# Plant Stress Physiology

Introductory article

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'Stress' in plants can be defined as any external factor that negatively influences plant growth, productivity, reproductive capacity or survival. This includes a wide range of factors which can be broadly divided into two main categories: abiotic or environmental stress factors, and biotic or biological stress factors.

Article Contents



## Stress Factors, Their Influence on Plant Metabolism, and Tolerance or Resistance to Stress

### Stress factors

The abiotic stress factors that most commonly influence plant performance include deficiencies or excesses of water (drought and flooding), extremes of irradiance, excessively low or high temperature, or deficiencies or excesses of several nutrients, including macro- and micronutrients, high salinity (i.e. excesses of $Na^+$, $Cl^-$ and/or $SO_4^{2-}$), and extremes of soil pH. Abiotic stresses may also include mechanical stresses (e.g. wind, hail, mechanical impedance of root growth in compacted soils, and wounding), and stresses associated with toxic, manmade chemicals, including gaseous pollutants (sulfur dioxide, nitrogen oxides, ozone), heavy metals and xenobiotics (e.g. herbicides). Biotic stresses include a wide range of plant pathogens (bacteria, fungi and viruses) and herbivorous animals. Because of the enormous diversity of these stresses, this article will focus only on the physiology of plant responses to the abiotic stresses.

### Interaction of stress factors

In many cases the abiotic stresses do not occur independently, and thus the stress environment may involve a complex of interacting stress factors. For example, acid stress may frequently be associated with aluminium toxicity. Aluminium is abundant in many soils, and under acidic conditions (pH $< 5.5$) the phytotoxic species, $Al^{3+}$, is solubilized to levels that inhibit plant growth. Both salinity and freezing stresses can induce water deficits; it is well established that a high concentration of dissolved solutes in the root zone lowers the soil water potential, creating a situation similar to soil water deficits, and that slow freezing of plant tissue resulting in extracellular ice formation will result in cellular dehydration. Consequently there are some common osmotic stress features of water deficit, salinity and freezing stresses. However, salinity stress involves both osmotic and specific ion effects (e.g. $Na^+$ and $Cl^-$ inhibition of photosynthesis and protein synthesis, and displacement of $Ca^{2+}$ from membranes, resulting in altered membrane permeability and $K^+$ leakage). Water excesses, or flooding stress, cause anoxia or hypoxia in the root zone; waterlogging fills soil pores and spaces, preventing oxygen diffusion to the root. The failure of waterlogged roots to undergo normal aerobic metabolism leads to inhibition of protein synthesis, and inhibition of ion acquisition and transport. This, coupled with alterations of water permeability of the roots in flooded soils, often leads to macronutrient deficiencies in the shoot, and ironically, shoot water deficits in response to flooding.

Because higher plants rely on water loss via transpiration for leaf cooling, water deficit stress can predispose plants to greater injury when exposed to high temperatures. Water deficits prevent evaporative cooling and associated heat loss by causing closure of stomates, which dramatically restricts water loss, carbon dioxide fixation, and hence growth. Chilling stress, and a number of other environmental stresses that disrupt membrane-bound electron transport systems, can predispose plants to high photon fluxes as a result of damage to the photosynthetic machinery. When such stress-injured leaves are exposed to high light intensities, this leads to production of toxic active oxygen species, including superoxide, hydrogen peroxide ($H_2O_2$), singlet oxygen and hydroxyl radical. Superoxide is formed by oxygen photoreduction by photosystem I in the chloroplast. Superoxide is then dismutated to $H_2O_2$. The hydroxyl radical can then form by the reaction of $H_2O_2$ with superoxide. Singlet oxygen forms by energy transfer from excited triplet state chlorophyll to oxygen. The chloroplast is particularly susceptible to these active oxygen species, whose synthesis is enhanced on exposure to excess excitation energy, particularly when carbon fixation is impaired. These active oxygen species denature proteins, damage nucleic acids and cause lipid peroxidation. Injury from these active oxygen species is common with damage from pollutants such as ozone, nitrogen oxides and sulfur dioxide, because

they generate active oxygen and other free radicals, with ultraviolet B (UV-B) radiation stress, and with post-anoxic injury, which occurs when roots are exposed to oxygen after a period of oxygen deficiency (waterlogging).

### Stress resistance versus tolerance

It is evident from the brief discussion above that stresses result in cellular injury or damage which frequently result from impaired or perturbed metabolism. The production of damaging active oxygen species as a consequence of impairments in the photosynthetic apparatus is an excellent example of this. Stress damage or injury is typically referred to as a 'strain'. By analogy with engineering terminology, stress resistance = stress/strain. The smaller the strain incurred by a specific stress, the greater the stress 'resistance'. The term stress 'tolerance' implies that the development of stress-induced strains can be tolerated and/or actively repaired during the stress or following stress relief.

## Physiological Correlations between Stress Sensitivity/Resistance and Reactions of Different Plants to Stress

### Constitutive adaptations

Many plants exhibit constitutively expressed traits that are recognized to confer resistance or tolerance to environmental stresses; these are frequently referred to as 'adaptations'. Examples of morphological adaptations to heat, light and water deficit stresses might include leaf hairiness and waxiness which influence light absorption and hence heat balance. An example of a combined anatomical and metabolic stress adaptation is $C_4$ photosynthesis, which reduces photorespiration, leading to a higher temperature optimum for photosynthesis and higher water use efficiency in comparison to $C_3$ photosynthesis. $C_4$ photosynthesis requires spatial separation of distinct metabolic functions between discrete mesophyll and bundle-sheath cells of the leaf (see 'Ecophysiological Adaptations – Examples', below). Constitutive crassulacean acid metabolism (CAM) allows for noctural carbon dioxide fixation into malate, and photoprotection as a result of high intracellular carbon dioxide concentrations generated by decarboxylation of malate during the day when stomates are closed. Like $C_4$ metabolism, CAM suppresses photorespiration, increasing the efficiency of photosynthesis at high temperatures in comparison to $C_3$ plants. The CAM pathway requires strict temporal control over malate synthesis and decarboxylation coordinated with control over stomatal aperture (see 'Ecophysiological Adaptations – Examples').

### Facultative/inducible adaptations

Many plant species, when exposed to a sublethal stress (such as a heat shock, or a period of chilling, or exposure to water deficits), acquire tolerance and/or resistance to a subsequent stress that would be lethal or severely damaging to nonpretreated plants. This phenomenon is known as 'acclimation' or 'hardening'. Acclimation responses are generally distinguished by their stress inducibility from the inherent, constitutively expressed tolerance or resistance mechanisms or 'adaptations' discussed above. However, the terminology is often used loosely in the literature. In our opinion, metabolic acclimation responses can best be regarded as 'facultative metabolic adaptations'. Several examples of facultative metabolic adaptations of plants to different stresses which are proposed to confer increased stress resistance/tolerance are summarized in **Table 1**. Asterisks denote that confirmatory evidence for the proposed function(s) have been obtained with mutants and/or transgenic plants.

An example of a morphological acclimation to anaerobic stress exhibited by certain plant species is the production of intracellular gas channels (aerenchyma) which improve aeration of the root. Aerenchyma formation accelerates the transfer of oxygen from the aerial tissues to the oxygen-deficient tissues of the stem base and root; however, in some flooding-tolerant species aerenchyma formation is constitutive, occurring in the absence of oxygen deficiency. An example of a facultative metabolic adaptation to anaerobic stress is the coordinated induction of glycolytic enzymes and enzymes of lactate and ethanol fermentation which allow continued energy production during anoxia or hypoxia (**Table 1**). Phosphate starvation may lead to a host of facultative metabolic adaptations including induction/derepression of phosphate transporters and alterations of pathways of glycolytic carbon flow and mitochondrial respiration to circumvent the adenylate- and inorganic phosphate ($P_i$) dependent reactions of respiration, facilitating continued respiration in $P_i$ deprived plants (**Table 1**). Osmotic adjustment, the active accumulation of solutes in response to drought or salinity stress, lowering solute potential, and facilitating maintenance of turgor, is an example of a facultative metabolic adaptation to drought and salinity stress (**Table 1**). The induction of heat-shock proteins, which may play roles as molecular chaperones, stabilizing proteins that are in a heat-induced unstable state, is an example of a facultative metabolic adaptation to high temperature stress that confers a higher level of thermotolerance, i.e. protection to a subsequent heat treatment that would be lethal to nonacclimated plants. There is growing evidence for cross-protection between heat stress, dehydration/drought, cold/chilling/freezing, heavy metal stress and oxidative stress in plants.

Certain facultative metabolic adaptations may in part serve to afford protection against injury when constitutive

**Table 1** Examples of facultative metabolic adaptations to environmental stresses in plants, and their proposed functions

| Stress | Facultative metabolic adaptation | Proposed function(s) |
|---|---|---|
| Aluminium toxicity | Excretion of organic acids (malate, citrate, oxalate) | Organic acids chelate toxic $Al^{3+}$, preventing its absorption* |
| Anaerobic (flooding) | Induction of glycolytic enzymes and enzymes of alcohol and lactate fermentation (e.g. alcohol and lactate dehydrogenases (ADH and LDH, respectively)) | Provides energy under $O_2$ deficiency; ADH and LDH recycle $NAD^+$ from NADH required to sustain glycolysis; ADH may divert flux away from lactate, delaying cytoplasmic acidification* |
| Anaerobic (flooding) | Low pH and/or $Ca^{2+}$/calmodulin activation of glutamate decarboxylase, leading to γ-aminobutyrate (GABA) accumulation | GABA synthesis is $H^+$-consuming, and may function in delaying cytoplasmic acidosis under anaerobic stress |
| Chilling | Induction of cold-responsive genes and genes encoding antifreeze proteins | Cold-responsive proteins may protect membranes from freezing/dehydration induced injury*; antifreeze proteins may inhibit intracellular ice formation |
| Chilling | Induction of fatty acid desaturases | Increased unsaturated fatty acid content of membranes may restore membrane fluidity and function at low temperatures* |
| Heat | Induction of heat-shock proteins (HSPs) | HSPs may serve as molecular chaperones, interacting with proteins rendered in an unstable state by heat stress, conferring thermotolerance* |
| Heat | Increased synthesis of isoprene | Isoprene may stabilize photosynthetic membranes at high temperatures |
| Heavy metals | Cadmium activation of phytochelatin synthase; copper induction of metallothioneins | Phytochelatins (PCs) may chelate and detoxify cadmium*, metallothioneins may chelate and detoxify copper |
| High light | Activation of a de-epoxidase that leads to synthesis of zeaxanthin from violaxanthin | Zeaxanthin may serve to quench chlorophyll fluorescence under intense illumination, preventing overexcitation and photodamage |
| Phosphate ($P_i$) deficiency | Induction/derepression of $P_i$ transporters; activation of alternative respiratory pathways | May facilitate increased $P_i$ uptake; may permit respiration via adenylate- and $P_i$-independent pathways |
| $K^+$ deficiency, osmotic and acid stresses | Induction of polyamine synthesis, primarily via induction/activation of arginine decarboxylase, leading to increased putrescine synthesis | Polyamines may function in charge balance in $K^+$-deficient plants; synthesis of putrescine may play a role in intracellular pH regulation |
| Numerous abiotic stresses | Induction of superoxide dismutase, ascorbate peroxidase, catalase, and monodehydroascorbate, dehydroascorbate and glutathione reductases | May function to scavenge reactive oxygen species* |
| Salinity and water deficits | Osmotic adjustment (OA); active accumulation of solutes, including compatible organic solutes (sugars, polyols, amino acids and onium compounds) | OA may restrict dehydration, and facilitate turgor maintenance*; organic solutes may function as osmo- and cryoprotectants*; certain organic solutes may also serve as antioxidants* |

**Table 1** – *continued*

| | | |
|---|---|---|
| Water deficits | Induction of dehydrins and late-embryogenesis abundant (LEA) proteins | May stabilize membranes and/or proteins during desiccation* and freezing |
| Xenobiotics | Induction of glutathione *S*-transferases (GSTs) | GSTs may conjugate the xenobiotics, rendering them nontoxic* |

* An asterisk denotes that the proposed function has been corroborated with mutants and/or transgenic plants.

defence mechanisms are overwhelmed. For example, carotenoid pigments and the action of the photorespiratory carbon oxidation (PCO) cycle in $C_3$ plants, afford a basal level of protection against excess light energy by rapidly quenching the excited state of chlorophyll, and dissipating excess adenosine triphosphate (ATP) and reducing power from the light reactions of photosynthesis, respectively. These constitutive defence mechanisms are supplemented by facultative/inducible mechanisms; a light activation of a de-epoxidase that converts violaxanthin to zeaxanthin via antheraxanthin, which may serve to quench chlorophyll fluorescence, and the stress induction of enzymes (e.g. superoxide dismutase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase) that function to scavenge reactive oxygen species (**Table 1**). Ascorbate provides the electrons for violaxanthin de-epoxidase, and so is particularly important in these facultative defensive tiers in the chloroplast. Only when all these defence systems are overwhelmed, may injuries result. The D1 protein of photosystem II (PS-II) is known to be particularly susceptible to reactive photoproducts, and its photodamage may be the primary cause of photoinhibition in stressed leaves. Damaged D1 proteins must be excised from the PS-II reaction centres, and replaced with newly synthesized D1 proteins. This repair will be inhibited if protein synthesis is impaired in stressed leaves.

# Interactions Between Different Stresses in Signalling and Metabolism

## Abiotic stress perception

As alluded to above, different stresses can interact in complex ways to lead to a wide variety of metabolic responses, including both facultative metabolic adaptations that may afford stress protection, and metabolic impairments or injuries; e.g. inhibition of photosynthesis, inhibition of protein synthesis, and protein and membrane damage. Many of the facultative metabolic adaptations involve coordinated changes in gene expression in response to environmental stimuli (**Table 1**). How do plants perceive these environmental signals and transduce them into changes of gene expression and/or metabolic rate?

Plants, like other organisms, must perceive environmental signals via specific receptors; these receptors then trigger a cascade of events leading to modification of cellular or metabolic activity, including regulation of the expression of specific genes. Ion channels, intracellular signalling proteins and second messengers play a key role in these signal transduction cascades. Although specific environmental stress receptors have not yet been identified in plants, it is reasonable to postulate that they may be membrane bound, as in other eukaryotes. Osmotic stresses may compress or stretch membranes to modulate directly or indirectly the activity of proton pumps and ion channels. Chilling, freezing and heat stresses directly influence membrane fluidity. Putative temperature stress sensors may detect such membrane fluidity or conformational changes. These sensors are postulated to be either histidine kinases or $Ca^{2+}$ channels. Early events in the response of plant cells to many environmental stimuli are known to involve membrane depolarization and elevations of cytosolic $Ca^{2+}$. Calcium may in turn regulate a host of enzyme activities via calcium-dependent protein kinases and calmodulin. Calcineurin (a $Ca^{2+}$- and calmodulin-dependent protein phosphatase) has recently been shown to play an important role in salinity stress signalling and salt tolerance in plants.

## Abscisic acid and stress signalling

One of the best studied examples of the cascade of signalling events in stress responses in plants is the process of stomatal closure, mediated by elevated concentrations of the plant stress hormone, abscisic acid (ABA), in guard cells. ABA is synthesized from carotenoid precursors in both leaves and roots; its synthesis is greatly stimulated by water deficits and other stresses. ABA synthesis increases markedly in the roots that are in contact with drying soil, and is transported from the root to the shoot via the xylem. This transport can occur before the low water potential of the soil causes any measurable decrease in water potential of the leaf. Under normal conditions the xylem sap is slightly acidic, favouring the uptake of the unassociated form of ABA by the mesophyll cells. During the early stages of water stress, however, the pH of the xylem increases. This alkalinization favours formation of the dissociated form of ABA, which is less readily taken up by the mesophyll cells; thus, more ABA reaches the guard cells. Activation of ABA receptors in guard cells leads to

$Ca^{2+}$-dependent and $Ca^{2+}$-independent signalling events that regulate ion channel activity in complex ways. The rapid activation of nonselective ion channels by ABA triggers membrane depolarization and allows $Ca^{2+}$ influx from the extracellular space. The increase of $Ca^{2+}$ and the production of other signalling intermediates, such as inositol 1,4,5-trisphosphate, then triggers $Ca^{2+}$ release from internal stores. Membrane depolarization, elevated cytosolic $Ca^{2+}$ and other signalling events activate anion channels resulting in anion release and long-term membrane depolarization. $K^+$ efflux through voltage-dependent outward $K^+$ channels is driven by membrane depolarization and enhanced by ABA-induced cytosolic alkalinization. Loss of $K^+$ and a parallel loss of $Cl^-$ and malate and/or conversion of malate to starch, leads to loss of guard cell turgor, and stomatal closure. Activation of anion channels may involve $Ca^{2+}$-independent signalling pathways. Phosphorylation is implicated in the activation of anion channels. The ABA-insensitive loci – *abi1* and *abi2* – in *Arabidopsis* encode type 2C protein phosphatases, which may function as negative regulators in ABA signalling. Protein farnesylation, a posttranslational modification process, which mediates the COOH-terminal lipidation of specific cellular signalling proteins, has recently been implicated in the ABA signal transduction pathway in plants. *Arabidopsis* mutants lacking the farnesyltransferase (FTase) β-subunit exhibit ABA hypersensitivity of guard cell anion channel activation and of stomatal closure. The FTase may function downstream to the ABI protein phosphatases.

ABA regulates the expression of a large number of genes that possess ABA response elements. Desiccation/dehydration may lead to induction of these genes as a result of water stress-induced ABA accumulation. Many of these desiccation/ABA responsive genes are also responsive to heat shock, chilling temperatures and salinity stress. Interacting ABA-dependent and -independent signal transduction pathways have been identified in both cold and osmotic signal transduction in plants. ABA appears to play a role in inducing freezing tolerance; thus, the ABA-insensitive mutant (*abi1*) of *Arabidopsis* is unable to undergo low-temperature acclimation to freezing. Cold acclimation in part involves induction of cold-responsive genes that encode hydrophilic polypeptides that may help to stabilize membranes against freeze-induced injury. These cold-responsive genes are regulated by the transcriptional activator, CBF1, which binds to a DNA regulatory element that stimulates transcription in response to both low temperature and water deficits. Constitutive overexpression of CBF1 induces expression of the cold-responsive/dehydration-responsive genes and results in enhanced freezing tolerance. The cold-induced proteins are homologous to the ABA and desiccation inducible late-embryogenesis abundant (LEA) proteins that are thought to play a role in dehydration tolerance of seeds, but which can also potentially contribute to freezing tolerance.

## Ethylene and stress signalling

The gaseous plant hormone, ethylene, is synthesized from the amino acid 1-aminocyclopropane-1-carboxylic acid (ACC), derived from methionine via *S*-adenosylmethionine (Adomet). ACC formation from Adomet is catalysed by ACC synthase. ACC oxidation to ethylene is catalysed by an oxygen-dependent ACC oxidase. A large number of stresses (including wounding, flooding, chilling, high temperature and osmotic stresses) induce ethylene biosynthesis primarily by modulating the expression of genes encoding ACC synthase. ACC synthase has a short half-life, and is the rate-limiting enzyme in ethylene biosynthesis. Ethylene production in response to hypoxia is implicated in the $Ca^{2+}$-dependent signal transduction pathway leading to cell death and lysis in the root cortex resulting in aerenchyma formation. Ethylene regulates numerous genes possessing ethylene response elements. This cascade likely involves an ethylene receptor, ETR1, a transmembrane protein that is similar to prokaryotic two-component histidine kinases. Binding of ethylene to this receptor is proposed to result in inactivation of a negative regulator, CTR1, a member of the Raf family of protein kinases. The inactivation of CTR1 then allows a channel-like transmembrane protein, EIN2, to become active. Activation of EIN2 may ultimately lead to the activation of a transcription factor, EIN3, that alters gene expression. It seems likely that other putative ethylene receptors that have been identified (ERS, ETR2 and EIN4) in plants, may have similar functions to ETR1; all three share sequence similarity to ETR1 and have a putative histidine protein kinase transmitter module. The different receptors may have tissue- or stage-specific functions.

## Oxidative stress signals

There is increasing evidence that the reactive oxygen species, hydrogen peroxide ($H_2O_2$), is perceived by plants as a signal of environmental change, acting as a diffusible signal-transduction molecule to alert metabolism to both abiotic and biotic stresses. As discussed above, increased production of active oxygen species is a common response of plants to a number of environmental stresses that result in impaired carbon dioxide assimilation at high light intensities. Pathogen attack also elicits an oxidative burst of superoxide production via activation of a neutrophil NADPH oxidase (plasmalemma-bound NADPH-dependent superoxide synthase). Apoplastic superoxide dismutase rapidly converts this superoxide to $H_2O_2$. Cell death results if $H_2O_2$ production overwhelms the antioxidative defence mechanisms (catalase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione peroxidase, glutathione reductase and glutathione *S*-transferase). Cell death triggered by the oxidative burst plays a key role in pathogen defence. There is growing evidence that osmotic

and UV-B stresses may also activate neutrophil NADPH oxidase, suggesting that certain abiotic and biotic stresses may share a common signalling pathway.

## Integrative Approaches to Understanding Stress Responses

Rapid advances in plant molecular genetics now permits detailed dissection of the signal transduction pathways involved in stress perception (e.g. the salt, osmotic, cold, ABA and ethylene signal transduction pathways described above). These advances also permit the modulation of the activity or abundance of specific transcription factors, regulatory proteins, transport proteins, proteins that are implicated in membrane or protein stabilization, or key enzymes of metabolism, in transgenic plants, in order to determine their effects on plant growth during or after recovery from a specific stress treatment. This progress is clearly shifting the balance of evidence away from correlations or associations between physiological responses to specific stresses, to firmer cause–effect relationships between response and resistance/susceptibility. The enhanced expression of cold-responsive genes in response to cold temperature stress, discussed above, is an excellent example of a physiological response that has long been recognized as correlated with the acquisition of cold (freezing) tolerance, and/or dehydration tolerance. Modulation of the expression of the transcriptional activator, CBF1, now provides direct evidence that these changes in gene expression do indeed contribute to increased freezing tolerance. Several other examples of these advances are noted with asterisks in **Table 1**.

Although genetics and molecular biology are becoming increasingly important tools in our understanding of plant stress responses, the tools of electrophysiology, enzymology, analytical biochemistry and classical plant physiology remain key to interpreting the consequences of specific genetic interventions on metabolism, ion fluxes, compartmentation, water relations, growth and overall plant fitness. More than ever before, integrative, interdisciplinary approaches are needed to probe the underlying mechanisms, and the complex interactions in stress signalling and metabolism. Although model organisms, such as *Arabidopsis*, have been central to the rapid advances described above, it is clear that the plant kingdom exhibits an enormous diversity of stress adaptations. Continued work on plant species uniquely adapted to environmental extremes is essential if we are to appreciate the full spectrum of adaptive strategies employed by plants.

## Ecophysiological Adaptations – Examples

The best studied examples of ecophysiological adaptations are those of carbon assimilation in plants. We will briefly compare and contrast $C_3$, $C_4$ and crassulacean acid metabolism (CAM) modes of photosynthesis to point out key features of these carbon assimilation pathways that determine relative fitness of $C_3$, $C_4$ and CAM plants for growth in cool/temperate versus hot/arid environments.

### $C_3$ photosynthesis

$C_3$ plants assimilate carbon dioxide ($CO_2$) by the Calvin cycle; the first reaction of this cycle is the condensation of $CO_2$ with ribulose-1,5-bisphosphate to generate two molecules of 3-phosphoglycerate, catalysed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). As the name implies, Rubisco also has an oxygenase function, and will utilize oxygen ($O_2$) in place of $CO_2$ as substrate, generating 2-phosphoglycolate. This must be recycled to 3-phosphoglycerate via a complex set of reactions known as the photorespiratory carbon oxidation (PCO) cycle. The PCO cycle results in $CO_2$ loss and $O_2$ consumption, and expends energy. In normal air, at 25°C, the ratio of carboxylation to oxygenation catalysed by Rubisco is between 2.5 and 3.0. However, as the temperature increases, the concentration of $CO_2$ in solution in equilibrium with air decreases more than does the concentration of $O_2$; consequently the ratio of $CO_2$:$O_2$ decreases as the temperature rises, driving greater flux via the PCO cycle. Photorespiration therefore results in inefficiency of $CO_2$ fixation, particularly at high temperatures. However, as noted earlier, it may play an important role in protecting $C_3$ plants against high light intensities at low $CO_2$ concentrations (e.g. during water stress when stomates are closed), functioning to dissipate excess ATP and reducing power from the light reactions of photosynthesis, preventing damage to the photosynthetic apparatus (photooxidation and photoinhibition).

### $C_4$ photosynthesis

$C_4$ plants suppress photorespiration by concentrating $CO_2$ in bundle-sheath cells where Rubisco and other enzymes of the Calvin cycle are exclusively localized. Fixation of $CO_2$ occurs via the carboxylation of phosphoenolpyruvate (PEP) in the mesophyll cells, to form the $C_4$ acid, oxaloacetate, which is then converted to either malate or aspartate, transported to the bundle-sheath cells and decarboxylated to generate the $CO_2$ for assimilation via the Calvin cycle. A $C_3$ acid (pyruvate) formed from the aforementioned decarboxylation is then recycled to the mesophyll cells to regenerate the PEP which serves as $CO_2$ acceptor in the cycle. Because the $CO_2$ concentrating

mechanism suppresses photorespiration, $C_4$ plants are more efficient than $C_3$ plants in $CO_2$ fixation at high temperatures. Moreover, PEP carboxylase has a higher affinity for $HCO_3^-$ than Rubisco has for $CO_2$, enabling $C_4$ plants to reduce stomatal aperture and conserve water while maintaining high $CO_2$ assimilation rates. $C_4$ plants are therefore better adapted to drier, hotter climates than $C_3$ plants, and show greater water-use efficiency. However, $C_4$ metabolism has a higher energy requirement than $C_3$ photosynthesis, consuming two extra molecules of ATP per $CO_2$ fixed. This renders $C_4$ plants less efficient in $CO_2$ fixation, particularly at low temperatures where the PCO cycle is suppressed in $C_3$ plants. Consequently $C_3$ plants have a competitive advantage over $C_4$ plants in cool, temperate climates.

## Crassulacean acid metabolism

Crassulacean acid metabolism (CAM) is another mechanism of concentrating $CO_2$ at the site of action of Rubisco that is found in numerous angiosperm species adapted to hot, arid environments. In CAM plants, $CO_2$ is captured by PEP carboxylase at night when stomates are open, generating oxaloacetate from which malate is then derived and stored in the vacuole. During the day when stomates are closed, malate is transported to the chloroplast, releasing $CO_2$ for assimilation via the Calvin cycle. The elevated $CO_2$ concentrations achieved by this mechanism effectively suppress photorespiration, as in $C_4$ plants. Closure of stomates during the day greatly restricts both water and $CO_2$ losses. CAM plants have specialized mechanisms to cope with the high leaf temperatures that can occur at peak irradiances in the absence of transpirational cooling. They rely on re-emission of infrared radiation and heat loss by convection and conduction for leaf cooling. A key regulatory mechanism in CAM metabolism is the diurnal phosphorylation and dephosphorylation of PEP carboxylase. The active night form of PEP carboxylase is phosphorylated; this phosphorylated form of the enzyme is insensitive to malate, permitting the large accumulation of malate at night. The inactive day form of the enzyme is dephosphorylated, and is sensitive to malate. Certain plant species (e.g. *Mesembryanthemum crystallinum*) exhibit facultative CAM, i.e. a switch from $C_3$ to CAM in response to salinity or drought stress; this requires coordinated expression of CAM genes, including the gene encoding PEP carboxylase.

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