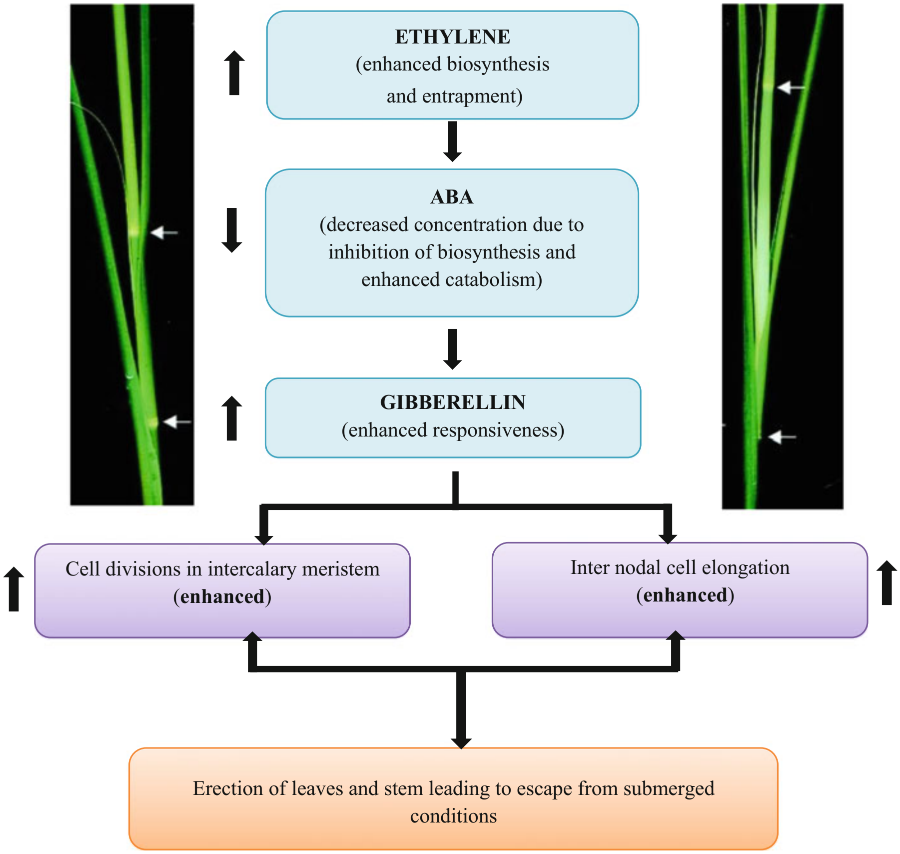


Fig. 31.34 Hormonal interaction accompanying flooding-induced elongation of rice plants. Flooding induces extension growth up to as high as 25 cm in 24-h growth cycle in some varieties of rice

31.9 Summary

- Environmental factors which result in stress may be divided into biotic and abiotic. **Biotic** stress is imposed by direct or indirect interactions with other organisms, whereas **abiotic** stress originates from excess or deficit in the physical, chemical, and energetic conditions to which plants are exposed. Various factors, such as genotype, developmental stage, species, and organ or tissue type influence the sensitivity or resistance of a plant to stress condition(s).
- *Stress tolerance* mechanisms enable the plants to withstand stress by development of resilient structures and physiological processes, whereas **stress avoidance** mechanisms prevent or minimize exposure to vulnerable stages of stress

conditions by adopting life cycle strategies which limit growth to favorable periods in a varying environment.

- A rapid rise in ROS concentration results in oxidative stress in the cells, known as **oxidative burst**. Rate of ROS production and cellular ROS levels both increase significantly in plants subjected to abiotic or biotic stresses. In order to overcome oxidative damage caused due to high ROS concentration and for maintaining **redox homeostasis**, plants have a well-established defense system which includes both nonenzymatic and enzymatic antioxidants. High concentration of ozone is one of the best characterized oxidative stressors.
- *Salinity* refers to an excessive accumulation of salts in soil solution. When the concentrations of the sulfates or chlorides of Na, Mg, and Ca in the soil are enough to produce an electrical conductivity of at least 4 $dS.M^{-1}$ in the soil extract and a sodium absorption ratio [SAR; based on $Na/(Ca^{+}Mg]$ of less than 13, it is referred to as **saline soil**. Plants develop mechanisms for salt exclusion or salt tolerance to deal with deleterious effects of salt stress. They are classified as halophytes or glycophytes, depending upon their capacity to survive on substrate containing high salt concentration. Elongation zone of the roots is highly susceptible to sodium influx due to higher absorption capability in this region. Salt tolerance in plants depends upon the following factors: 1. accumulation and sequestration of Na^+ and Cl^-, 2. maintenance of K^+ and Ca^{2+} pools as essential macronutrients in the presence of high Na^+ levels, 3. regulation of long-distance Na^+ transport and its accumulation in different aerial organs following transpiration, 4. accumulation of suitable osmolytes to prevent desiccation, and 5. modulation of efficient antioxidant machinery.
- Some plants maintain a high water potential in the tissues to avoid water stress by reducing the loss of water to minimal level under conditions of acute water deficit. Many desert plants, like cacti, are included in the category of water stress avoiders. Water stress avoidance is crucial for plants especially in arid habitats. Succulents store water to tolerate water stress conditions. Moreover, succulent plants exhibit crassulacean acid metabolism, low transpiration rate, and thick cuticles to prevent water loss. Under severe water stress, the reaction center of photosystem II degenerates, and the proportion of chloroplast proteins may decline as well. The decrease in photosynthetic activity may result in shortage of nutrients, thereby hindering plant growth. Certain pressure sensor proteins on the plasma membrane, **osmosensors**, are involved in the primary perception of water deficit. Several drought-tolerant plant cells accumulate solutes to regulate their solute potential and, hence, lower ψ during transient or extended periods of water stress. This capacity is called **osmotic adjustment**. Two processes are involved in osmotic adjustment: one takes place in the vacuole and the other in the cytosol. The accumulation of ions is majorly limited to vacuoles so as to keep ions out of contact with cytosolic enzymes. Accompanying accumulation of high concentration of ions in the vacuole, certain solutes called **compatible solutes or osmolytes** accumulate in the cytosol in order to maintain the water potential equilibrium between the two compartments. Some compatible solutes like proline may act as **osmoprotectant** as well. Osmoprotective effects include stabilization

of protein and membrane structure, scavenging of free radicals and hence protecting against oxidative damage, and providing cellular source of carbon and nitrogen to the cell when conditions normalize. In addition to solutes, late embryogenesis abundant (LEA) proteins also accumulate in plant cells exposed to water stress.

- Temperatures over 45 °C are lethal for most plants, and temperatures over 30 °C are usually stressful. **Thermal death point** of a plant is the heat-killing temperature depending upon the time of exposure. Thermal motion of molecules is increased causing enhanced membrane fluidity and extreme fluidity, which are detrimental for cell. High transpiration rate helps in dissipating heat as water evaporates and hence leads to cooling of leaves and protects against heat stress-induced injury. In hot, dry climates, some plants possess thick corky bark to reduce absorption of heat and also to provide insulation to the phloem and cambium from water loss. In response to high temperature stress, a new set of proteins known as heat shock proteins (HSPs) are synthesized as a generalized physiological response. HSPs provide protection to organisms from acute damage, assist in acclimation by allowing metabolic and cellular activities to resume, and also increase the temperature tolerance range for survival. The synthesis of HSPs in response to high temperature stress is controlled by regulation of HSP gene transcription, and the regulatory mechanism is similar in all eukaryotes.
- Temperate plants are usually able to survive chilling temperatures. However, plant species adapted to warm climates are damaged by low, non-freezing temperatures and hence are **chilling-sensitive**. During ice formation in extracellular compartments, the cellular water moves across the plasma membrane toward the extracellular ice, down the water potential gradient. The majority of injury due to freezing stress takes place at the plasma membrane, and the subsequent damage involves membrane destabilization as a result of dehydration of plant cells. Varied forms of injuries can occur in plants due to membrane destabilization, such as loss of osmotic responsiveness and expansion-induced lysis. Prior exposure to low but non-freezing temperatures confers the ability to survive low temperature (below freezing) in plants, and this process is known as **cold acclimation**. Post-cold acclimation, the cold hardiness in plants is retained as long as the temperatures remain below freezing. During cold acclimation, the proportion of phospholipids (phosphatidylserine and phosphatidylcholine) is enhanced, while that of cerebrosides (lipids composed of ceramide and a sugar residue) is declined in the plasma membrane. During cold acclimation, expression of certain proteins is induced to stabilize the membranes by bringing down the incidence of phase transition. A group of genes, called **cold regulated genes** (COR), are induced during cold acclimation, and many of COR genes are induced during water-deficit stress as well. Certain protein called as **antifreeze proteins (AFPs)** are also produced during cold acclimation. AFPs are secreted into the extracellular space/apoplast and prevent the nucleation of ice crystals or re-formation of ice crystals post a freeze-thaw cycle. **Molecular chaperones (chaperonins)** like HSP90 and HSP70.12 are also encoded by some COR genes to prevent protein denaturation during freezing. Proteins involved in low temperature signaling, like

calmodulin-related proteins, certain transcription factors, and MAP kinase (MAPK) and MAP kinase kinase kinase (MAP3K), are also encoded by some COR genes. During low temperature, a rapid increase in the concentration of cytosolic free calcium is evident, primarily due to influx from extracellular sources. This abrupt rise in cytosolic calcium is essential for induction of COR genes and hence freezing tolerance.

- *Flood-tolerant plants* exhibit only temporary anoxic conditions under flooding. With the continued extension of roots in oxygen-deficient soil, continuity of aerenchyma allows oxygen movement up to the apical region. Additionally, suberized and lignified cell walls prevent oxygen diffusion outward to the soil. **Flood-sensitive plants** get injured in response to anoxia because of cytoplasmic acidification. The cells of root meristem of flooded sensitive plants exhibit reduced rate of protein synthesis, inhibition of cell division, degradation of mitochondria, cell death, and disruption of ion transport, in response to flooding. Such plants do not develop root aerenchyma and do not generally survive for more than 24 h. The chemistry of flooded soil varies with its composition and microbial interactions. There is an increase in the metabolic activity of **methanogens** under anoxic conditions, leading to enhanced production of methane. The rate of methane release from wetlands into the atmosphere is, thus, determined by the combined metabolic activities of methanogenic and methanotrophic microbes in soil. Pneumatophores have well-developed aerenchyma as long and continuous columns in the straightened cortex of the root system. Extensive lenticels present on the surface of pneumatophores at the distal end facilitate entry of oxygen into the cortex from the atmosphere. Thus, pneumatophores both provide structural support and facilitate gaseous exchange in wetlands. Plants exhibit an internal oxygen gradient if roots are growing in an anoxic environment in waterlogged soil in contrast with shoots exposed to oxygen-rich environment. Porosities of root cells as high as 40–50% have been observed in some wetland species. The porous tissue of waterlogging-tolerant plants is referred to as **aerenchyma**. Aerenchyma can develop as a result of cell death and dissolution (lysigeny), separation of cells without collapse (schizogeny), or a combination of lysigeny and schizogeny (schizolysigeny). A number of wetland plant roots have hypodermis—a layer of dense, hexagonally placed cells beneath the epidermis. Roots adapted to hypoxia also show much reduced stele. In some plants which are not adapted to flooding, waterlogging can lead to epinastic growth. The epinastic strategy may be adopted by plants during flooding by which downward directed leaves act as sails in wind. Such a bending of leaves facilitates faster removal of water vapors just below them, thereby facilitating faster aeration of top soil layer. Both epinasty and hyponasty are triggered by enhanced ethylene available to the leaves. In waterlogging-sensitive plants, onset of hypoxia decreases flow of electrons to O_2, but glycolysis still occurs, using ADP and NAD^+ to produce some ATP and NADH. Another early response to excess water is the modulation of gating of PIP_2 (plasma membrane intrinsic protein 2) aquaporin by cytosolic acidosis. This leads to a decrease in water uptake in response to flooding. Many plants which are non-adapted to waterlogging exhibit ion toxicity caused by a reduction in their ability to select ions during uptake to the stele. Anaerobic stress in plant roots caused by flooding leads to enhanced ethylene levels in plants by one or more of the following reasons: (1) ethylene does not diffuse away from roots; (2) anoxic conditions stimulate expression of genes for ethylene biosynthesis; and (3) anoxic conditions promote enhanced ethylene synthesis. Oxygen is required for the activity of ACC oxidase which is one of the enzymes (the other being ACC synthase) required for ethylene biosynthesis. Under hypoxic conditions, however, ethylene levels increase due to ACC oxidase activity.

Multiple-Choice Questions

1. Example of stress avoidance mechanism represented by desert plants:
   (a) Presence of deep root system
   (b) Crassulacean acid metabolism
   (c) Presence of spines
   (d) Presence of succulent tissues
2. A *catastrophic response* is observed when:
   (a) System bounces back and regains its former state.
   (b) System is deformed and settles on a new stable configuration.
   (c) System is deformed and leads to decrease in entropy and ultimately system dies.
   (d) System enters into a stage of incoherence, entropy enhances, and the living system dies.
3. Evolutionary improvements, which enhance the fitness of the organism and occur over many generations and across entire populations, are termed as:
   (a) Adaptations
   (b) Acclimation
   (c) Gene alterations
   (d) Both b and c
4. What happens to GSH concentration under stressed condition?
   (a) GSH concentration usually increases, and redox state becomes more reduced.
   (b) GSH concentration usually declines, and redox state becomes more oxidized.
   (c) GSH concentration usually declines; thus, redox state becomes more reduced.
   (d) GSH concentration remains the same though GSSG concentration increases.
5. Why Na^+ and K^+ ions compete for uptake via transporters under saline conditions?
   (a) Both have similar ionic radii and ion hydration energies.
   (b) Both have same transporters.

   (c) Na^+ ions have small ionic radii compared to K^+ ions.
   (d) Both b and c.
6. Compartmentalization of Na^+ into vacuoles is carried out by?
   (a) Nonselective cation channels (NSCCs)
   (b) SOS3-SOS2 complex
   (c) Na+/H+ exchangers (NHXs)
   (d) P-type ATPases
7. Cellular osmolarity in the shoots of halophytes is 2–3 times higher than the osmolarity of soil solution however the cytoplasmic concentration of ions is maintained at nontoxic levels. How?
   (a) Action of tonoplast-localized Na+ and Cl− importers
   (b) Accumulation of organic solutes in the cytoplasm
   (c) Active sequestration of Na+ into the vacuoles by Na+-H+ antiporters
   (d) All of the above
8. Bladder cell is characteristic feature of:
   (a) Desert ephemerals
   (b) Saprophytes
   (c) Halophytes
   (d) None of the above
9. In which categories of plants minimum thermal death point has been observed?
   (a) Desert plants
   (b) Hydrated organs of temperate plants
   (c) Aquatic and shade plants
   (d) Both b and c
10. Biosynthesis of heat shock proteins (HSPs) is induced in response to:
   (a) High temperature stress
   (b) Cold stress
   (c) Drought stress
   (d) All of the above
11. In plants molecular chaperons are:
   (a) Histidine kinase Hik33
   (b) HSPs
   (c) LEA proteins
   (d) HSPs and LEA proteins
12. Role of antifreeze proteins (AFPs) under freezing stress:
   (a) Prevent protein denaturation
   (b) Act as a osmoprotectants
   (c) Prevent the nucleation of ice crystals
   (d) Prevent electrolyte leakage by stabilizing plasma membrane
13. miRNAs and siRNAs play important role in certain plant responses against environmental stressors via:
   (a) Post-translational gene silencing
   (b) RITS (RNA-induced transcriptional silencing) formation
   (c) Enhanced gene expression by ribonuclease complex formation
   (d) Both a and b

14. ABA and cytokinins exhibit opposite effects with respect to:
    (a) Stomatal closing and photosynthesis
    (b) Nutrient deficiency
    (c) Stomatal opening, transpiration, and photosynthesis
    (d) Photosynthesis
15. Flood-sensitive plants get injured in response to anoxia due to absence of:
    (a) Cell division
    (b) Aerenchyma
    (c) Ion transport
    (d) Protein synthesis
16. Both epinasty and hyponasty are triggered by enhanced production of:
    (a) ABA
    (b) GA
    (c) Ethylene
    (d) Jasmonic acid

Answers

1. a 2. d 3. a 4. b 5. a 6. c
7. d 8. c 9. c 10. d 11. d 12. c
13. b 14. c 15. b 16. c

Suggested Further Readings

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