Encyclopedia of
APPLIED PLANT SCIENCES

Editors in Chief
Brian Thomas
Brian G Murray
Denis J Murphy

SECOND EDITION

ENCYCLOPEDIA OF
APPLIED PLANT SCIENCES

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APPLIED PLANT SCIENCES

SECOND EDITION

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PREFACE

Since The Encyclopedia of Applied Plant Sciences was first published in 2003, it has become an invaluable resource for researchers, teachers and agricultural practitioners. The aim of the work was, and still is, to provide a friendly and comprehensive portal to the vast amount of knowledge we have on plants and how we make use of them. The background to the production of the first volume has not changed. As we said in the introduction to the first edition, plants have a huge impact upon human existence. All of our food and much of our other energy needs are ultimately derived from the products of plant photosynthesis. Plants also have their impact through the social and economic dimensions of human existence. All agriculture, which provides us with our food, is ultimately based on plant production, be it directly through arable crops such as cereals, horticultural crops including fruits and vegetables or indirectly through pasture, forage and plant derived animal feedstuffs. Industrial crops such as fiber crops or woodlands and forests are a vital source of renewable materials such as for clothing, building and recreation that we take for granted in everyday life. Flowers and ornamental plants delight us with their beauty, fragrance and their seemingly infinite variety.

However, since the last edition was published the role of applied science in agricultural production has been brought into greater focus as fluctuations in global food production feed through into prices and availability to consumers. The perfect storm of demands for energy, increasing population and climate change are manifested in concerns about food security. At the same time, technological advances are changing the way plant science is done. Crop genome sequencing has become routine during the preparation of this second edition of the Encyclopedia and increasingly quantitative systems approaches to understand fundamental processes have become commonplace. The need to incorporate these key developments has been a major motivation in publishing a second edition. The revised edition provides additional coverage on: plant science and its role in food security, genome sequencing and its impact on plant breeding and crop improvement; new methods of genetic modification; climate change and its impact on crop production. Chapters that deal with basic mechanisms, e.g., how growth regulators work, have been revised to include new information leading to greater understanding. In addition a number of new chapters have been included on specific crops.

The structure of the work has been revised to make it easier for users to locate articles of interest and related chapters. The major change is that the second edition will be available both as hard copy and as an online resource. Abstracts and keywords have been added to make searching for relevant chapters easier and quicker. The printed version of the Encyclopedia is now organised into three thematic volumes, namely (1) Crop Systems and Products; (2) Plant Physiology and Development; and (3) Breeding, Genetics and Biotechnology.

As for the first edition, the Editors are hugely indebted to our excellent International Editorial Board, who have helped to source chapters and used their expertise to ensure the chapters submitted provide an authoritative introduction to the subject and a portal to the wider literature available. Pulling together over 200 separate articles, almost all of which have been rewritten or updated since the first edition, is a substantial logistical exercise and the Editors are highly appreciative of the support we have been given by the Editorial team at Elsevier. In particular, we would like to thank Simon Holt, who was influential in getting the project off the ground and Blerina Osmanaj, whose persistence and dedication in chasing authors and articles was pivotal to completing the second edition on schedule. We must also acknowledge the collective input of over 200 authors in providing the substance of the encyclopedia. As with the first edition, we believe that the end result is a unique assemblage of the key information in this important field and are

privileged to have been involved in the project. The value in the work is down to the quality of the contributors. Omissions or lack of balance are entirely the responsibility of the Editors. We hope that the work proves to be not only of practical value but can act as a catalyst for readers to gain entry to the exciting world of Plant Sciences.

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ABIOTIC STRESS

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Cold Stress

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This article is a revision of the previous edition article by J. Sutka, G. Galiba, volume 1, pp. 1–9, © 2003, Elsevier Ltd.

Nomenclature

Abscisic acid (ABA), Jasmonic acid (JA), Salycilic acid (SA), gibberellic acid (GA) Plant growth regulators
Chilling Suboptimal temperature stress above 0 °C
Cold acclimation Nonheritable modification of structures and functions by cold temperatures in order to minimize cold-induced injuries
Cold stress The effect of suboptimal temperature resulting in structural and functional injuries
COR proteins Cold-regulated proteins
Freezing Temperature stress below 0 °C

Introduction

Apart from the availability of water, low temperature (chilling (suboptimal temperature stress above 0 °C) and frost) is the most important environmental factor that limits the productivity and geographical distribution of plants in large areas of the world. Cold temperatures can affect the development of plants in almost every phase, from germination through to seed set. The sensitivity of a plant species in a particular environment is determined by the limit to which its metabolic processes continue to function under low temperature stress or the point at which it may suffer permanent injuries that finally bring about death. The cold tolerance of a plant species is evolutionarily determined and depends on the climate of the area the plant originated from. According to their cold tolerance, plants are divided into three categories:

1. Chill susceptible: damaged by temperatures below 12 °C.
2. Chill tolerant but freezing (temperature stress below 0 °C) susceptible; able to acclimate to temperatures below 12 °C but unable to survive freezing.
3. Freezing tolerant (frost tolerant); able to acclimatize to survive temperatures significantly below freezing.

As shown in Table 1, tropical plants like banana (*Musa sapientum*), papaya (*Carica papaya*), etc., belong to the chill susceptible category. Several subtropical plant species such as paprika (*Capsicum frutescens*), potato (*Solanum tuberosum*), and tomato (*Lycopersicon esculentum*) which are cultivated in temperate regions are classified as chill tolerant but freezing susceptible. The herbaceous or woody plants characterized as freezing tolerant originated mostly from temperate climates. There are substantial differences among the plant species classified as freezing tolerant. Most of the herbaceous plants survive only moderate freezing, between −7 °C and −30 °C. However, there are some much hardier woody species, which can tolerate temperatures below −80 °C. The effect of cold on plants is not only determined by the magnitude of the drop of the temperature, but is to a large extent dependent upon the season, developmental stage, and for how long the low temperature persists.

Table 1 Examples of cold-sensitive (tender) and freezing-tolerant (hardy) plants, including some important crop species

Family	*Species*	*Range of temperature causing injury (°C)*	
Musaceae	*Musa* spp. (banana)	+10	+12
Lauraceae	*Persea* spp. (avocado)	+6	+8
Caricaceae	Carica spp. (papaya)	+4	+10
Poaceae	*Oryza sativa* (rice)	+12	+15
	Zea mays (maize/corn)	+2	+12
	Avena sativa (oat)	−5	−10
	Hordeum vulgare (barley)	−7	−12
	Triticum aestivum (bread wheat)	−9	−18
	Secale cereale (rye)	−15	−30
Solanaceae	*Lycopersicon esculentum* (tomato)	+2	+5
	Capsicum annuum (paprika/pepper)	−2	+4
	Solanum tuberosum (potato)	−2.5	0
	Solanum acaule (wild potato species)	−6	−8.5
Brassicaceae	*Arabidopsis thaliana*	−9	−14
Rutaceae	*Citrus* spp. (orange and lemon)	−2.2	−10
Myrtaceae	*Eucalyptus* spp. (eucalyptus)	−8	−16
Cupressaceae	*Juniperus* spp. (juniper)	−25	−45
Pinaceae	*Pinus* spp. (pines)	−20	−60
Rosaceae	*Prunus* spp. (plum)	−20	−80

Cold Acclimation of Plants

Due to the sessile nature of the plants, they must adapt to the seasonal and daily changes of temperature and light conditions of the prevailing environment. Additionally, plants growing in the temperate regions of the northern hemisphere must cope with the additional environmental constraint of freezing temperatures during winter. There are three environmental cues which signal temperate zone perennial plants to prepare for the upcoming frosty conditions: decreasing temperature, day length, and alteration in light spectra in the autumn. Plants adapt their physiological processes to the daily fluctuation of temperature and light intensity with the help of a circadian clock. This synchronizes gene expression, protein synthesis and activity, and the synthesis and degradation of various compounds to the regular daily alterations in environmental conditions, consequently ensuring fitness and optimal growth. Plants also have to adapt to weekly, seasonal, and annual changes in the environment and to achieve the appropriate mass production necessary for successful reproduction. The seasonal alterations in growth and development are probably regulated by changes in day length and temperature, leading to the reprogramming of their metabolism.

The growth habit of plants determines their strategy to protect their very sensitive reproductive tissues (flower primordia) to ensure the reproduction. In the case of spring annuals, germination, reproduction, and senescence occur during the warm seasons. In contrast, winter annuals set seed and germinate in the autumn, overwinter in a vegetative growth state, and flower in the spring. To avoid freezing damage to the reproductive organs, varieties with a winter growth habit require long exposures to cold temperatures to be transformed from vegetative to reproductive phase and initiate flowering (known as a vernalization requirement) whereas those with a spring growth habit do not have such a requirement. Plants are able to cold acclimate (a hardening process) only in their vegetative developmental phase. Under natural conditions, the cold hardening (acclimation) takes place in autumn when the temperature gradually decreases to 0 °C over several weeks. A nonacclimated rye (*Secale cereale*), for instance, is killed by freezing at about −5 °C, but after a period of exposure to low nonfreezing temperatures can survive freezing down to about −30 °C.

It became obvious as early as the 1920s and following many decades of research, that boreal trees and shrubs survive the winter by sensing shortening day length in early autumn, initiating developmental programs that result in growth cessation, and reaching the state of dormancy (endodormancy). The timing of this growth cessation is critical to enable the development of frost tolerance. As the season continues and the temperature becomes colder, plants sense the low temperature and respond by additional increases in freezing tolerance.

Molecular and Physiological Changes Associated with Cold Acclimation and Cold Tolerance

Plant Hormone Interactions during Different Phases of the Cold Stress Response

Cold acclimation (nonheritable modification of structures and functions by cold temperatures in order to minimize cold-induced injuries) is a dynamic time-dependent process in which the plant hormones play a central role. It is important to emphasize that the role of different plant hormones differs substantially among the individual stress phases (Figure 1). The alarm phase (cold shock, first days of cold treatment) is associated with a decrease of hydraulic conductivity of roots, which results in the decrease of water potential in leaves. Abscisic acid (ABA) content increases transiently in the early stage of the cold response governing stomata closure and thus adjusting water homeostasis. ABA stimulates the expression of a number of stress-related genes, including transcription factors which play important roles in the reprogramming of the metabolism. Increased levels of ABA were found to coincide with downregulation of other stress hormones, salicylic acid (SA) and jasmonic acid (JA) during an early phase of wheat response to cold stress (the effect of suboptimal temperature resulting in structural and functional injuries), which indicates the antagonistic character between ABA and the other stress hormones. SA is a stress hormone predominantly associated with biotroph infection, while JA is associated with the response to wounding and necrotroph attack. ABA can play role in inhibiting plant growth, downregulating gibberellic acid (GA) biosynthesis. Cytokinins (CKs) are involved mainly in stimulation of cell division, lateral bud or shoot growth, and the prevention of senescence. The levels of active cytokinins are downregulated during the alarm phase. The other growth-promoting hormone, auxin (indole-3-acetic acid), showed a decrease in the wheat leaves during the alarm phase as well.

The characteristic features of the acclimation phase are an increase in acquired frost tolerance, accumulation of protective proteins, especially dehydrins, downregulation of ABA levels, and an elevation of positive regulators of cell division and growth (i.e., cytokinins, gibberellins, and auxin). These changes indicate that the readjustments of metabolic activity to the cold

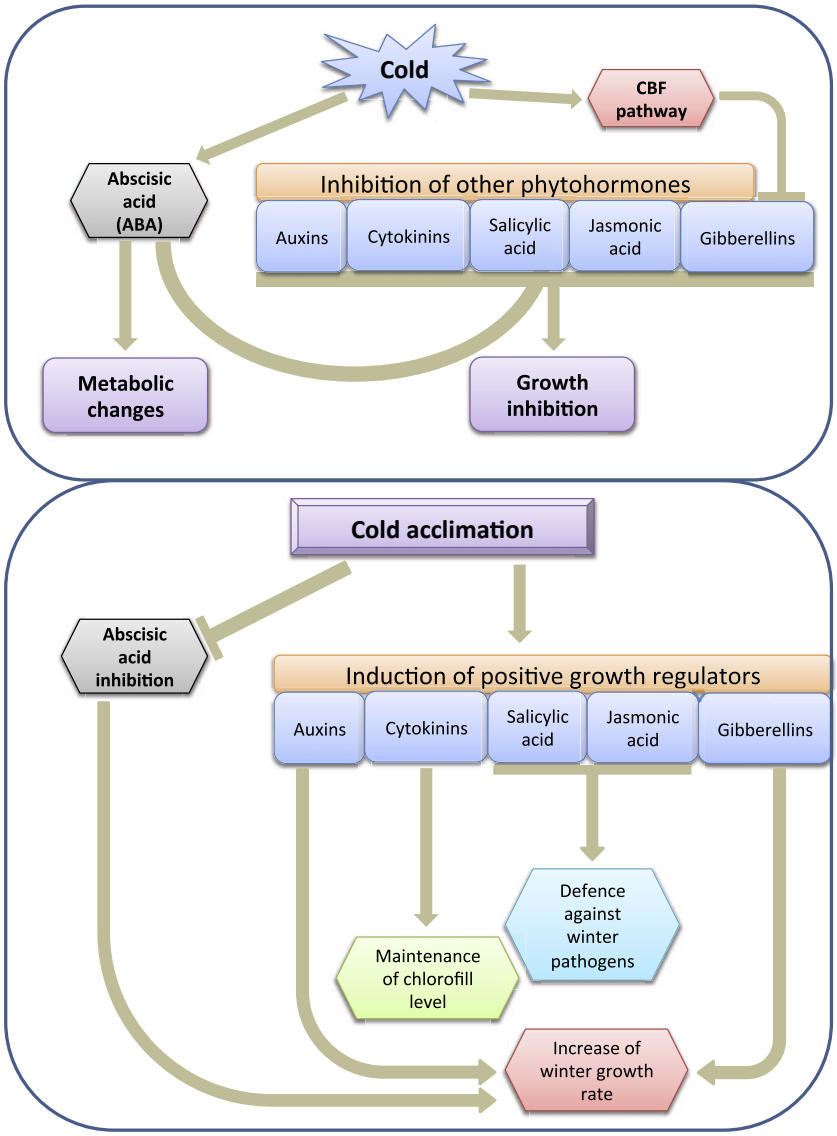

Figure 1 Interaction of plant growth regulators involved in the different phases of cold response.

conditions have been completed. The increased cytokinin levels improve the maintenance of chlorophyll levels resulting improved photosynthetic performance. Elevation of active gibberellins (GA1 and GA4) and SA was also found during the adaptation phase. Elevation of SA might relate to SA functioning in the regulation of reactive oxygen species (ROS) evolution. JA was reported to increase during the acclimation phase and even more during longer term exposure to cold. During the same period, there was also increased resistance of winter wheat to snow mold, powdery mildew, and stripe rust pathogens. These changes show the well-orchestrated interaction between abiotic and biotic stress responses.

Molecular Changes Associated with Cold Acclimation and Freezing Tolerance

During cold acclimation, one of the most characteristic phenomena is the reprogramming of gene expression resulting in accumulation not only of protective proteins, but also of hundreds of other metabolites, some of which are known to have protective effects. The best known cold regulatory system with a key role in frost tolerance is the CBF/DREB (C-repeat *binding factor* or *d*ehydration *r*esponsive *e*lement *b*inding factor) pathway, which has been well studied both in monocot and dicotyledonous plant species (Figure 2). These genes were discovered simultaneously in the USA and Japan in *Arabidopsis thaliana* (the key model plant for plant molecular biology), which is why this gene family has two names. The CBFs belong to the AP2/EREBP transcription factor family and possess a plant-specific AP2 DNA domain that binds to the C-repeat elements (A/GCCCGAC) present in the promoters of cold-regulated (*COR*) genes. The expression of *CBF* genes is ABA independent and they switch on after 15 min of cold treatment. In *Arabidopsis*, six CBFs have been identified and three of them have a primary role in cold acclimation. Large CBF families are present in cereals and are subdivided into four phylogenetic

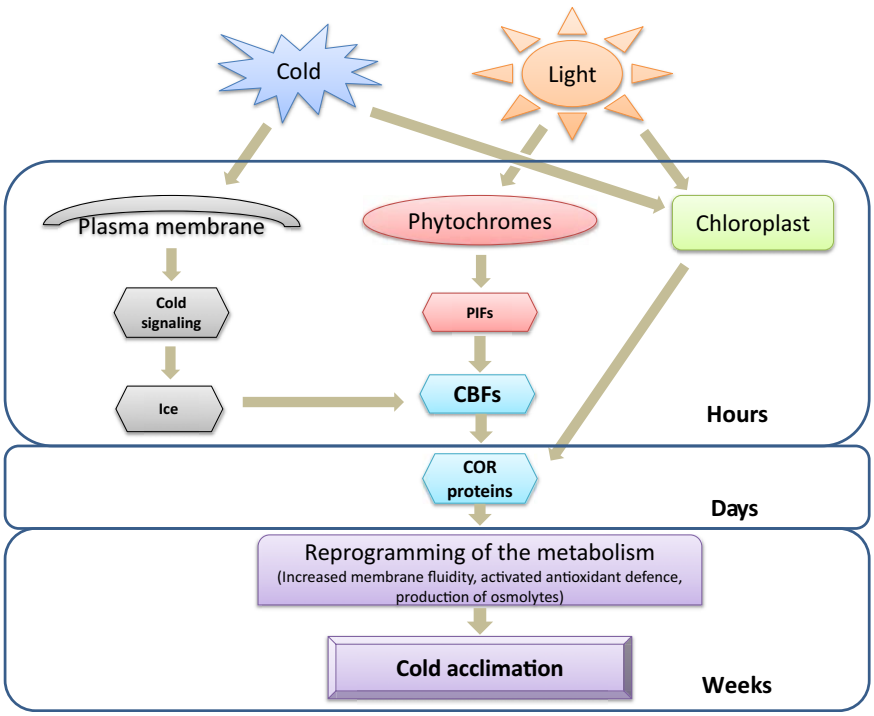

Figure 2 Summary of the activation of the CBF regulatory system.

groups. In the economically important cereals, several CBFs have been characterized, including 20 from barley (*Hordeum vulgare* L.), 13 from *Triticum monococcum*, and 37 from common wheat (*Triticum aestivum* L.). *CBF* genes are positioned in clusters on the homeologous group 5 chromosomes of the Triticeae and coincide with the FR-2 quantitative trait locus (QTL) for freezing tolerance. The regulation of *CBF* expression has been only partly elucidated as yet. The expression of *Arabidopsis CBF3* is positively regulated by the ICE1 protein (*inducer* of CBF *e*xpression 1), which is the product of the constitutively expressed *ICE1* gene. At room temperature the ICE1 protein is located in the cytoplasm and degraded by other enzymes. However, when Arabidopsis plants are exposed to low temperature conditions, the ICE1 is activated by conformation changes and, in turn, activates *CBF3* expression. Multiple regulatory elements exist in the *CBF* gene promoters: so for example the expression of *CBF* genes is also regulated by the circadian clock, moreover some of them are short-day and light regulated. The regulation and the interaction of the multiple *CBF* genes in cereals is still poorly understood.

The role of the *CBF*-regulon (regulon means the whole set of genes which are up- or downregulated by the transcription factor) has been revealed using *CBF*-overexpressing transgenic plants. Overexpression of *CBF* genes in *A. thaliana*, canola (*Brassica napus* L.), tomato (*Solanum lycopersicum* L.), barley (*Hordeum vulgare*), and poplar (*Populus balsamifera* subsp. *trichocarpa*) increased their freezing tolerance. However, the increased freezing tolerance of *CBF*-overexpressing plants is associated with a dwarf phenotype, delayed flowering and enhanced photosynthetic performance. Thus, the *CBF* regulon–induced freezing tolerance is associated with phenotypic changes that are analogous to growth and development events that are controlled by plant hormones and photoreceptors such as phytochromes.

Some results indicate that the *CBF* genes regulate not only cold acclimation but also induce the state of dormancy as well. CBF cold regulation of dormancy induction may be mediated through *DAM* (*D*ormancy *A*ssociated *M*ADS-Box) genes which contain a CCGAC motif in their promoters (a known binding site of CBF protein). *DAM* genes have been directly linked to dormancy induction using the evergreen peach mutant. Downstream effects on growth cessation and dormancy induction may be occurring via upregulation of the gibberellin 2-oxidase gene, which degrades GA. The role of *CBF* genes in the regulation of dormancy was also directly proven by ectopic expression of a peach (*Prunus persica*) *CBF* gene in apple (*Malus* × *domestica*), which resulted in short-day–induced dormancy and increased cold hardiness. In apple, growth cessation and the onset of dormancy are normally relatively unresponsive to short-days and instead require low temperature. Perennial trees are particularly vulnerable to climate change since varying annual temperature may alter timing of dormancy induction and therefore, timing of cold hardiness. If we fully understand the role of *CBF* and *DAM* genes in induction of dormancy, it might be possible to engineer the timing of dormancy according to the predicted climatic conditions.

Light-Dependent Regulation of Cold Acclimation and Freezing Tolerance

More and more information has been accumulated, mostly by using the *Arabidopsis* model system, that other factors than low

temperature especially day length and light quality also significantly affect the degree of freezing tolerance. It became obvious as early as the beginning of the 1920s that trees and shrubs survive the winter by sensing shortening day length during early autumn and initiate developmental programs that result in the cessation of growth and the state of dormancy. This also results in increased frost tolerance. As the season continues and the temperature becomes colder, plants sense the low temperature and respond by additional increases in freezing tolerance. The molecular basis for photoperiodic regulation of freezing tolerance is most likely the action of phytochromes. Phytochromes mainly absorb red (R) and far-red (FR) light, while cryptochromes and phototropins absorb blue and UV-A light, respectively. Apart from woody plants there was only limited knowledge about photoperiodic regulation of freezing tolerance in herbaceous plants. However there was a recent breakthrough in 2012 from work using *Arabidopsis* as a model plant. It was found that *Arabidopsis* plants increase in freezing tolerance in response to a short-day photoperiod, and that this regulation involves photoperiodic regulation of the CBF pathway. The regulation of the CBF pathway is mediated by the PHYB photoreceptor.

Apart from temperature and light intensity, the spectrum of the daylight also changes on a daily basis. The red:far-red (R:FR) ratio of daylight is around 1.15 and varies little with weather conditions or time of year. Reductions in R:FR also occur naturally at dawn and dusk periods known as twilight. The twilight spectrum is relatively enriched in the blue and the FR regions, but relatively poor in the orange-red regions. Thus, the onset of sunrise and dusk are both associated with a significant drop in R:FR ratio from about 1.15 to about 0.7–0.8. There is preliminary evidence that at high latitudes where twilight duration is extended, the change in light quality is used to provide seasonal information. So, a plausible hypothesis is that at northern latitudes lower temperatures and longer twilight periods during autumn induce *COR* genes, which protect plants against further decreases in temperature. Indeed in support of this theory expression of *Arabidopsis CBF* genes and freezing tolerance can be activated by a low red-to-far-red (R/FR) ratio when plants are grown at 16 °C under a 12-h photoperiod and *CBF* expression is under the influence of the circadian clock, as discussed above, and repressed by phytochromes B and D. Responses to low R/FR result from conversion of phytochromes B, D, and E to the inactive Pr form. When grown at 16 °C, monogenic mutants deficient in phytochromes B and D showed increased expression of *COR15a*. *COR15a* is regulated by *CBF* genes. Loss of phyE had little additional effect, suggesting that repression of the *CBF*-regulon in high R/FR is mediated by phyB and phyD in a nonredundant manner.

Molecular Bases of the Interactions between Vegetative and Reproductive Developmental Phases and Freezing Tolerance

It is obvious from the previous sections of this article that freezing tolerance is an inducible process and that it increases gradually under cold conditions. In the case of cereals, the maximum freezing tolerance is achieved after several weeks, when the temperature stays slightly above 0 °C. But the hardened phase is not maintained indefinitely and the plants lose their cold hardiness in spring. When researchers studied the reason for the loss of frost hardiness, they found that it coincided with the change of developmental phase from vegetative to reproductive. Inspection of the shoot apex of the plantlets at different times during winter and early spring revealed that when the shoot apex reached the so-called double ridge phase (indicating that the shoot apex was switching to a flower primordium) the plants lost their freezing tolerance. The molecular background of this process has now been revealed.

Two gene families namely the photoperiod (*PPD*) and the vernalization (*VRN*) genes are the most important genes regulating the initiation of the reproductive phase. *VRN* genes are of particular interest as they are regulated by long exposures to cold but nonfreezing temperatures, the same conditions required for plant acclimation to freezing temperatures. There are three *VRN* genes, of which *VRN1* is the most important in determining both the vernalization requirement and freezing tolerance. In case of winter growth habit cereals, (wheat, barley, rye, oats) several weeks of cold treatment (vernalization) are necessary to switch on the *VRN1* gene. When the *VRN1* gene is fully expressed, it promotes the transition of vegetative shoot apex into a flower primordium and at the same time, the expression of *COR* genes including the *CBF*-regulon which are positively associated with freezing tolerance are downregulated and the plant becomes freezing sensitive. In the case of spring growth habit, the *VRN1* gene is constitutively expressed without any cold treatment and hence they are freezing sensitive even after prolonged cold treatment.

Consequently, if a plant breeder would like to improve the productivity of crops used in an area where winter damage is prevalent, it is necessary to consider not only the maximum freezing tolerance of the genetic stocks used for prebreeding the developmental pattern of the genotypes, but also be considered which must fit to the local agrometeorological conditions.

Biochemical and Metabolic Changes during Cold Acclimation

As described above, upon sensing the temperature drop, the transcriptome (the full range of gene transcripts present in a plant) undergoes reorganization with thousands of genes involved being up- or downregulated. These transcripts serve as informants on the changing environment and being translated into COR proteins (cold-regulated proteins) they will significantly change the nature of the plant tissues. This transition is needed to avoid the frost damage.

Cold Perception and Signaling

In plants, cold perception and cold signaling is studied mostly in model organisms. According to the most recent results, cold perception in plants may occur through multiple pathways. One major hypothesis is that the decreasing temperature changes the fluidity of the cell membranes and this might transform membrane-linked proteins (ion channels/kinases) into a conformational active form triggering signaling cascades. However, there is evidence that besides membranes, other sensors are involved in the cold perception as well. Temperature-dependent conformational remodeling of the

cytoskeleton and the nucleosomes appears to be important in certain cold-dependent responses. Although, the temperature sensor is still not clarified in plants, an elevated intracellular calcium concentration is common and a crucial early event in the cold signaling. At the next level, the Ca influx is decoded by calcium responsive proteins and then the signal results in altered expression of cold responsive genes.

Membranes

The survival of a cell highly depends on its membrane integrity. Nonfreezing chilling stress directly affects the cell membranes causing the formation of gel (or solid phase) by lipids in biological membranes and inducing damage to the plant tissues that can lead to the death of the plant. Freezing, however, often acts indirectly, damaging the cells by dehydration. As temperatures drop below 0 °C, ice formation is generally initiated in the cellular spaces, due in part to the extracellular fluid having a higher freezing point (lower solute concentration) than the intracellular fluid. Because the water potential of the extracellular ice is less than the water potential of liquid water within the cells, there is movement of unfrozen water down the water potential gradient from inside the cell to the intercellular spaces. At −10 °C, more than 90% of the osmotically active water typically moves out of the cells causing shrinkage of the cell volume. When the thaw is setting in the water, it moves back to the cytoplasm causing expansion-induced lysis and resulting cell death. Dehydration in the absence of cold has the same effect, confirming that damage is a consequence of the freezing-induced desiccation. Thus, a key part of cold acclimation is to stabilize membranes against freezing-induced injury.

Acclimation of plants to temperatures below their respective normal growth temperatures generally results in changes in composition of membrane phospholipids and the unsaturation of fatty acids in membrane lipids. The extent of unsaturation has a considerable effect on the fluidity of membrane lipids. When organisms are exposed to low temperatures, the fluidity of their membrane lipids decreases. Such exposure enhances the expression of genes for fatty acid desaturases, which introduce double bonds into the fatty acyl chains of membrane lipids, thereby compensating for the decrease in membrane fluidity. As a result, the original physical properties of the membranes are restored and can support the functions of the membrane-associated proteins and their complexes.

Oxidative Stress

Chilling and cold injury is mediated, in part, by oxygen free radicals (singlet oxygen, superoxide radicals, or hydrogen peroxide) as agents causing damage to the proteins, nucleic acids, and membranes. The activation of oxygen by the photosystems in the presence of excessive light is probably the major site of production of free radicals in leaves but other electron transport systems, including those in the mitochondria or plasmalemma, may also contribute especially in nonphotosynthetic tissues. The development of the symptoms of chilling injury is frequently coincident with peroxidation of fatty acids. In this way, lipid peroxidation alters the physical properties of membrane lipids, thereby inhibiting the function of membrane-bound proteins and contributing to the development of visual symptoms of injury caused by cold temperatures. Therefore, it is clear that the capability of the plants to enhance the free radical scavenging capacity by increasing the endogenous level of antioxidants, e.g., carotenoids, tocopherol, ascorbate, superoxide dismutase, glutathione, etc., is an important part of the plant's defense mechanism under cold stress conditions.

Cold-Inducible Proteins (COR)

As described above, a temperature decline leads to a decrease in plasma membrane fluidity with changes in composition of membrane phospholipids, which also affects conformation of several transmembrane protein complexes. At the protein level, changes in the composition of plasma membrane proteins in response to cold were also verified. For example, the increase in ERD10, ERD14, and 270 COR47 dehydrins indicated protection of membrane-associated proteins against dehydration and denaturation. The increase in outer membrane proteins belonging to the lipocalin family was associated with their important role in membrane biogenesis and repair. During cold acclimation, freezing-tolerant plants accumulate antifreeze proteins (AF) in anticipation of the arrival of freezing conditions. These are chitinases, glucanases, and thaumatin-like proteins, which accumulate in the apoplast (xylem-lumen, cell wall, and intercellular spaces), and they are highly similar to pathogen-related proteins. AF proteins have the capacity to bind to ice crystals and inhibit their growth. The chaperone protein family assists in the covalent folding or unfolding and the assembly or disassembly of other macromolecular structures. Several proteins, for example the heat shock proteins (HSP), exhibit chaperone function. An increased abundance of HSP70 proteins and a decreased abundance of HSP90 proteins were found in cold-treated winter wheat. HSP90 is known to conserve allele variation due to its role in protein folding. A decrease in HSP90 abundance could lead to an increased variation in protein conformation, which may be advantageous upon stress. RNA chaperones can prevent the formation of secondary structures during cold stress and regulate transcription and translation. For example, the *Arabidopsis* cold shock domain chaperone protein 3 (AtCSP3), is involved in the acquisition of freezing tolerance in plants. AtCSP3 complemented the cold-sensitive phenotype of the *Escherichia coli* CSP mutant. This is a good example proving that, in many cases, the gene or protein itself is known to be cold responsive in a number of species, indicating at least a partial uniformity of response to cold among unrelated organisms. It is also important to emphasize that several cold-inducible genes are responsive to a variety of stresses, not just cold, or are also expressed during seed development in the nonstressed plant, such as late embryogenesis abundant hydrophilic (*LEA*) genes. Thus, though their expression is often related to low cytoplasmic water content, they are not uniquely expressed in stressed plants. The differences in acquired frost tolerance between spring and winter wheat genotypes are mirrored in the relative abundance levels of several COR/LEA proteins, namely the LEA-II WCS120 proteins and their barley homologue DHN5 and LEA-III COR14b protein. The expression profile of stress-related genes has been used recently to select frost tolerant genotypes in wheat and barley breeding programs.

Osmotically Active Solutes

Low temperature–induced changes in the transcriptome and proteome finally result in the reconfiguration of metabolomes. The active accumulation of the hydrophilic organic compounds during cold stress in cytoplasm and vacuoles results in osmotic adjustment between the cellular solute and the extracellular fluid avoiding intracellular ice formation and cellular dehydration. Alterations in the level of osmolytes (carbohydrates and free amino acids), antioxidants, polyamines, and other metabolites are important in the cold acclimation process, and result in an increased freezing tolerance. They may function as cryoprotectants and many of these compounds may also stabilize membranes and serve as an energy source during cold acclimation.

Metabolomics

Metabolomics is a tool in 'system biology,' an emerging science of measurement and analysis of total metabolites available in the studied tissue or cell using analytical and statistical methods. The main point is that an organism's metabolome – its full and unique cocktail of metabolites – changes in response to stimuli and environmental conditions. A coordinated increase in the concentration of amino acids derived from pyruvate and oxalacetate, of polyamine precursors and compatible solutes was observed during cold shock experiments in *Arabidopsis* (*A. thaliana* is the model plant of molecular research). The role of the *CBF*-regulon was confirmed also at metabolite level in this model plant, since the cold acclimation–induced extensive changes in the metabolome could be mimicked by constitutive overexpression of *CBF3* gene.

Free Amino Acids

Cold acclimation alters both the composition and amount of free amino acids in overwintering plants. The ratio of amino acids belonging to the glutamate family increased and the ratio of those of aspartate family decreased in cereals. Considering the individual amino acids, Asp, Glu, Gln, and Pro levels were greatly induced by cold, and these changes were also observed at gene expression level in the case of Pro and Glu. Significant positive correlations between proline level and frost tolerance have been found in a broad spectrum of plants. The pattern of changes is not only species-dependent, but also influenced by the circadian rhythm moreover it is a transient feature during the period of cold acclimation. Consequently, to predict the frost hardiness of a plant by measuring its three amino acid level can lead to misjudgments.

Polyamines

The cold-induced increase in the amount of the amino acids, especially Glu and Arg which are precursors of polyamines, is also associated with an increased polyamine synthesis. At physiological pH, polyamines (putrescine (Put), spermidine (Spd), spermine (Spm)) are positively charged compounds, which can interact electrostatically with negatively charged proteins, including ion channels in a charge-dependent manner ($Spm^{4+} > Spd^{3+} > Put^{2+}$). Like hormones, polyamines are involved in the process of replication, transcription, translation, cell division, and elongation, membrane stabilization and plant development. Put is produced directly from ornithine or indirectly from arginine. The formation of Put from arginine is usually associated with the plant response to stresses such as drought, cold, salinity, potassium deficit etc. The importance of Put in the response to low temperature stress was demonstrated in tomato leaves, in which exogenous Put decreased the cold-induced electrolyte leakage, while the inhibition of Put synthesis increased membrane damage in 2002. More recent studies using either transgenic overexpression or loss-of-function of PAs in plant responses support this protective role of PAs in plant responses to abiotic stress. Indeed, heterologous overexpression of key genes of polyamine biosynthesis from different animal and plant sources in rice, tobacco, and tomato has shown tolerance traits against a broad spectrum of stress conditions. Enhanced tolerance always correlated with elevated levels of Put and/or Spd and Spm. So, metabolic regulation of polyamines has now emerged as a promising approach to practical applications.

See also: **Abiotic Stress:** Drought Stress; Free Radicals, Oxidative Stress and Antioxidants. **Plant Breeding and Genetics:** Control of Gene Expression: Regulation of Transcription; Plant Genomes. **Regulators of Growth:** Abscisic Acid; Auxins; Cytokinins; Gibberellins; Jasmonates; Photoperiodism; Phytochromes and Other Photoreceptors; The Regulation of Circadian Rhythms in Plants; Vernalization. **Reproduction and Biodiversity:** Flower Development.

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Drought Stress

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Physiological and Molecular Responses to Drought Stress

Drought stress is one of the most severe environmental stresses that limits crop production. Global warming can cause drought in many places and damage crop production. Understanding molecular mechanisms involved in plant drought stress tolerance is crucial for the maintenance of agricultural productivity under climate change. Plants respond and adapt to drought stress to survive water-deficit conditions. Plant responses to water deficit include dehydration avoidance and dehydration tolerance. Extensive physiological and molecular studies of model plants and crops have been performed to elucidate the mechanisms involved in dehydration tolerance under severe drought stress. Recently, phenotyping analysis has been used to identify mechanisms involved in dehydration avoidance under mild or moderate drought stress (Figure 1).

When confronted with mild water stress, plants respond and adapt by enhancing water uptake and reducing water loss (Figure 1(a)). Greater water uptake is achieved by modifying root system architecture and increasing intracellular solute accumulation, which functions to maintain cell turgor and reduce cell osmotic potential. To reduce plant water loss, stomata are closed, shoot growth is inhibited, and leaf senescence is accelerated. Stomatal closure during water-deficit conditions is induced by abscisic acid (ABA). Recent work using Arabidopsis indicates that ABA is synthesized primarily in leaf vascular tissues and transported to guard cells to induce stomatal closure under water-deficit conditions.

When confronted with severe water stress, dehydration tolerance protects cells from desiccation damage (Figure 1(b)). ABA-mediated signal transduction cascades are activated to induce expression of protective proteins and accumulation of metabolites involved in dehydration tolerance. ABA mediates responses to mild and severe drought.

Signaling Cascades Involved in Dehydration Responses

A root-derived hydraulic signal induced by water deficit results in local water potential changes, reduced turgor, higher solute concentrations, and mechanical forces exerted at the cell wall−plasma membrane interface and at the cell wall. Sensor perception of the hydraulic signal induces the chemical messenger ABA, which mediates plant adaptive responses. Recent biochemical and genetic analyses clarified the signal transduction pathways involved in hydraulic signal sensing, ABA perception, and protein phosphorylation cascades involved in dehydration stress responses (Figure 2). A pioneering study determined that an *Arabidopsis*, histidine kinase1 (AHK1), localized at the plasma membrane functions as an osmosensor or turgor sensor. The plasma membrane–localized receptor-like kinases (RLKs) are also involved in hydraulic sensing. The RLK extracellular domain is variable, including an LRR extension-like domain and a cysteine-rich domain, and is believed to possess carbohydrate-binding activity. RLKs are thought to transduce hydraulic signaling into intracellular signaling cascades and responses. The *Arabidopsis* wall-associated protein kinases (WAKs) bind pectins in the cell wall. WAK2 mediates cellular water homeostasis by regulating cell expansion. Several studies indicate that L-type lectin RLKs and proline-rich extension-like receptor kinases (PERKs) are potential candidates for cell wall integrity sensors. PERKs act at points where hydraulic signaling and ABA signaling intersect, and PERK4 regulates ABA-inducible Ca^{2+} oscillations (Figure 2).

ABA is one of the major phytohormones and is required for drought stress resistance. Several recent studies reported an ABA perception and transduction model consisting of the following three core components: pyrabactin resistance/pyrabactin resistance-like/regulatory component of ABA receptors (PYR/PYL/RCARs), protein phosphatase 2Cs (PP2Cs), and SNF1-related protein kinase2s (SnRK2s) (Figure 2). These three components coordinate ABA signals by regulating SnRK2 activity. Genetic analyses revealed that the subclass III SnRK2s SRK2E/OST1/SnRK2.6, SRK2D/SnRK2.2, and SRK2I/SnRK2.3 play essential roles in ABA signaling and activate downstream targets including transcription factors, membrane proteins, and ion channels. The ABA-responsive element (ABRE)–binding protein/ABRE-binding factor (AREB/ABFs) families of proteins, a group of basic region leucine zipper (bZIP)–type transcription factors, are phosphorylated by SnRK2s on the conserved motifs R/K-X-X-pS/pT and/or pS/pT-X-X-X-X-D/E. Phosphorylated AREB/ABFs induce expression of many stress-inducible genes whose products mediate stress resistance mechanisms in vegetative tissues. ABA-responsive kinase substrates (AKSs) are basic helix-loop-helix (bHLH) transcription factors. AKSs control stomatal opening by regulating expression of the potassium channel *KAT1* gene in response to blue light. ABA-activated SnRK2s phosphorylates AKSs, which represses their activities and promotes ABA-induced stomatal closure. The S-type slow anion channel SLAC1 is another SnRK2s target in guard cells. SLAC1-mediated anion transport is a crucial pathway in ABA-responsive stomatal closure, and it is regulated by SnRK2s in an ABA-dependent manner (Figure 2). The SLAC1 homolog SLAH3 and the R-type anion channel QUAC1 are both phosphorylated by SnRK2s. The NADPH oxidase RbohF and the potassium transporter KUP6/8 are also the targets of SnRK2s that maintain stomatal aperture under stress conditions.

Phosphoproteomic analyses indicate that the SnRK2 pathway functions in coordination with other phosphorylation

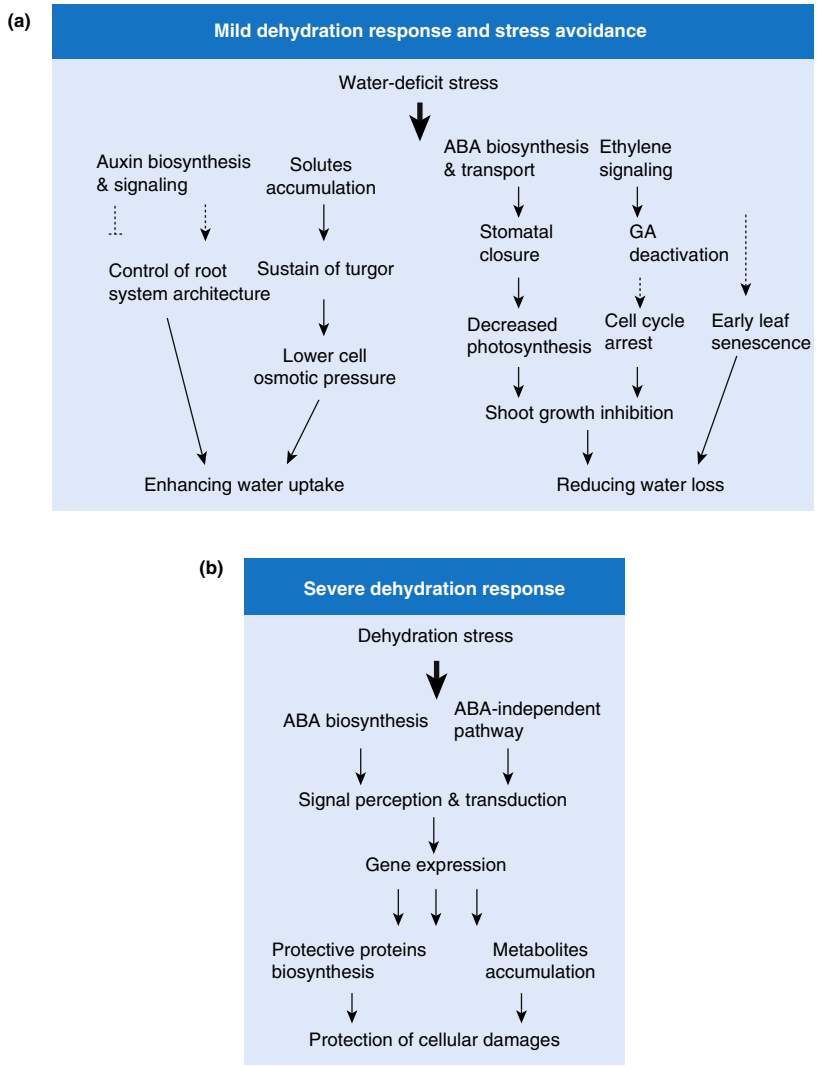

Figure 1 Physiological and molecular responses to drought stress in plant cells. (a) Plants respond and adapt to water-deficit conditions by enhancing water uptake and reducing water loss. Changes in root system architecture and solute accumulation sustain turgor and reduce cell osmotic potential to enhance water uptake. Auxin biosynthesis and signaling regulate root system architecture to absorb water from deep soil layers. Stomatal closure, shoot growth inhibition, and accelerated leaf senescence reduce water loss. The accumulation of abscisic acid (ABA) induces stomatal closure. Shoot-growth inhibition is induced by cell cycle arrest and indirect effects of stomatal closure. Ethylene signaling and deactivation of gibberellin (GA) biosynthesis contribute to growth inhibition under mild dehydration stress. (b) Plants induce cellular and molecular changes to protect cells against severe dehydration stress–induced damage. The activation of signal transduction and gene expression results in the accumulation of protective proteins and metabolites involved in dehydration tolerance. ABA regulates these molecular responses under severe dehydration stress.

signals, including Ca^{2+}-dependent protein kinases (CDPKs) and mitogen-activated protein kinases (MAPKs) (Figure 2). CDPKs phosphorylate AREB/ABFs, SLAC1, and KAT1 in response to an ABA-dependent Ca^{2+} increase. CDPKs recognize the phosphorylation target motif R/K-X-X-pS/pT similar to SnRK2s, suggesting that CDPKs act coordinately with SnRK2. MPK1 and MPK2 phosphorylation is regulated by SnRK2s. MPK9 and MPK12 activate SLAC1 in guard cells in response to ABA and Ca^{2+}. Quantitative trait locus (QTL) mapping revealed that MPK12 regulates guard cell size, stomatal movement, and improves water use efficiency (WUE). During the last decade, many studies have shed light on signal transduction pathways involved in drought stress responses. However, further system analysis is required for a full understanding of drought stress perception and signaling.

ABA Biosynthesis, Catabolism, and Transport under Water-Deficit Conditions

The endogenous ABA level plays a key role in ABA-dependent stress responses. ABA biosynthesis and catabolism is well characterized in numerous plant species. Here, we focus primarily on ABA biosynthesis, catabolism, and transport in *Arabidopsis* (Figure 3). Genetic analyses using ABA-deficient *Arabidopsis* mutants have identified key steps in ABA

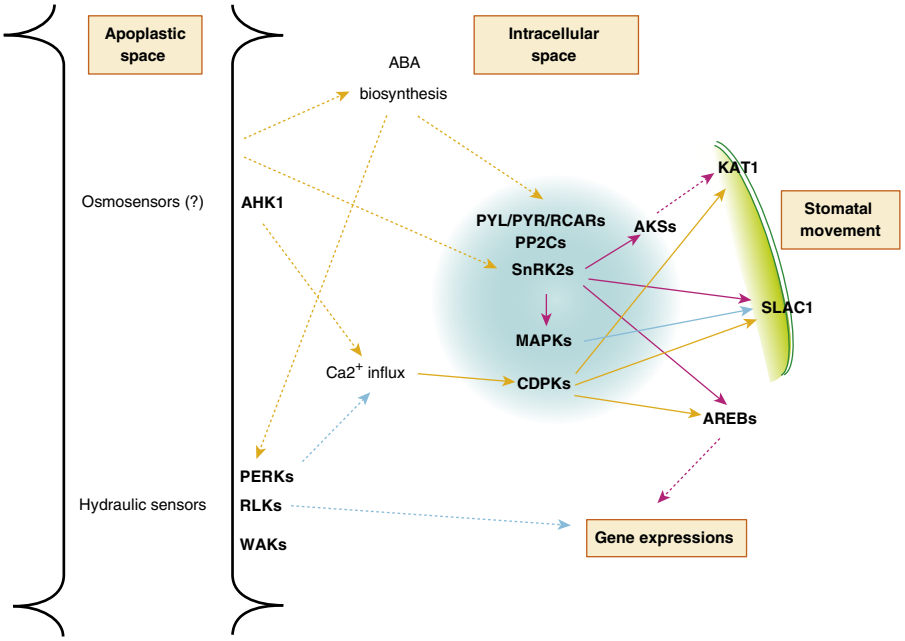

Figure 2 Signal perception and transduction pathways under dehydration stress conditions. Plants perceive water-deficit signals at the plasma membrane. Several plasma membrane–localized sensor proteins transmit those signals into the intercellular space to induce physiological responses including ABA biosynthesis, Ca^{2+} influx, and protein phosphorylation. ABA-dependent PYL/PYR/RCARs, PP2Cs, and SnRK2s form core components and phosphorylate key proteins such as AREBs, AKSs, KAT1, and SLAC1. CDPKs and MAPKs are involved in ABA-induced protein phosphorylation networks. ABA, abscisic acid; PYL/PYR/RCARs, pyrabactin resistance-like/pyrabactin resistance/regulatory component of ABA receptors; PP2Cs, protein phosphatase 2Cs; SnRK2s, SNF1-related protein kinases 2; AREBs, ABA-responsive element–binding proteins; AKSs, ABA-responsive kinase substrates; KAT1, potassium channel; SLAC1, S-type slow anion channel; CDPKs, Ca^{2+}-dependent protein kinases; MAPKs, mitogen-activated protein kinases.

biosynthesis. ABA is derived from carotenoid synthesized in plastids. Zeaxanthin epoxidase (AtABA1) catalyzes the first two steps of zeaxanthin epoxidation via antheraxanthin to all-*trans*-violaxanthin. The *Arabidopsis aba4* is an ABA-deficient mutant; AtABA4 is involved in the conversion of violaxanthin to neoxanthin. The cleavage reaction of epoxycarotenoids to produce xanthoxin by 9-*cis*-epoxycarotenoid dioxygenase (NCED) is a key step in ABA biosynthesis. Maize *VP14* is the first proposed NCED, and induction of *NCED* expression has been observed in several species. In *Arabidopsis*, five *NCED* genes (*AtNCED2*, *3*, *5*, *6*, and *9*) are involved in ABA biosynthesis in response to physiological changes. Of these five, *NCED3* is a key gene in ABA biosynthesis under water-deficit conditions. Transgenic plants overexpressing *NCED3* have greater drought tolerance compared with that of wild type, whereas an *NCED3* knockout mutant is more sensitive to drought stress. The last two biosynthetic reaction steps from xanthoxin occur in the cytosol. AtABA2 catalyzes xanthoxin conversion to abscisic aldehyde. ABA is synthesized by AAO3 oxidation of abscisic aldehyde. The *aao3* mutant shows a drought stress–sensitive phenotype similar to that of *nced3*. AtABA3 catalyzes molybdenum cofactor biosynthesis that is required for abscisic aldehyde oxidation. The *aba3* mutant displays an ABA-deficient phenotype.

ABA is catabolized to phaseic acid via hydroxylation at the 7′, 8′, or 9′ position. The *Arabidopsis* CYP707A family encodes a cytochrome p450 that catalyzes 8′-hydroxylation of ABA to produce 8′-hydroxy ABA (Figure 3). *Arabidopsis* has four *CYP707A* genes; *AtCYP707A3* mediates ABA degradation that occurs in response to rehydration after dehydration stress. Cytochrome p450 is localized in endoplasmic reticulum (ER), and this is the site of ABA hydroxylation. ABA glucosyl ester (ABA-GE) may be a storage form of ABA, which could be hydrolyzed to ABA under water-deficit conditions. The *AtBG1* gene product is a candidate for mediating this conversion, due to its ER localization and *in vitro* biochemical function. *AtBG2* is localized to vacuole, and it hydrolyzes ABA-GE to ABA *in vitro*. UDP-glucosyl transferase (*UGT71B6*) glucosylates ABA to ABA-GE *in vivo* and *in vitro*, and *UGT71B6* knockdown plants display an ABA-hypersensitive phenotype. *UGT71B6* is localized in the cytosol. The physiological roles of ABA homeostasis mediated by AtBG1, BG2, and UGT71B6 under water-deficit conditions have not been completely elucidated.

ABA transport is important for determining the ABA concentration at the site of action. The localization of AtNCED3, AtAAO3, and AtABA2 indicates that ABA is primarily synthesized in vascular tissues. To mediate stomatal closure, ABA is exported to the apoplastic area and then imported into guard cells under dehydration stress. Genetic screens strongly suggest that the ABC transporters AtABCG25 and AtABCG40 function as ABA transporters (Figure 3). AtABCG25 functions in ABA efflux from vascular cells, from where ABA would diffuse into apoplastic areas. AtABCG40 functions in ABA influx into guard cells and subsequent stomatal closure. The nitrate transporter NRT1.2 functions as an ABA-IMPORTING TRANSPORTER 1 (AtAIT1). A unique yeast two-hybrid system determined that the *AtAIT* gene product induced interactions between the ABA receptor PYR/PYL/RCAR and PP2C protein phosphatase under low ABA

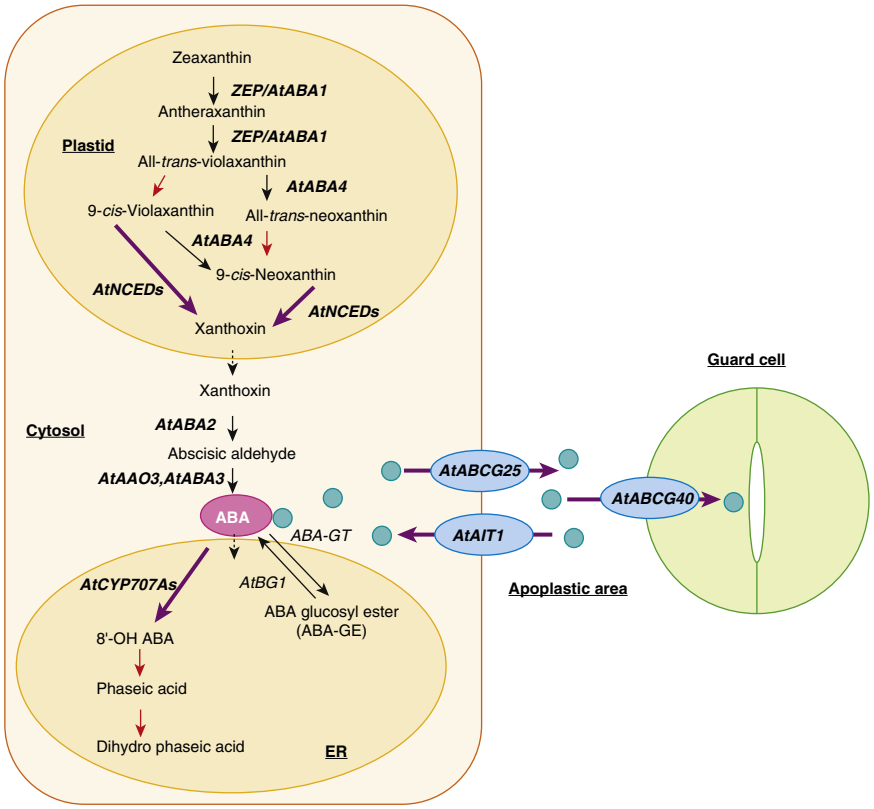

Figure 3 Abscisic acid (ABA) metabolism and transport pathways in *Arabidopsis*. ABA is synthesized in vascular tissues and transported to guard cells in response to dehydration stress. ABA is derived from plastid-synthesized carotenoid. Epoxycarotenoid cleavage to xanthoxin by 9-*cis*-epoxycarotenoid dioxygenase (NCED) is a key step in ABA biosynthesis. AtABCG25 exports ABA from vascular cells and AtABCG40 imports ABA into guard cells to facilitate stomatal closure. AtAIT1 functions as an ABA importer from the apoplastic area to the cytosol. ABA is catabolized to phaseic acid in the endoplasmic reticulum (ER) during rehydration after dehydration stress. CYP707A catalyzes the key step of ABA catabolism. ABA glucosyl ester (ABA-GE) is thought to be an ABA storage form that might be hydrolyzed to ABA under water-deficit conditions. *AtBG1* and ABA glucosyltransferase *UGT71B6* (ABA-GT) are candidate genes involved in ABA homeostasis. Identified genes encoding enzymes and transporters are indicated in italics, key steps are indicated by bold arrows, unidentified steps are indicated by red arrows, and ABA is indicated as cyan circles.

concentrations. AtABCG25 and AtABCG40 are thought to function in leaf stomatal closure, whereas AtAIT mainly functions in inflorescence stems.

Regulation of Drought-Responsive Gene Expression

Transcription factors are master regulators of gene expression. Here, we discuss recent crystal structure analyses, transcriptome analyses, and promoter analyses that have identified transcriptional mechanisms and representative transcriptional pathways activated under water-deficit conditions (Figure 4).

ABA triggers transcriptional activation under water-deficit conditions. In *Arabidopsis*, more than 70% of dehydration-inducible promoters contain ABREs (PyACGTGG/TC). ABRE is highly conserved in dehydration-inducible promoters of crops, including rice, soybean, and wheat. Dehydration- or ABA-inducible gene expression requires more than one ABRE, or an ABRE with a coupling element as a functional promoter (Figure 4). ABRE was first identified in the promoter sequences of wheat *Em* and rice *RAB*. Several bZIP factors that bind ABREs have been isolated from *Arabidopsis*, rice, tobacco, maize, and soybean. The *Arabidopsis* AREB/ABF family contains nine members; of these, *AREB1/ABF2*, *AREB2/ABF4*, and *ABF3* are induced by dehydration or exogenous ABA in vegetative tissues. ABA-mediated SnRK2-dependent phosphorylation of AREB/ABFs is required for transcriptional activation. Gain-of-function AREB/ABF mutants show enhanced dehydration stress tolerance, whereas the *areb1 areb2 abf3* triple mutant shows reduced dehydration stress tolerance compared with that of control plants. These effects are accompanied by functions of dehydration-inducible downstream targets of AREB/ABFs. Therefore, AREB/ABFs and ABRE-dependent gene expression likely plays a major role in ABA-dependent transcriptional regulation during dehydration stress (Figure 4).

The dehydration-responsive element/C-Repeat (DRE/CRT; A/GCCGAC) is a highly conserved motif in dehydration-inducible *Arabidopsis* promoters and functions in ABA-independent gene expression (Figure 4). Around 40% of dehydration-inducible *Arabidopsis* promoters contain single or multiple DRE/CRT sequences. DRE/CRT was first identified in the *Arabidopsis rd29A* promoter. DRE-binding protein (DREB) or CRT-binding factor (CBF), DREB1B/CBF1, DREB1A/CBF3, and DREB2A encode an ethylene-responsive element-binding factor/APETALA2 (ERF/AP2)-type transcription factor. Expression of three *DREB1/CBF* genes is induced by cold but

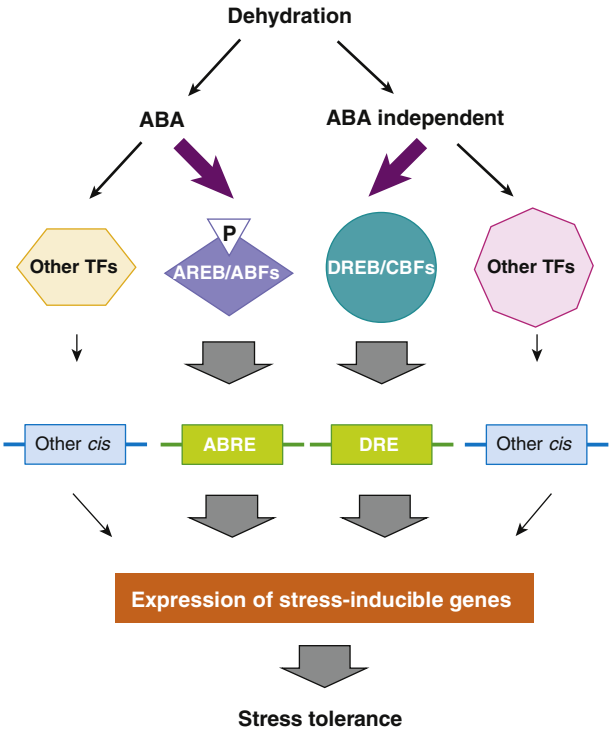

Figure 4 Transcriptional regulation by *cis*-acting elements under water-deficit conditions. ABA levels significantly increase in response to dehydration. Under dehydration conditions, AREB/ABFs–ABRE-dependent gene expression likely plays a major role in ABA-dependent transcriptional regulation. The DREB1/CBFs–DRE-dependent transcriptional pathway plays an important role in ABA-independent gene expression. ABA, abscisic acid; ABRE, ABA-responsive element; AREB/ABFs, ABRE-binding protein/ABRE-binding factors; DRE, dehydration-responsive element; DREB, DRE-binding protein; CBF, CRT-binding factor.

not by dehydration, whereas *DREB2A* is induced by dehydration, high salinity, and heat stress. DREB1/CBF and DREB2A proteins bind to DRE, but DREB1A/CBF3 and DREB2A have different DNA-binding specificities. DREB1A/CBF3 has highest affinity for A/GCCGACNT, whereas DREB2A preferentially binds ACCGAC. Transcriptional analyses using transgenic *Arabidopsis* overexpressing constitutively active DREB2A show that DREB2A-dependent downstream gene targets overlap with some drought-inducible genes. These results suggest that DREB2A−DRE-dependent transcriptional regulation has a specific role in *Arabidopsis* dehydration-inducible gene expression (Figure 4).

The CATGTG motif is involved in drought- and salinity-inducible gene expression. The CATGTG motif was identified in the dehydration-inducible promoter of *Arabidopsis ERD1*. The binding proteins with CATGTG motif in *ERD1* promoter, ANAC019, ANAC055, and RD26/ANAC072 encode NAM, ATAF, and CUC (NAC) transcription factors. There are more than 100 NAC transcription factors in *Arabidopsis*; the seven-member subfamily that includes ANAC019, ANAC055, and RD26/ANAC072 are induced by dehydration or exogenous ABA in vegetative tissues. Transgenic *Arabidopsis* overexpressing *RD26/ANAC072* is highly sensitive to ABA, whereas RD26 repression in transgenic *Arabidopsis* shows low ABA sensitivity. These results suggest the involvement of ABA in the NAC-CATGTG transcriptional pathway under dehydration stress. Other *cis*-acting elements [e.g., coupling elements1 (CE1), coupling elements3 (CE3), and motif III] involved in dehydration stress responses have been isolated. The *cis*-acting elements are not highly conserved in dehydration-inducible promoters. These results suggest that these *cis*-acting elements represent a specific pathway that functions in a dehydration stress response (Figure 4).

Epigenetic regulation as well as genetic regulation is thought to play some roles in gene networks under dehydration stress. Dehydration stress up- or downregulates transcriptional units from thousands of unannotated nonprotein-coding regions. There is a significant and linear correlation between the expression ratios of sense and antisense transcripts. Stress-responsive antisense transcripts have been identified on antisense strands of stress-responsive *RD29A* and *CYP707A1*. The biological functions of antisense transcripts have yet to be established. RNA-dependent RNA polymerase might be involved in the generation of antisense transcripts. The modulation of epigenetic status is important to alter gene expression in response to abiotic stresses. The trimethylation of histone H3 lysine 4 (H3K4me3) is enriched in coding regions of dehydration stress-responsive genes such as *RD29A*, *RD29B*, and *RD20* under dehydration stress. Atypical levels of H3K4me3 after recovery from dehydration stress suggest possible roles of H3K4me3 in plant stress memory.

Cellular Metabolites Involved in Drought Tolerance

Recent metabolite analysis methods have greater accuracy due to advances in mass spectrometry equipment. Using multiple instruments, it is possible to measure many different metabolites including carbohydrates, amino acids, organic acids, fatty acids, secondary metabolites, and phytohormones. Correlation analysis of metabolites can be performed by multivariate statistical techniques such as hierarchical clustering, principal component analysis, and self-organizing maps. Plant metabolites extensively quantified using multiple devices can be compared using multivariate statistical analysis.

Metabolites involved in water-deficit stress responses have been identified in plants. The levels of carbohydrates, oligosaccharides, amino acids, and polyamines are significantly higher in plants exposed to water-deficit conditions compared with those in control plants. The levels of several metabolites under water-deficit conditions correlate with expression levels of their biosynthetic genes. Genetic engineering strategies targeting metabolite accumulation for enhancing drought tolerance have succeeded in several plants (Table 1). Regulation of glycine betaine level is one of the most useful strategies for enhancing drought tolerance in plants (Table 1). Glycine betaine has the highest cellular osmoprotective efficiency under water-deficit conditions. Glycine betaine is accumulated in halophilic organisms, but not in most glycophytes.

Water-deficit conditions stimulate expression of several genes encoding enzymes involved in starch degradation and sucrose metabolism, and these changes correlate with accumulation of glucose, fructose, and sucrose. Transcript levels of genes encoding starch-degrading α-amylase, β-amylase, glucan-water

Table 1 Genetic engineering of metabolites for enhancing drought tolerance in plants

Metabolites	*Gene*	*Selected plants*	*Parameters*
Fructan	*BsSacB*	Sugar beat	Biomass production
Galactinol	*AtGolS2*	*Arabidopsis*	Survival rate
		Brachypodium	Survival rate
Glycine betaine	*ApCOX*	*Arabidopsis*	Plant growth
		Canola	Plant growth
		Tobacco	Plant growth
	AhGSMT + AhDMT	*Arabidopsis*	Plant growth
	MpGSMT + MpDMT	*Arabidopsis*	PSII activity, plant growth
Mannitol	*EcmtlD*	Wheat	Plant growth, biomass production
Ononitol	*McIMT1*	Tobacco	PSII activity
Polyamine	*CfSPDS*	*Arabidopsis*	Plant growth
Proline	*VaP5CS*	Rice	Plant growth
	AtP5CS	Petunia	Survival rate
	OsP5CS	Petunia	Survival rate
Trehalose	*ScTPS1*	Tobacco	Biomass production, survival rate
	EcOtsA, *EcOtsB*	Tobacco	Biomass production
	EcTPSP (OtsA + OtsB)	Rice	PSII activity, plant growth
	ScTPS1	Tomato	Plant growth

At, *Arabidopsis thaliana*; *Ah*, *Aphanothece halophytica*; *Ap*, *Arthrobacter pascens*; *Bs*, *Bacillus subtilis*; *Cf*, *Cucurbita ficifolia*; *Ec*, *Escherichia coli*; *Mc*, *Mesembryanthemum crystallinum*; *Mp*, *Methanohalophilus portucalensis*; *Os*, *Oryza sativa*; *Sc*, *Saccharomyces cerevisiae*, *Va*, *Vigna aconitifolia*.
COX, choline oxidase; DMT, dimethylglycine methyltransferase; GolS, galactinol synthase; GSMT, glycine sarcosine methyltransferase; IMT, myo-inositol O-methyltransferase; mtlD, mannitol-1-phospahate dehydrogenase; OtsA, TPS, trehalose-6-phosphate synthase; OtsB, TPP, trehalose-6-phosphate phosphatase; P5CS, Δ1-pyrroline-5-carboxylate synthase; SPDS, spermidine synthase.

dikinase, and phosphoglucan-water dikinase correlate with glucose and fructose accumulation. Gene transcript levels of alkaline/neutral invertase and sucrose synthetase correlate with sucrose accumulation. Galactinol and raffinose act as osmoprotectants that stabilize cellular membranes and scavenge reactive oxygen species to protect the chloroplastic photosynthetic complex during water-deficit conditions. The *galactinol synthase* transcript levels are significantly higher during water stress in *Arabidopsis*, tobacco, maize, cucumber, soybean, and rice. Galactinol synthase overexpression results in higher endogenous galactinol and raffinose levels and improved stress tolerance (Table 1). Therefore, galactinol synthase likely plays a key role in generating galactinol and raffinose under water-deficit conditions.

Proline accumulates in plants in response to dehydration, salinity, and cold. The level of proline accumulation correlates with stress tolerance. The expression of Δ1-pyrroline-5-carboxylate synthetase (P5CS) and proline dehydrogenase (ProDH) correlates with proline accumulation. P5CS overexpression results in higher proline levels and enhanced stress tolerance (Table 1), whereas P5CS knockout mutants have reduced proline levels and hypersensitivity to high-saline conditions. Expression of antisense ProDH in transgenic *Arabidopsis* results in higher proline levels and enhanced stress tolerance.

Polyamines (PAs) are small organic compounds and include putrescine, spermidine, and spermine. PA levels are significantly elevated under water-deficit conditions. There is some debate regarding the function of PAs under stress conditions. Higher PA levels under water-deficit conditions positively correlate with stress tolerance. The overexpression of *arginine decarboxylase* in transgenic *Arabidopsis* results in higher putrescine levels and enhanced stress tolerance, whereas knockout mutants have reduced putrescine levels and are hypersensitive to stress. Overexpression of *spermidine synthase* in transgenic *Arabidopsis* results in higher spermidine levels and enhanced tolerance to dehydration, cold, and high salinity (Table 1).

Enhancing Plant Performance under Drought Stress

The genetic improvement of drought stress resistance in crops is important for sustainable food and agriculture. Genetic modification (GM) and plant breeding are strategies for improving plant stress resistance. Gene transfer by GM is a powerful and efficient approach for generating drought-resistant crops. Several dehydration-inducible genes encoding enzymes, protective proteins, and transcription factors have been introduced in crops and evaluated in the greenhouse and field. The overexpression of transcription factors can effectively improve dehydration tolerance by activating many target genes. *AtDREB1A* has been used to generate genetically modified crops, although constitutive expression causes growth defects. Most traits associated with drought resistance have dual effects that are positive in very severe conditions and negative in milder or unstressed conditions. The use of conditional or tissue-specific promoters is a general alternative used to bypass the negative effects. Overexpression of *AtDREB1A* under control of the *Arabidopsis* stress-responsive *RD29A* promoter improves drought resistance of rice, wheat, peanut, and soybean. *AtDREB2A* functions as a master gene for transactivation of gene networks in response to dehydration and heat stress, which commonly occur together in field conditions caused by drought, high light, high temperature, and reduced evaporation due to stomatal closure. The overexpression of the active DREB2A form, *AtDREB2ACA*, under control of the *RD29A* promoter effectively enhances drought tolerance in several crops.

Plant breeding programs are focusing on ensuring food security under global warming and more frequent drought conditions. These programs seek to develop new varieties that are higher yielding and drought resistant. Rice is a major crop in Asia and Africa, but it is particularly susceptible to drought stress due to shallow-rooting architecture. Several drought-resistant QTLs have been reported in rice, but a QTL for a deep root system that enables water absorption from deep soil layers is essential to avoid water-deficit stress. The introduction of DEEPER ROOTING 1 (DRO1), a QTL controlling root growth angle, into a shallow-rooting rice cultivar improves water stress tolerance by increasing deep rooting and enables high-yield performance under water-deficit conditions. The regulation of root growth and root architecture under water-deficit conditions is a conserved process among several plant species; auxin

biosynthesis, transport, and signaling are important for this regulation. GM using drought-resistant QTL and dehydration-inducible genes can contribute to generate drought-tolerant crops.

Several commercial higher-yielding and drought-tolerant crops are available for field planting. A number of drought-tolerant maize varieties are available, such as Syngenta's Agrisure Artesian and Pioneer's Optimum AquaMax. In 2013, Monsanto launched the first drought-tolerant genetically modified maize called DroughtGard. It is a transgenic hybrid line expressing *CspB*, an RNA chaperone from the soil microbe *Bacillus subtilis*. *CspB* enhances plant adaptation to drought stress, improves WUE, and reduces yield loss from drought.

Plant scientists are discovering novel approaches to enhance drought tolerance traits in plants. As water resources become scarce and global grain demand continues to increase, plant research must identify new strategies to maintain crop yields. New breeding technologies such as zinc finger nucleases, transcription activator-like effector nucleases, and RNA-dependent DNA end-nuclease Cas9 of the clustered regularly interspaced short palindromic repeat system will achieve precise genome editing to improve grain yield and WUE in agriculturally important crops.

Phenotyping Systems for Monitoring Plant Growth under Mild Drought Stress

Inhibition of plant shoot growth is a physiological response to water-deficit conditions. This growth defect is caused by active arrest of cell cycle machinery and indirect effects of stomatal closure. Recent molecular analyses in *Arabidopsis* reveal that ethylene signaling and gibberellin biosynthesis deactivation contribute to growth inhibition by cell cycle arrest under mild drought stress (Figure 1(a)). Plants actively maintain shoot growth to balance development and survival under mild drought stress. Phenotyping systems can be developed to estimate plant growth under mild drought stress conditions. These phenotyping systems enable high-throughput and nondestructive estimations of plant growth under mild and severe drought stress. These systems incorporate irrigation systems that automatically weigh pots and adjust for water evaporation and control soil water content. These systems combine camera systems to monitor shoot biomass development, photosynthetic activity, and leaf temperature. These phenotyping systems accelerate QTL mapping analysis in natural variants of *Arabidopsis* and near isogenic lines of crops to identify important genes for drought resistance. In-depth phenotyping systems such as optical coherence microscopy, optical projection tomography, and high-resolution X-ray computed tomography (HRXCT) explore the microscopic phenotype and 3D structures under stress conditions. HRXCT is a useful tool for the visualization of the hydropatterning of root system architecture in dry soil. Magnetic resonance imaging quantifies water flow in xylem and phloem using the nuclear magnetic resonance of water protons. The 3D information provides novel insight into the relationship between temporospatial regulation of water status and physiological changes caused by water-deficit stress at the cellular level.

Plant phenotyping systems have not combined high-throughput phenotyping and in-depth analysis of microscopic traits because development of the image processing technologies began only recently. Continued development of advanced imaging technologies in different research areas will accelerate the development of plant phenomics and provide greater understanding of plant physiological traits.

Perspectives

Tissue-specific analyses of ABA metabolism and transport have identified the sites of ABA biosynthesis and action under water-deficit conditions in *Arabidopsis*. Recent work focuses on ABA dynamics at the single cell level using biosensors. Live cell metabolomics can be analyzed using mass spectrometry techniques. It will be important to develop methods to analyze drought stress–induced spatiotemporal regulation of small molecules such as RNAs, plant hormones, metabolites, and peptides. Plant phenotyping platforms have been developed to measure plant growth and water demand under water-deficit conditions. Future work will perform systematic analyses of complex physiological and molecular data from precise phenotyping platforms. Integrated transcriptome and phenotype analysis in agricultural fields will provide details on the interaction of drought avoidance and drought tolerance mechanisms. Statistical data analysis and modeling are necessary for understanding the complex relationship between genotype and phenotype.

Acknowledgments

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See also: **Physiology:** Basic Water Relations; Phloem; Stomata; Xylem. **Plants and the Environment:** Global Warming Effects. **Regulators of Growth:** Abscisic Acid.

Further Reading

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Free Radicals, Oxidative Stress and Antioxidants

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Introduction

Plants, as all aerobic organisms, are continuously exposed to reactive oxygen species (ROS) production. ROS include molecules such as hydrogen peroxide, ions such as the superoxide anion, or radicals such as the hydroxyl radical. However, not all ROS are free radicals. For instance, singlet oxygen, one of the most unstable ROS is not a free radical. These unstable and highly reactive molecules, which are summarized in Table 1, present a challenge to all plants. If left without control, ROS can cause oxidative injury by initiating chain reactions that disrupt membrane lipids, denature proteins, or damage DNA, ultimately leading to cell death. ROS production is more likely to occur in plants exposed to high light, extreme temperatures, or water deficit, particularly if exposure to these stressors occurs suddenly or if the plant is not adapted to them. Plants are, however, tolerant to several abiotic stresses. Every plant species has adapted to a particular habitat and among several mechanisms of adaptation, the development of an adequate antioxidant machinery is essential for survival, and as is discussed here particularly in plants usually exposed to excess light.

Abiotic Stresses Increase ROS Formation

Abiotic stressors lead to a series of physiological adaptations in plants (Table 2). The most frequent abiotic stresses that affect productivity both in agronomic and forestry systems are the following: water availability (drought), excess of incident radiation (high light), high ionic concentration (salinity), and extreme temperatures (both cold and heat stress). Such stressful conditions lead to suboptimal CO_2 assimilation rates and consequently light absorption can exceed the demand of reduced molecules (NADPH) and ATP that would be needed for carbon fixation in the Calvin cycle. Therefore, one of the most affected compartments during abiotic stress is the chloroplast, as extra energy in the electron transport chain can lead to photosystem II (PSII) photoinhibition followed by a sequence of harmful alterations (mainly oxidation) in the molecules that are in both membranes and the stroma of the chloroplast.

Table 1 Examples of free radicals and reactive oxygen species in plant cells

Name	*Formula*
Diatomic oxygen	O_2
Singlet oxygen	$^1O_2$
Superoxide	$O_2^{\cdot-}$
Hydroxyl	$OH^{\cdot}$
Hydrogen peroxide	H_2O_2

Plants use several strategies to mitigate the effects of excess light that would lead to such oxidation processes (Figure 1). The first strategies to minimize PSII photoinhibition are the movement of leaves, and the chloroplasts inside the mesophyll cells to avoid excess light. Plants have also evolved solar radiation screening mechanisms, including the accumulation of phenolic compounds or anthocyanins, which reduce the amount of specific wavelengths – such as UV and blue light – that will arrive to the chloroplast. Once the light reaches the

Table 2 Examples of primary reactive species and putative mechanisms for their overproduction in response to environmental, anthropogenic, and biotic stressors

Stressor	*ROS*	*Mechanism(s)*
High light	$O_2^{\cdot-}$, H_2O_2, $^1O_2$	Enhanced Mehler activity; photorespiration; triplet chlorophyll excitation
Heat	$O_2^{\cdot-}$, H_2O_2, $^1O_2$	Impairment of photosynthetic and mitochondrial electron transport; enzyme inhibition; increased membrane permeability
Cold	$O_2^{\cdot-}$, H_2O_2, $^1O_2$	Enhanced Mehler activity; suppression of Calvin cycle enzymes; reduced antioxidant activity; decreased membrane fluidity
UV radiation	$OH^{\cdot}$, $O_2^{\cdot-}$, H_2O_2, $^1O_2$	Inhibition of PSII reaction center enzymes; possibly fission of H_2O_2
Drought	$O_2^{\cdot-}$, H_2O_2, $^1O_2$	Inhibition of Rubisco; uncoupling of electron transport from ATP synthesis; enhanced Mehler activity; photoinhibition; inhibition of mitochondrial antioxidants; enhanced root respiration
Mechanical injury	$O_2^{\cdot-}$, H_2O_2, $^1O_2$	Elicitation by cell wall fragments; interference with redox systems on plasma membrane
Salinity	$O_2^{\cdot-}$, H_2O_2, $^1O_2$	Stomatal closure causing $NADP^+$ deficit and O_2 reduction in mitochondria
Pathogens	$O_2^{\cdot-}$, H_2O_2, $^1O_2$	Activation of membrane-bound NADPH oxidase or cell wall peroxidase
Herbicides	$O_2^{\cdot-}$, H_2O_2, $^1O_2$	Interference with photosynthetic electron transport; photoactivated herbicide interactions with O_2; inhibition of antioxidants
Heavy metals	$OH^{\cdot}$, $O_2^{\cdot-}$, H_2O_2, $^1O_2$	Direct uptake from contaminated soils; Haber–Weiss reactions; Fe-dependent photosensitization

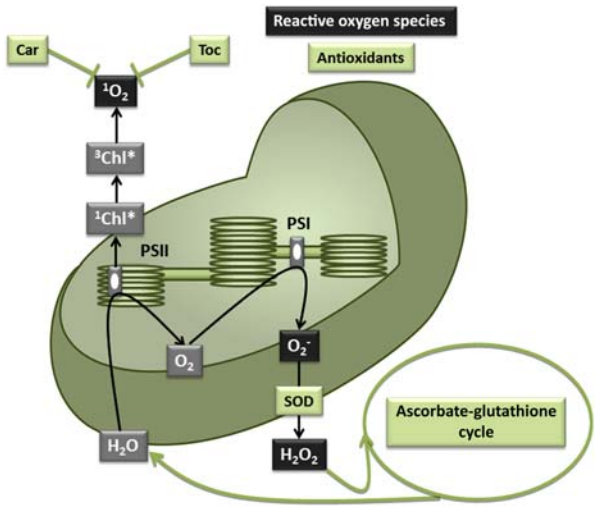

Figure 1 Production and elimination of reactive oxygen species (ROS) in chloroplasts. Toc, tocopherols; Car, carotenoids; Chl, chlorophyll; SOD, superoxide dismutase; PS, photosystem.

reaction centers in the photosystems, the accumulation of singlet excited chlorophyll *a* (^{1}Chl*) can be dissipated through nonphotochemical quenching (NPQ) by the xanthophyll cycle. However, if there is not enough deexcitation of ^{1}Chl* the generation of the excited triplet chlorophyll (^{3}Chl*) will be unavoidable. In PSII, ^{3}Chl* can pass excitation energy to molecular oxygen forming singlet oxygen ($^1O_2$), and in the electron transport chain, a higher pool of reduced ferredoxin in thylakoids will increase the chance of O_2 receiving electrons from PSI and become overreduced forming superoxide radicals (O_2^-). $^1O_2$ is highly reactive and can oxidize lipids as well as other molecules inside chloroplasts. O_2^- is rapidly converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD), but if not rapidly detoxified, H_2O_2 can give rise to the highly reactive hydroxyl radical ($OH^\bullet$).

The formation of all these ROS will start an oxidation chain that can affect several macromolecules (including lipids, proteins, and nucleic acids) both inside and outside the chloroplasts (hydrogen peroxide can move across membranes by diffusion), and will lead to the activation of oxidative signaling cascade mechanisms. This phenomenon is known as *photooxidation*, and it occurs very frequently when plants grow under stressful conditions as well as in leaves at advanced stages of ontogeny. ROS molecules have important roles on stress signaling in the affected plant cell. Here starts the bumpy road of oxidative stress.

Oxidative Stress Activates Antioxidant Protection

To control effects of photoinduced ROS production inside and outside the chloroplast, plant cells will activate photoprotection mechanisms. These work to avoid and tolerate the possible consequences of excess light by preventing ROS formation and scavenging the unavoidably formed ROS pool. In other words, antioxidants appear on the scene to protect from excessive oxidation of the whole plant cell and to maintain an adequate reduction/oxidation (redox) balance (Figure 2). Photooxidative stress is a transient or sustained production of ROS (not counterbalanced by antioxidant defenses) that will induce

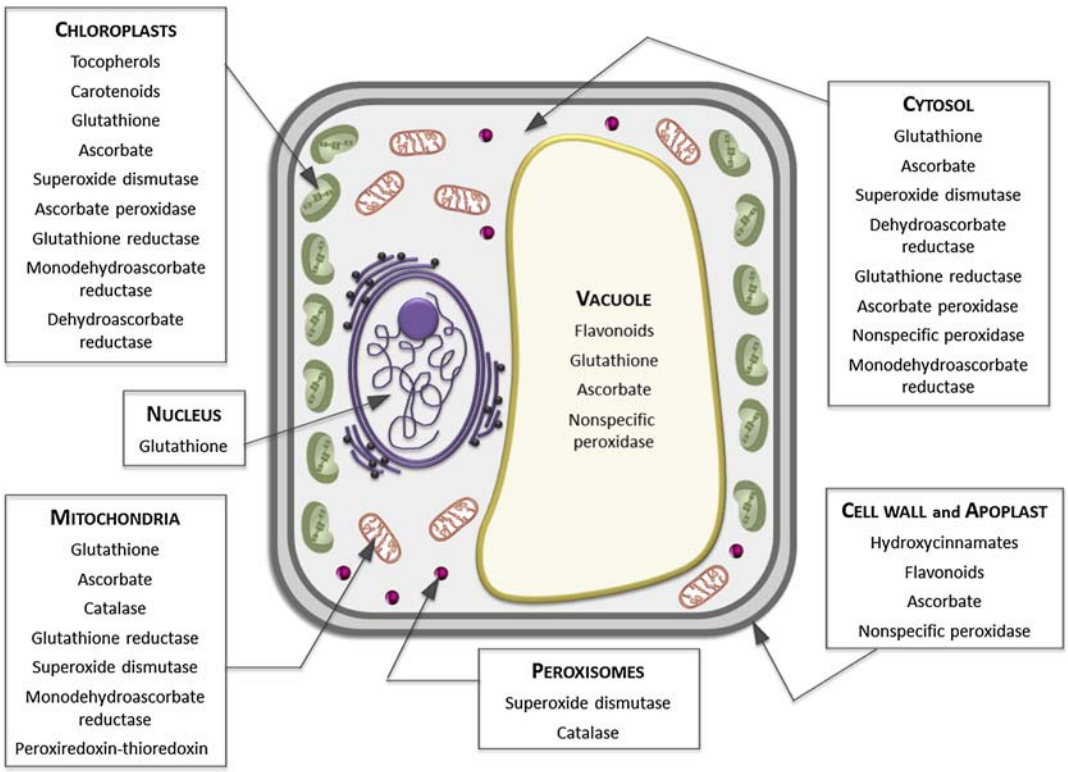

Figure 2 Localization of enzymatic and low molecular–weight antioxidants in plant cells.

photooxidation processes. If the oxidative stress is controlled by the endogenous antioxidant system, it will be temporary and will play a positive role activating a plethora of defense-related genes that will help with the defense and acclimation of the individual plant to the new environmental conditions.

Plants have developed several antioxidant systems that reduce ROS (Figure 1). A first step occurs close to the photosystem II (PSII), where ^{3}Chl* and $^1O_2$ are produced. Carotenoids direct one of the most important reactions at reaction centers (β-carotene) and light harvesting antenna complexes (PsbS proteins and xanthophylls) to allow a physical quenching of ^{1}Chl*, ^{3}Chl*, and $^1O_2$ by excitation transfer and harmless thermal dissipation. In the PSII reaction center, β-carotene additionally scavenges $^1O_2$ forming β-cyclocitral. Furthermore, tocopherols quench and scavenge $^1O_2$ in PSII and chloroplast membranes, while hydrophilic antioxidants (such as ascorbate and glutathione) together with enzymatic antioxidants (like ascorbate peroxidase and glutathione reductase) scavenge the H_2O_2 produced in PSI. When water photolysis in PSII is coupled to superoxide formation in PSI, and the latter is converted to H_2O_2 by superoxide dismutase and finally to water by the ascorbate–glutathione cycle, the so-called water–water cycle and excess energy dissipation occur. Xanthophyll cycle-dependent energy dissipation, $^1O_2$ formation and its subsequent elimination by carotenoids and tocopherols, and the water–water cycle represent three safety valves for excess energy dissipation in chloroplasts. Therefore, carotenoids, tocopherols, and the ascorbate–glutathione cycle are essential components of the antioxidant machinery in chloroplasts, which serve an essential function in the control of ROS production and excess energy dissipation.

However, if antioxidants are not produced at sufficient amounts and are therefore not able to reduce oxidative reactions, ROS will be produced at high concentrations and sustained in time. These molecules will overwhelm the antioxidant defense system and lead to irreversible damage to essential cellular components, from the photosynthetic apparatus to the nuclear gene expression machinery. ROS therefore play a dual role in the regulation of plant responses to oxidative stress. On the one hand, a transient ROS production is needed to trigger leaf antioxidant defenses. On the other hand, sustained ROS accumulation can lead to cell death. Consequently, tools to identify sustained ROS production and activation of the adequate antioxidant responses will undoubtedly help us to elucidate the degree of oxidative stress imposed by abiotic stresses in leaves.

Oxidative Stress Markers

Scientific technology has been developing interesting methodologies to estimate and quantify all the groups of photooxidative molecules, their quenchers/scavengers, and their targets. Using these tools, researchers can follow the extent of oxidative stress and the mechanisms evolved by plants to withstand abiotic stresses.

Reactive Oxygen Species

Most of the ROS present in the plant cell under abiotic stresses are formed in the chloroplast during photosynthesis. Detecting them under optimal conditions is extremely difficult because they are present at very low concentrations. This is because: (1) ROS are highly reactive with other molecules within a maximum of a few milliseconds (or even faster in the case of $^1O_2$ or $OH^{\bullet}$); (2) antioxidant systems are present in the chloroplast to quench and scavenge them very quickly; (3) ROS (most particularly H_2O_2) can diffuse outside the chloroplast becoming an important cellular signal. However, when abiotic stresses increase ROS concentration in the chloroplast, they can be quantified. H_2O_2, for example, is usually found at low concentrations in chloroplasts due to the scavenging of the ascorbate–glutathione cycle. However, if this cycle does not work properly or if there is a deficiency in ascorbate (vitamin C) or glutathione, H_2O_2 levels can rise rapidly. Although chloroplasts are one of the most important generators of ROS within the cells, other compartments such as peroxisomes, mitochondria, and the apoplast can also significantly contribute to overall cellular ROS formation within the cell. In peroxisomes, the glycolate oxidase (GOX) oxidizes glycolate, just arrived from the chloroplast, producing H_2O_2 molecules, which will break down by the catalase enzyme (CAT) ($2H_2O_2 \rightarrow 2H_2O + O_2$). At high photorespiration rates, peroxisomes show very high H_2O_2 levels. In the mitochondria, O_2^- is the main ROS as product of the respiration electron transport chain in complex I and complex III. As usual, the unstable O_2^- will be quickly transformed by the SOD to H_2O_2. Mitochondrial ascorbate–glutathione cycle or the peroxiredoxin–thioredoxin system will convert the H_2O_2 to H_2O. In the apoplast (the extracellular space), two different ROS are formed by extracellular peroxidases (H_2O_2) and by plasma membrane NADPH oxidases (O_2^-). Close to the cell wall, O_2^- can be transformed to H_2O_2 both spontaneously or by the SOD. Therefore, two of the main plant ROS to understand oxidation dynamics are $^1O_2$ and H_2O_2.

All techniques to quantify $^1O_2$ are destructive and comprise direct methods of quantification (1–3 below) or indirect methods which work by quantifying specific products generated due to $^1O_2$ oxidation (4–6 below). The key methods are: (1) spin and chemical trapping; (2) phosphorescence; (3) fluorescence imaged by confocal laser scanning microscopy; (4) quantification of β-carotenoid oxidation products by HPLC coupled to mass spectrometry (HPLC-MS/MS); (5) quantification of the tocopherol oxidation product (tocopherol quinone, TQ) by HPLC; and (6) quantification of the specifically $^1O_2$ oxidized PUFAs by HPLC-electrospray ionization-MS/MS.

Quantification of H_2O_2 in whole tissues is based on the oxidation of different substances to obtain products altered with spectral characteristic which are measured by light emission methods (such as fluorescence or luminescence) or light absorbance measurements. *In situ* H_2O_2 quantification can be performed with probes, detected by optical microscopy, or transmission electron microscopy, or fluorescent probes detected by confocal laser scanning microscopy.

Antioxidants

When researchers aim to quantify the antioxidant capacities of any plant tissue, they should keep in mind that the most important antioxidant systems present a reduced and an oxidized form, the so-called redox pair. Therefore, not only the total concentration but also the ratio between the

oxidized/reduced forms (oxidation state) will be necessary for a correct elucidation of the effects of abiotic stress in plants. Quantification of antioxidant molecules can be performed by either spectrophotometry or by HPLC, depending on the redox pair being investigated and the degree of confidence needed in the measurements.

Lipid Peroxidation

Furthermore, malondialdehyde (MDA) is one of the most widely measured nonenzymatically formed lipid peroxidation products (i.e., oxylipins) that researchers tend to correlate with oxidative stress, as it is easy to measure spectrophotometrically. Oxylipin quantification also includes gas or liquid chromatography coupled to mass spectrometry (HPLC-electrospray ionization-MS/MS). Oxidized lipids can also be detected by spontaneous ultraweak emitted autoluminescence or by thermoluminescence.

Conclusions and Perspectives

Abiotic stresses have become a central issue to government financed priorities. Agricultural crops and forestry, very important for sustainability, are dependent on appropriate environmental conditions to be successfully productive. Global climate change is changing, and most often reducing, the available resources worldwide by reducing precipitation (drought), causing more frequent extreme temperature events (freezing in winters and too hot in the mid-summer), or changing the ocean's limits (salinity), among others. Researchers have the role of trying to understand plant stress responses and transmit the acquired knowledge to stake holders, forest managers, and farmworkers. Here, we have provided some clues to better understand how plants respond to the changing environment and some of the mechanisms they have evolved to survive adverse climatic conditions. ROS production in chloroplasts is a means evolved by plants to dissipate excess energy, but at the same time it can lead to significant damage to plant cells and reduce productivity if the antioxidant machinery is not working properly. A delicate balance between ROS and antioxidants must therefore be finely regulated to ensure survival in plants. Oxidative stress markers are essential to monitor changes in this delicate balance to understand whether a transient ROS production is activating adequate responses in the antioxidant machinery, or by contrast a sustained ROS accumulation is inflicting damage and productivity loss.

See also: **Abiotic Stress:** Cold Stress; Drought Stress; Salt Stress. **Photosynthesis:** C_3 Plants; Photoinhibition; Photorespiration. **Plants and the Environment:** Global Warming Effects. **Secondary Products:** Anthocyanins.

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Mechanical Stress and Wind Damage

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Glossary

Compression wood Compression wood is the reaction wood produced in most conifer species when trees are tilted. Compression wood exhibits internal forces: it is in compression within the living tree. It is generally produced on the lower side of a tilted tree and it enables to right-up the tree by 'pushing' it up. Anatomically, it is characterized by round shape tracheids with a thicker cell wall than normal wood tracheids. The microfibril angle of the S2 layer of tracheids cell wall is higher than the one of normal wood.

Flexure wood The term flexure wood has been used to characterize a special wood found in trees that has been mechanically solicited. This wood is not yet well characterized but in hardwoods, it shares some features of tension wood.

Tension wood Tension wood is the reaction wood produced in most hardwood species. Tension wood exhibits internal forces: it is in tension within the leaving tree. In gravitropic and phototropic responses it is produced on one side of the axes and 'pulls' the axes to right up or to move toward light. In a high proportion of angiosperms the fibers of tension wood are characterized by the presence of a layer almost purely cellulosic, which replaces the S3 layer of the cell wall. The microfibril angle of the S2 layer of fibers cell wall is lower than the one of normal wood.

Introduction

It has been known since Darwin's work in 1894 that plants can sense mechanical signals. Some plant reactions to mechanical signals are spectacular like the rapid closure of the Venus fly trap, the folding of leaflets of the sensitive plant (*Mimosa pudica*) after touch, or the immediate curling of tendrils of climbing plants after they encounter a support. But other plants also react to mechanical signals by modifying their growth considerably in a less spectacular fashion. There was a resurgence of interest in this response to mechanical signals in the 1960s to 1970s, and it was named thigmomorphogenesis by Jaffe.

In nature, one of the main sources of mechanical signals is the wind. However, understanding and quantifying the effect of wind on plant growth is a difficult issue because wind induces mechanical signals due its multiple swaying of plant axes, but it can also modify gas exchange around the leaf. Several questions arise then immediately: does the wind modify plant growth because of a mechanical effect and/or by modifying the gas exchange around the leaves? If wind acts as a mechanical signal, do all wind sways lead to a growth response? The plant sways in the wind but what does it sense, perceive? What is the force of wind? What is the intensity of the wind sways? What is the frequency of wind sways? Does the plant experience some refractory periods during wind sways? Does the plant acclimate to repeated wind sways? This article is dedicated to offering some answers to these different questions. The first section presents the physiological changes induced by wind including growth. The second section is focused on knowledge of the effects of mechanical signals on plants in controlled conditions. The third section is dedicated to the perception of mechanical signals and transduction events after perception. The article ends with an overview of the damage caused by wind in relation to tree architecture, mechanosensing and cultural practices.

Mechanical Effect of Wind on Plant Physiology

This section presents the effect of wind on plants' (especially trees) physiology in terms of growth, differentiation of tissues, and gas exchange (respiration).

Mechanical Effect of Wind on Plant Growth and Shapes

The majority of the effects of wind on plants have been reported on trees. A well-known work on *Larix* demonstrated that trees prevented from wind sways were taller and thinner demonstrating that wind induces important quantitative changes in tree growth. Trees free to move in the wind exhibited a different taper (evolution of diameter with height) than protected trees: they were more conical (Figure 1).

More recently a study on wild cherry trees compared the growth (height and diameter) and biomasses (shoot/root ratio) of trees free to move in the wind, trees protected by individual plastic shelters and trees protected by individual shelters but regularly and artificially bent within the shelter. After 1 month of treatment, trees exposed to wind exhibited a lower height (−50%) and increased diameters (+30%) and a reduced shoot/root ratio in comparison with sheltered trees. The artificial bending leads to reduced height of sheltered trees, increased growth in girth and enabled a reallocation of part of the biomass toward the root (Figure 2). Concerning the effect of wind on root growth and architecture, the work of Stokes and collaborators on trees from which tap roots were removed showed that there was a 60% increase in the growth of roots on the windward

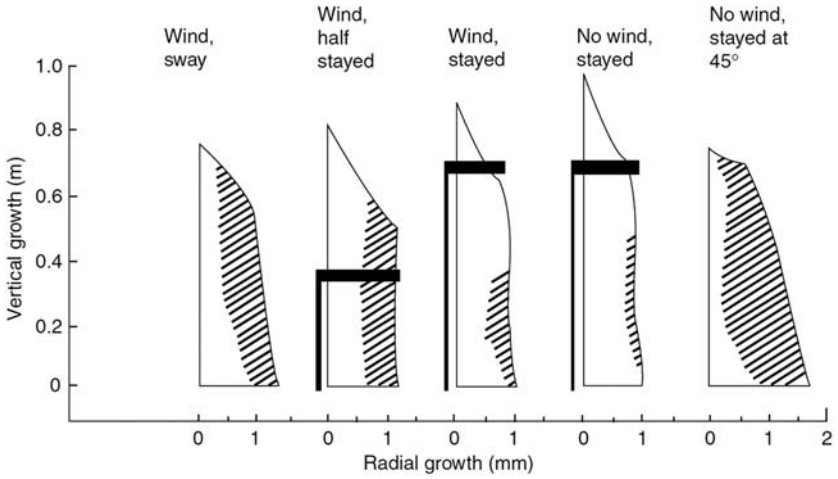

Figure 1 The influence of mechanical support on the distribution of wood increment in the stem of young trees (*Larix laricina*). The solid bars show how the plant was restrained using 'sways.' The shaded areas denote thick-walled (exceeding 5 μm) tracheids. Reproduced with permission from Larson, P.R., 1965. Stem form of young *Larix* as influenced by wind and pruning. Forest Sci. 11, 412–424.

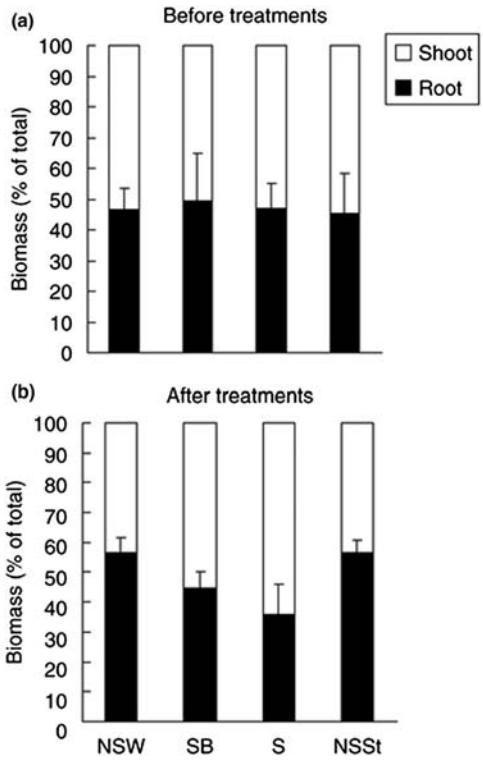

Figure 2 Biomass partitioning between shoots and roots before and after treatments, expressed as percentage of the total biomass of the tree. (a) Biomass partitioning at the date of planting: there was no significant differences in the biomass partitioning between the four sets of trees. On average, the shoot biomass represented 53% of the total biomass. (b) Biomass portioning after treatments at the end of August, by which there were differences between, SN, NSW, and NSSt. Artificial bending in the shelter induced an allocation of the biomass toward the roots. There was no difference between the NSW and NSSt treatments.

side and a 45% increase in the growth of roots on the leeward side, relative to growth at right angles to the prevailing wind.

Wind has thus an effect on growth in dimensions of tree axes (shoots and roots) which tends to make them more resistant to wind. In addition to this active control of axes dimensions, depending on the species, the tree crown can also geometrically reconfigurate within the wind flow (the tree crown shape changes and becomes more aerodynamical) so that it reduces the drag force, thus reducing the risk of failure. This reconfiguration can be transient or permanent: it is well known that in very windy environments with prevailing wind, the tree crown can take on a permanent flag shape (Figure 3). This reconfiguration of the tree crown shape is thought to be an ecological strategy to reduce the wind drag force and the area of the crown intercepted by the wind and so to reduce the breakage risk.

Figure 3 Photo of a *Pinus sylvestris* growing in prevailing wind. Reproduced with courtesy of Dr Catherine Lenne.

Mechanical Effect of Wind on Plant Tissues

The effect of wind on wood formation was also demonstrated in the experiments on *Larix*: trees not protected from wind-induced movements developed wood on one side of the trunk in which tracheids were thicker (Figure 1). However, in this study it was not detailed whether these tracheids were rounded (as seen in characteristic compression wood) or still rectangular. Compression wood is the reaction produced mainly by conifers and is known to be a response to gravity. In most deciduous trees reaction wood is called tension wood and in temperate species, it is characterized by the presence of an additional cell wall layer called the G layer. It is mainly composed of crystalline cellulose. The fact that wind-exposed trees differentiate a wood that is close to reaction wood raises questions about perception of mechanical signals. This is detailed later on in the third section of this article about perception.

After considering the effect of wind on growth, the next section provides information about gas exchange in order to check if wind has a mechanical effect or an effect mediated by modification of gas exchange around the leaves. The next section has been written by J Grace.

Effect of Wind on Respiration

The stomatal pores are distributed over the leaf surface, and for most plants they are usually open for all or part of the day and closed at night. It is through these stomata that carbon dioxide enters the leaves by diffusion from the atmosphere to the sites of photosynthesis. At the same time, water vapor inevitably diffuses from the substomatal cavities within the leaves to the outside, being driven by the difference in water vapor pressure between the inside and outside of the leaf. This vapor pressure difference is influenced by the humidity of the air and the thickness of the boundary layer surrounding the leaf. However, it is also influenced by the leaf temperature because the air in the substomatal cavity is water saturated, and the saturated vapor pressure of water in air is a steep function of temperature.

Early authors believed that one of the inevitable effects of wind was to increase the rate of transpiration. This belief would only be true if the leaf remained at a constant temperature, but it does not. In bright sunlight and light breezes, a leaf may be several degrees warmer than the air, but when wind speed is increased the leaf temperature is reduced to be closer to air temperature. This fall in leaf temperature makes the saturated vapor pressure of water inside the leaf also fall, and so the driving gradient for transpiration falls. Hence, the effect of wind speed on transpiration is rather complex, depending on factors that influence the thickness of the boundary layer (size of leaf and wind speed), and also on the energy balance of the leaf. In 1995, researchers carried out wind tunnel experiments and calculations based on the leaf energy balance, which showed that often the effect of moderate wind on transpiration is negligible, and sometimes wind actually decreases the transpiration rate. Here, the effect of wind on cooling the leaf may be substantial, especially in large leaves, so that the reduced vapor pressure of water inside the leaf causes a reduction in transpiration rate. There is also a possibility that an increase in wind speed may cause stomata to close, although the evidence for this phenomenon is rather slight.

The occasions where wind does cause water stress are those where there is the complication of mechanical abrasion of the cuticle. When the cuticle is damaged, the elevated transpiration continues after the wind has finished, and it may also continue at night (if there is a positive leaf–air gradient of water vapor pressure to drive it). The realization that wind does not necessarily increase transpiration rate (and sometimes decreases it) generally leads us to abandon the idea that the dwarfing of plants in windy places (especially mountains) is something to do with water stress. It exerts a notable impact on the temperature of the plant organ. In bright sunshine and light wind, an increase in the wind speed cools the leaf, bringing it closer to the air temperature. This effect can be large (for large leaves that have thick boundary layers) or small (for small leaves with small boundary layers). As we saw above, there are corresponding influences on the rate of evaporation, because the falling temperature reduces the saturation vapor pressure inside the leaf.

All these observations tend to suggest that the wind has a mechanical effect and not an effect on gas exchange except in the case of leaf abrasion. Now that the mechanical effect of wind has been demonstrated, the next section focuses on the knowledge acquired by studying the effect of mechanical signals in controlled conditions, from growth modifications to perception of mechanical signals.

Mechanical Effect of Mechanical Solicitations in Controlled Conditions

This section presents reports on plant responses in terms of growth and tissue differentiation and then focuses on the perception process of mechanical signals.

Effects on Plant Growth

In controlled conditions, it has been shown across a wide range of species that plants subjected to different kinds of mechanical solicitations (touching, brushing, shaking, and so on) exhibit a common growth response which is a reduction in growth in height and a stimulation of growth in girth that leads to a more compact aspect (reviewed by Biddington, 1986). This first demonstrated that mechanical solicitations have an impact on plant growth and second that the growth response after mechanical loading is a widespread phenomenon in plants. In order to quantify the mechanosensing phenomenon, studies of biomechanics were developed. Experiments were conducted on young tomato plants which were subjected to a single transitory bending applied in the basal part of stems and where elongation was continuously monitored at the top of the stem before and after the bending. The results showed a single transitory bending lead first to an arrest of elongation for an hour (very similar between tested plants) and then that elongation restarted but could remain far lower than the 'normal' growth (the elongation rate observed before bending) until it recovered the 'normal rate.' The recovery of control elongation rate was variable between plants (from 10 min to 10 h). Concerning growth in girth, young poplar stems were subjected to a single controlled bending, and growth in girth in the bent part was monitored continuously by a displacement

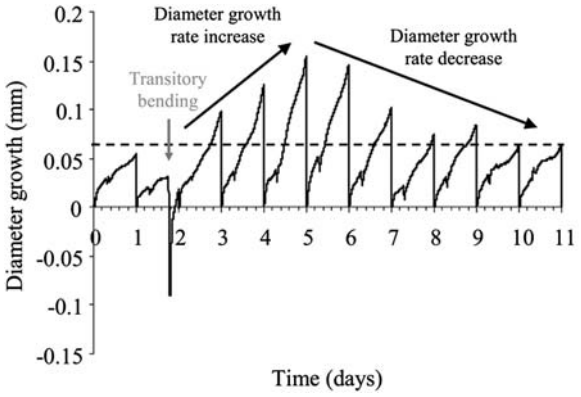

Figure 4 Representative example of the effect of a single transitory bending on stem diameter growth. Before the application of a transitory bending, the diameter growth rate was stable. The application of a transitory bending (gray arrow) led to an increase in diameter growth. The daily diameter growth rate increased for 3 days and then decreased for several days before returning to its prebending value.

sensor (LVDT). As in the case of elongation, the bending induced first a growth arrest but continues for several hours. Then the growth in girth increased for 2 or 3 days and then progressively returned to a 'normal' rate (Figure 4).

The effect of multiple bending was then studied: poplar plants were subjected to one bending treatment per day for 9 days and growth in girth was monitored. The results show that up to three bending treatments result in stimulation of girth growth, but after the third bending treatment, despite the stem being bent regularly the growth in girth returned to control values: hence a phenomenon of acclimation took place after the third bending treatment. The response of a plant is thus dependent on its mechanical history. The time necessary for a plant to recover its full sensitivity was studied by varying the time between two successive bendings. The sensitivity recovery was shown to be progressive and 7–10 days were necessary for the plant to recover its full sensitivity in terms of growth.

Effects on Plant Tissues

In work on *Abiès fraseri*, it was reported that flexed stems exhibited more xylem cells than unflexed stems and that the lumen of these cells was smaller. Furthermore, microfibril angle in these cells reached the less extreme values found in compression wood. The flexure wood also had a greater density than normal wood but flexure wood was different from compression wood even if it has more in common with compression wood than with normal wood. In deciduous species, changes in mechanical tissues have been reported for trees grown in windy conditions, but the anatomical structure of the wood has not been studied.

Mechanoperception and Transduction of Mechanical Signals

Mechanical signals can be brief or more long lasting. In this section, we will firstly detail the perception in the case of brief mechanical signals like transient bending for example and secondly the effect of long lasting mechanical signals due to gravity. Then, considering these different perceptions, we will return to the case of mechanoperception of a plant in the wind. But before moving on to our knowledge about perception of mechanical signals, a brief reminder of structure mechanics will be provided to introduce the notions of inclination, curvature, strains, and stresses that are necessary to understand the perception process.

A Brief Reminder on Structure Mechanics

As shown on (Figure 4), a stem can be tilted without being curved. In contrast a stem can be curved (and so tilted also) so that there is a gradient of inclination: the local inclination is not constant along the stem. When a homogenous symmetrical stem is bent, it curves; one side of the stem is in tension, the other side is in compression and the middle line is undeformed (it is called the neutral line). A stem can be seen as a pile of slices of thickness, dS. When the stem is curved under the effect of a lateral force (F), there is a bending moment and the slices rotate from each other of an angle dθ. The relative amount of rotation dθ/dS is called the curvature. Each little element of tissue of one slice S will expand on the tensed side and shrink on the compressed side. The product of the curvature of the slice S by the distance to the neutral axis is called the longitudinal strain. The product of the strain by the elastic modulus of the tissue is called the longitudinal stress.

Perception and Transduction of Transient Mechanical Signals

In this section, the focus is given on the perception of short mechanical signals (i.e., signals that are very brief from a time scale point of view). In the work on tomato plants, the plants were subjected to a controlled transient bending and elongation growth was monitored continuously. The strength of these assays was that all the mechanical variables describing the mechanical state of the bent stem were measured or could be calculated (force, displacement, curvature, bending moment, strains, and stress fields) with the aim of identifying the perceived variable. In these assays, the lateral displacement of the stem base was the same for all plants, but as stems were not all the same in diameter and in mechanical properties with the same displacement, the force applied was not the same between plants. A mechanical model was then built. In this model (1) each little piece of stem tissue perceives the mechanical variable to which it is submitted and generates a little signal, (2) these little signals sum up to generate the thigmomorphogenetical signal that leads to the growth response. Applying this model to the different types of variables revealed that there was no correlation between the duration of the growth response and the force or stresses, but there was a very significant relationship (of log form) between the duration of the growth response and the sum of strains. This demonstrated that the perceived variable was not the force (or stresses) but the strains. The hypotheses of the model were validated by studying two local responses: the growth in girth and the expression of a mechanosensitive gene (see perception section).

Sensors of mechanical signals are being intensively studied (Figure 5), several structures have been discovered, some of them are mechanosensitive channels located in the plasma membrane (MSL, SAC) directly activated by tension of the plasma membrane, others are structures that link the cell wall, the plasma membrane and the cytosol (WAK, THE1, lectin-like RLK).

The transduction phase is far from being understood. One of the first events detected after plants are submitted to mechanical signals is the movement of Ca^{2+} ions. This has been discovered thanks to plants transformed with a protein that becomes fluorescent when linked to Ca^{2+} ions (aequorin): a few seconds after the application of a mechanical signal, plants become fluorescent. Concerning genes, studies revealed the existence of *TOUCH* genes whose expression is regulated by mechanical signals. A recent study from Lee and coworkers revealed that in *Arabidopsis* more than 2.5% of the *Arabidopsis* transcriptome is modified after a single touch. More recently another gene has been discovered: it corresponds to a transcription factor and is called *Jr-ZFP2* in walnut and *Pta-ZFP2* in poplar. This gene is overexpressed in response to different stresses but its level of expression is far greater in the case of mechanical signals, which makes it a good marker of mechanoperception. Interestingly, the level of expression of this gene is closely linked to the level of strain applied to the plant and its level of expression was also regulated by multiple bending: its level of expression decreased very strongly as soon as the second bending was applied and only recovered its sensitivity to successive bending after about 3–4 days.

Perception and Transduction of Gravity

Gravity is a force that is exerted on all structures on Earth. Plants are able to sense that they are tilted in the field of gravity and to respond to this signal by modifying their shapes. Until very recently the perception of gravity was thought to be a perception of inclination in the field of gravity. Very recent studies have demonstrated, however, that the perception of gravity is not

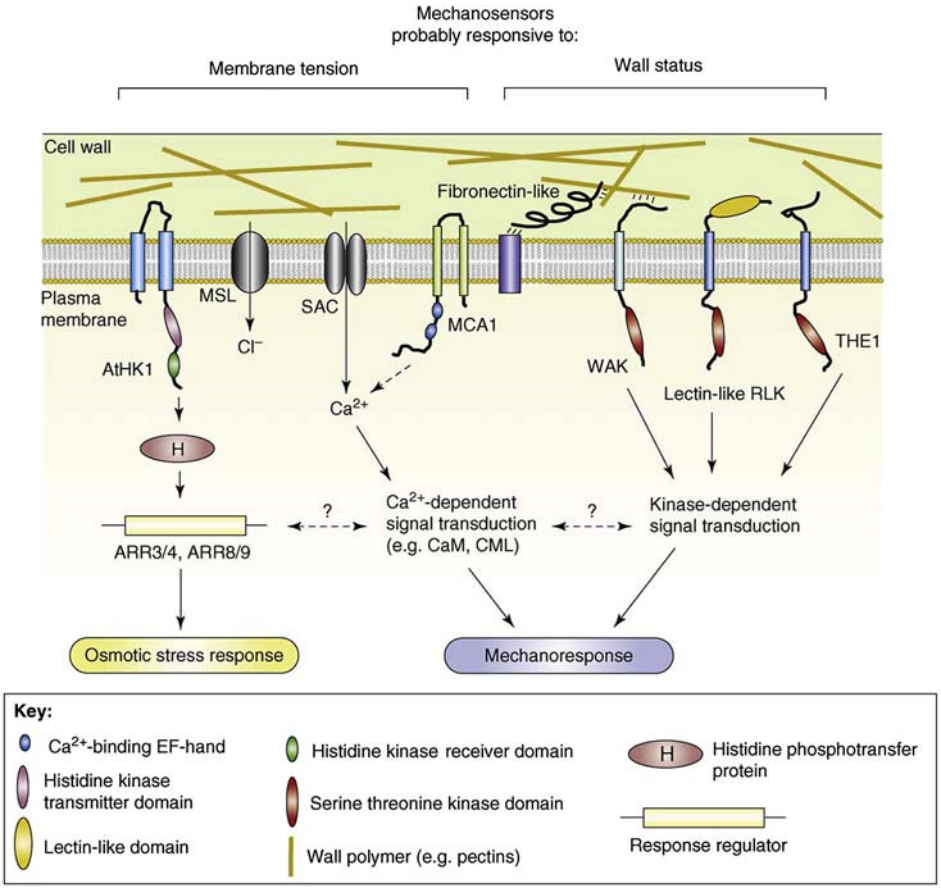

Figure 5 Action of possible mechanoresponsive elements. Plant mechanosensors probably fall into two broad classes: those activated by tension in the membrane, as exemplified by the MSL family of Cl^--permeable channels, and those monitoring wall status and/or sheer between the wall and the plasma membrane. SAC represents the stretch (activated Ca^{2+}-permeable conductance identified through electrophysiology but yet to be cloned. AtHK1 operated in an osmosensing pathway that potentially functions through a phosphorelay cascade that used the antagonistic response regulators ARR3/4 and ARR8/9. The RLKs such as WAKs, THE1, and the lectin domain containing RLK provide models for how a wall sensor might operate. They are likely to elicit protein-kinase-dependent signals that could relay mechanical information directly to mechanoresponsive gene expression for example, or interact with the Ca^{2+}-dependent signaling cascade that, to date, remains the best characterized mechanically induced signal transduction event in plants. Reproduced with courtesy of ASPB as copyright holder.

solely through the perception of inclination but also by the perception of curvature or more precisely associated strains. Gravity sensors are still not fully understood, but the identification of the perceived variables indicates that there are at least two different sensors: one can hypothesize that strains associated to curvature are perceived by the same or similar structures as those involved in short mechanical signals. Concerning the perception of inclination the most accepted hypothesis concerning the sensors are the sedimentation of statoliths (starch grains) within specialized cells called statocysts.

The transduction events from gravity perception to plant movements are also not fully identified but one of the first events after perception is the movement of calcium ions. As found by Monshausen and coworkers, it should be noted that this ion displacement is not the same as that elicited by short mechanical signals: in response to gravity they exhibit a shoulder shape whereas brief mechanical signals induce a transient sharp peak. Concerning genes involved specifically in gravity sensing, some have been identified and seem to be specific to the gravity signal (reviewed by Molas and Kiss, 2009), whereas others are also implicated in the transduction pathway of other signals. But the novel molecular biology techniques ('omics') should enable us to identify the missing links in the future.

One other important feature is that earlier studies revealed that the presentation time in the case of short mechanical signals is very short (in the order of 1 min to detect a change in elongation and in the order of a second to detect calcium ion movements), whereas the presentation time to detect a gravitropic response (movement) is about 20 min. If the response considered is the movement of calcium ions, the presentation time falls to 41 s but remains longer than the presentation time for ionic movements in the case of brief mechanical signals.

Perception of Mechanical Signals Induced by Wind

Wind is characterized by a large range in intensity and frequency and triggers a wide range of plant movements: from very brief to long-lasting deformations of plant axes in the case of continuous wind, and from small to very large displacements of plant axes depending on wind velocity and plant architecture and rigidity. Wind can thus trigger a wide range of mechanical signals including signals related to what we called short mechanical signals and to long-lasting mechanical signals that lead to the inclination of plant axes in the gravity field for a certain time. Thus, it may be hypothesized that the two types of signals are generated in the wind. This could explain why in some cases, wind-induced plant responses resemble more the responses described in controlled conditions in the case of short mechanical signals and, in some cases, resemble more (or at least encompass) some characteristics of responses induced by perception of gravity. A unified hypothesis of mechanosensing has been proposed.

Damage Induced by Wind in Relation to Mechanosensing and Tree Architecture

Although mechanosensing and thigmomorphogenesis can be seen as ways for plants to scale their axes to resist wind sometimes, when a wind becomes stormy, large-scale mechanical damage takes place, involving uprooting and breakage of mature trees. This is an enormous problem in Silviculture which will probably be amplified by climate changes as explained by Gardiner and colleagues. Interestingly, in most cases, trees inside a stand can be blown down but the trees at the border of the stand (Figure 6) remain standing. It can be hypothesized that trees in the border did not fall down because they are already acclimated to wind whereas trees from the center of the stand never experienced wind effects. Research on tree acclimation to wind is currently being developed. In addition, an important research effort is underway to decipher the optimal stand design to minimize the risks of windthrow. In particular, there is a focus on planting pattern to avoid thinning the forest at a later stage, and planting densities are often smaller than in the past. Research is currently being conducted in order to check the influence of planting densities on tree movements under 'regular' winds within the stands and their consequences on tree growth, trunk tapering by mechanosensing and trees' acclimation to 'regular' winds.

To protect individual trees, staking helps to maintain trees vertical but as we have seen staking can also reduce root growth as well as the radial growth of the trunk. The techniques have evolved and nowadays it is common to see that stakes have been redesigned: trees are maintained vertical by means of

Figure 6 Photo of a stand after storm of December 1999, at Chapelle au bois (Vosges region, France).

three or four wood poles linked to the trunk by elastic belts which not only keeps the tree vertical but also enables some movement of the trunk ensuring sufficient growth of roots and radial growth of the trunk. This solution works well for most conditions but not all: in very windy areas, a stake tightly attached to the tree is necessary to avoid the movement of the collar of the tree to maintain the soil in contact with the roots. So staking must be used with extreme care, and wind conditions in the area must be taken into account to make the right choice of staking.

Finally, it should be noted that some studies have been conducted with the aim of using mechanical signals as an innovative means to control and modify plant growth and architecture in horticulture. Recent work demonstrated that mechanical treatments also reduce plants' sensitivity to bioagressors. Probably, these questions will reappear soon in the context of innovative agroecological practices and sustainable agriculture.

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Oxidative Stress

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Introduction

Global climate change will have significant influences on crop productivity over the course of the current century. Increased atmospheric concentrations of CO_2 and other greenhouse gases are predicted to result in a mean average temperature increase ranging from 1.8 to 4 °C between 2000 and 2100. Changes in global and regional temperatures, combined with changes in land use and water demand will have significant impacts on the hydrological cycle. Changing rainfall patterns are likely to exacerbate both drought and flooding with summer Asian monsoon rainfall expected to increase while many parts of Africa are expected to become drier. At the same time, groundwater stores which represent over 40% of the water used for irrigation are being depleted globally. In coastal regions, sea level rise will increase the risk of flooding of agricultural land and salinization of coastal aquifers. While the increased atmospheric CO_2 concentration is expected to benefit plant productivity in the short term, emissions of other atmospheric pollutants are likely to have a harmful effect. For example, ground-level ozone, which is currently present at concentrations of approximately 40 ppb and causes 5–15% yield loss in sensitive agricultural crops, is anticipated to rise up to 70 ppb by 2100 as a result of the photochemical degradation of nitric oxides released from the burning of fossil fuels. In the absence of mitigation, all of these environmental changes are expected to have adverse impacts on agricultural production by increasing oxidative stress in crop plants. It is estimated that in the absence of mitigating circumstances, adverse environmental conditions occurring as a result of global change will lead to significant falls in crop production in many of the primary global crop-producing areas. At the same time, global population is expected to peak at over 9 billion people by 2050 requiring an estimated increase in food availability of between 70% and 100% as people demand an increasingly varied diet containing more animal protein.

The requirement to produce more food in the face of a more extreme and changeable environment has been described as the biggest challenge facing humanity this century. It has been recognized that the challenge will require a multifaceted approach that addresses demand and supply as well as reducing waste and improving governance. On the supply side, a combination of approaches and technologies will be required to allow sustainable intensification of crop production in different environments. GM technology represents one of the methods that could have significant benefits, and, within this context, genetic modification for the alleviation of oxidative stress has the potential to maintain food output. Here I will outline the primary sources of reactive oxygen species (ROS) production in plants and present an overview of the concepts of redox signaling and oxidative stress. I describe how GM technology has been used within an experimental context to improve our understanding of redox signaling and will outline the potential for the engineering of crops with enhanced tolerance to oxidative stress to address food security in a more extreme and changeable global environment.

Sources of Reactive Oxygen in Plants

Terrestrial plants live in an oxygen-rich atmosphere that arose as a result of the evolution of oxygenic photosynthesis approximately 2.5 billion years ago. This evolutionary milestone had an enormous impact on the development of terrestrial ecosystems allowing organisms to take advantage of the O_2/H_2O redox couple providing huge increases in energy yields compared with nonoxygenic pathways of respiration. Successful utilization of this high energy redox couple requires the simultaneous transfer of four electrons ($O_2 + 4H^+ + 4e^- \leftrightarrow 2H_2O$) and hence oxygen-utilizing organisms have evolved sophisticated electron transport mechanisms both to allow the tetravalent transfer of electrons and for the utilization of the electrochemical energy generated. Despite at least 2.5 billion years of evolution, both the respiratory electron transport (RET) and photosynthetic electron transport (PET) chains contain sites in which electrons are transferred univalently to oxygen generating in the first instance superoxide ($O_2^{\cdot -}$), a short-lived but highly reactive species containing unpaired electrons that is rapidly converted to the longer lived hydrogen peroxide (H_2O_2) via a spontaneous dismutation reaction. Univalent electron transfer occurs under all metabolic conditions, and hence a significant source of ROS in leaves are the PET and RET chains which in the light account for the estimated generation of approximately 4000 and 200 nmol m^{-2} s^{-1} H_2O_2, respectively, under nonstress conditions. In C3 plants, peroxisomes also contribute an estimated 10 000 nmol H_2O_2 m^{-2} s^{-1} in illuminated leaves as a result of their function in the photorespiratory pathway (Figure 1).

As the most significant sources of reactive oxygen generation within photosynthetic organisms are the core metabolic functions of photosynthesis and respiration, the capacity to sense ROS and cellular redox balance represents a mechanism for optimizing metabolism to the prevailing environment. The signaling function of ROS has evolved into a complex system that interacts with other signaling pathways such as those involving hormones, calcium, and kinases. Emerging research suggests that the signaling function requires the capacity to spatially and temporally recognize specific ROS or ROS signatures within the cell, and plants have subsequently evolved a range of enzyme systems that generate reactive oxygen either for their signaling function or in response to developmental or environmental cues. Examples of such enzymes include class III peroxidases, oxalate oxidases, amine oxygenases, lipoxygenases, quinone reductases, and NADPH oxidases, otherwise known as respiratory burst oxidase homologs. As the activities of these enzymes are tightly controlled, it is unlikely that they contribute significantly to the induction of oxidative stress in

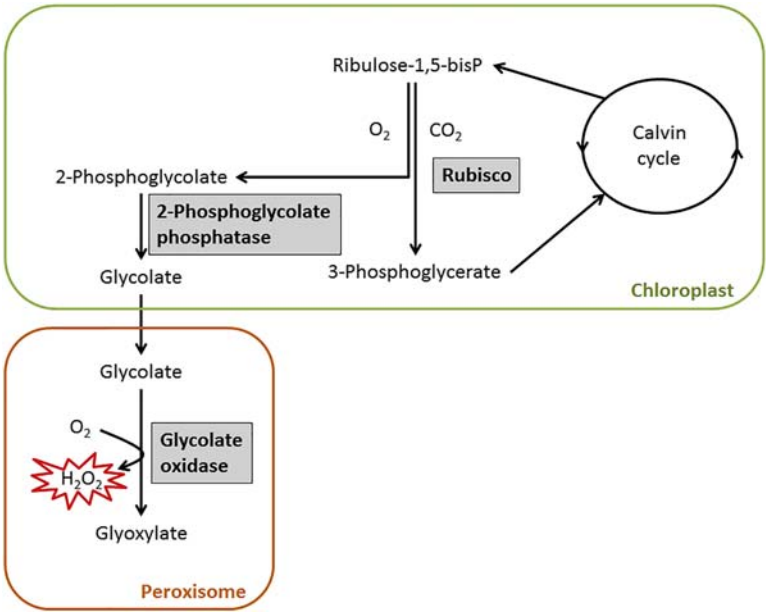

Figure 1 Photorespiratory generation of H_2O_2. Ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) has the capacity to fix CO_2 via carboxylation to generate 3-phosphoglycerate that enters the Calvin cycle from where the carbon can be diverted to a broad range of primary and secondary metabolic pathways. The oxygenation reaction generates 3-phosphoglycerate and toxic 2-phosphoglycolate that is dephosphorylated and transported to the peroxisome. Here, glycolate oxidase produces glyoxylate in a reaction that generates large quantities of H_2O_2.

plants; however, through their signaling function they can have profound effects on plant growth, development, and responses to biotic and abiotic stresses.

Plant Antioxidant Systems

As ROS production is an inevitable consequence of plant metabolism, they have developed a range of systems to prevent ROS accumulation, to repair ROS-induced damage and to neutralize ROS via conversion into less reactive compounds. All of these processes could be considered parts of the plant antioxidant system and almost all of these systems have been manipulated using GM approaches.

Plants contain a series of enzymatic and nonenzymatic antioxidants that neutralize ROS primarily by their reduction to less-reactive compounds (Table 1). Significant quantities of superoxide are produced by univalent electron transfer to oxygen at several sites in both the PET and RET chains. Although this radical is short-lived (<1 ms) and does not chemically modify biological macromolecules, it is a progenitor of highly reactive hydroxyl radicals through its interaction with iron and copper centers present in a range of redox active enzymes and additionally generates reactive nitrogen species through reaction with nitric oxide. In order to rapidly and efficiently remove superoxide, plants contain superoxide dismutases (SODs) that catalyze the dismutation of $O_2^{\cdot -}$ to H_2O_2 and oxygen. Several isoforms exist in plants using either a manganese (MnSOD), iron (FeSOD), or copper/zinc (Cu/ZnSOD) cofactor and they are found in the chloroplasts, mitochondria, peroxisomes, and the cytosol although their antioxidant function in the cytosol may be redundant as loss of activity of the major cytosolic FeSOD in *Arabidopsis* had little impact on plant resistance to oxidative stress.

$O_2^{\cdot -}$ is rapidly converted to H_2O_2 in plant tissues which can accumulate at concentrations up to 5 μmol gFW^{-1} in leaves. Plants have numerous systems for the removal of H_2O_2 operating across a range of subcellular compartments. All plant tissues studied to date have high catalase activity catalyzing the dismutation of H_2O_2 to O_2 and H_2O. Catalases exhibit a low affinity for H_2O_2 with K_M values estimated in the range 40–400 mM; however, they exhibit rapid enzymatic turnover with a single catalase protein capable of conversion of 6 million molecules of H_2O_2 to O_2 per minute. High catalase activity has been detected in peroxisomes and while activity has been detected in other cellular organelles and in the cytosol, there is still debate concerning whether measured activity is an artifact of cellular fractionation protocols.

Peroxiredoxins, glutaredoxins, and thioredoxins (Table 1) are a group of enzymes that form an antioxidant network based on thiol/disulfide exchange reactions in active cysteine residues. They are found in almost all cell compartments including the apoplast. In addition to direct removal of H_2O_2 by the peroxiredoxins, they perform important functions in the sensing of ROS and the control of metabolic activity within the context of cellular redox status. Their antioxidant functions have been most extensively studied in the chloroplasts and mitochondria. In the chloroplasts, 2-cys peroxiredoxins reduce H_2O_2 to water with the subsequent oxidation of the active site–cysteine residue and formation of a disulphide bridge between two enzyme subunits. This disulfide bridge is subsequently reduced by thioredoxins which in turn become oxidized and are themselves regenerated by electrons donated from the electron transport chain either via ferredoxin–thioredoxin reductase or NADPH thioredoxin reductase. Although less well characterized, a similar system is present in plant mitochondria where in *Arabidopsis*, a single type II peroxiredoxin (Prx IIF) is targeted

Table 1 Major antioxidant components of plant cells

Component	*Cellular location*	*Antioxidant functions*
Enzymatic antioxidants		
Superoxide dismutase	Chloroplasts, mitochondria, peroxisomes, cytosol	Catalyzes the reaction $2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$
Catalase	Peroxisomes, mitochondria[a], cytosol[a]	Catalyzes the reaction $2H_2O_2 \rightarrow 2H_2O + O_2$
Ascorbate peroxidase	Chloroplasts, mitochondria, peroxisomes, cytosol	Catalyzes the reaction $H_2O_2 + 2AsA \rightarrow 2H_2O + 2MDHA$
Monodehydroascorbate reductase	Chloroplasts, mitochondria, cytosol	Catalyzes the reaction 2MDHA + NAD(P)H → 2AsA + NAD(P)
Dehydroascorbate reductase	Chloroplasts, mitochondria, cytosol	Catalyzes the reaction DHA + 2GSH → AsA + GSSG
Glutathione reductase	Chloroplasts, mitochondria, cytosol	Catalyzes the reaction GSSG + NAD(P)H → 2GSH + NAD(P)
Peroxiredoxins	Chloroplasts, mitochondria, nucleus, cytosol	Reduce peroxides with the concomitant oxidation of active site–cysteine residues
Glutaredoxins	Chloroplasts, mitochondria, cytosol, apoplast	Glutathione-dependent reduction of oxidized cysteine residues in target proteins
Thioredoxins	Chloroplasts, mitochondria, nucleus, cytosol, apoplast	Ferredoxin/NADPH-dependent reduction of oxidized cysteine residues in target proteins
Nonenzymatic antioxidants		
Ascorbate	Soluble antioxidant found in all cellular compartments	Ascorbate–glutathione cycle, direct scavenging of $O_2^{\cdot -}$, $OH^{\cdot}$, regeneration of tocopheroxyl radical
Glutathione	Soluble antioxidant found in all cellular compartments	Ascorbate–glutathione cycle, reduction of oxidized protein cysteine residues
Carotenoids	Lipid soluble antioxidants located in plastids	Scavenging of $^1O_2$
Tocopherols	Lipid soluble antioxidants located in plastids, vacuoles, nuclei, oil bodies	Scavenging of $^1O_2$, quenching lipid peroxides

[a]Evidence for mitochondrial and cytosolic catalase localization remains uncertain.

to the organelle. *In vitro* studies have demonstrated that the oxidized form of the enzyme can be reduced directly by glutathione, by an NADPH thioredoxin reductase-dependent thioredoxin or by glutathione-dependent glutaredoxin (Figure 2).

A key antioxidant system is the ascorbate–glutathione (AsA-GSH) cycle that couples the reducing power of NAD(P)H to H_2O_2 reduction via the major soluble redox couples ascorbate and glutathione, and the enzymes ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Table 1; Figure 3). In comparison with catalase, APX has a much higher affinity for H_2O_2

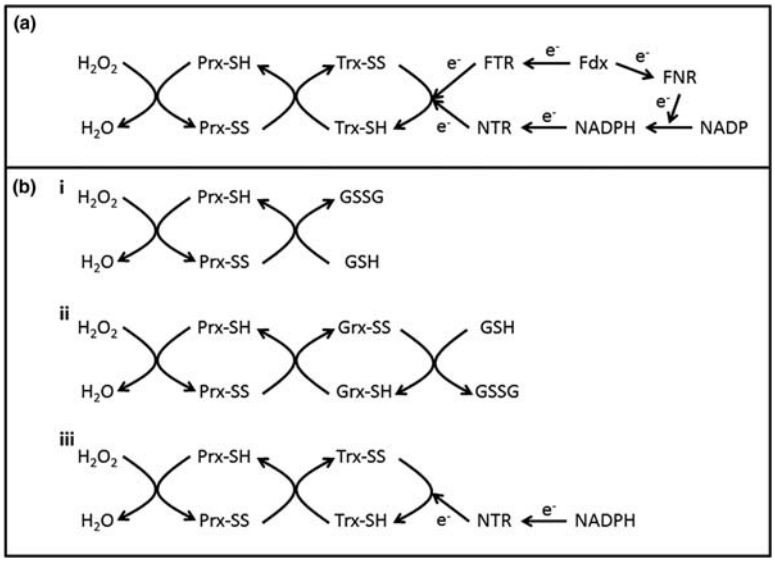

Figure 2 Thiol/disulphide-dependent pathways of ROS removal in chloroplasts and mitochondria. Peroxiredoxins (Prx) reduce H_2O_2 to water with the concomitant oxidation of the active site serine residue. In chloroplasts (a), oxidized Prx are regenerated by thioredoxins (Trx) which in turn are regenerated by electrons donated from the electron transport chain via ferredoxin (Fdx), catalyzed by ferredoxin–thioredoxin reductase (FTR) or NADPH thioredoxin reductase (NTR) that uses ferredoxin-NADPH reductase (FNR) to generate reducing power from the electron transport chain. In mitochondria (b), three potential pathways of Prx regeneration have been proposed from *in vitro* experiments. Directly via glutathione (GSH)-driven reduction (i), via glutaredoxin (Grx)-dependent regeneration (ii), or by thioredoxin-dependent regeneration using the reducing power of NADPH to regenerate Trx via the activity of NTR (iii).

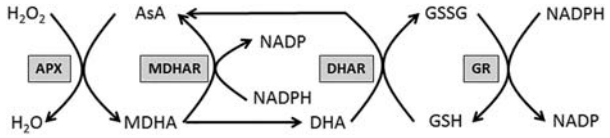

Figure 3 The ascorbate–glutathione cycle. H_2O_2 is reduced to water by the activity of ascorbate peroxidase (APX) using ascorbate (AsA) as a cofactor. Monodehydroascorbate (MDHA) can be reduced back to AsA through the activity of MDHA reductase (MDHAR) using NADPH to provide the reducing power. Alternatively, two molecules of MDHA disproportionate to dehydroascorbic acid (DHA) and AsA. The latter is reduced using reduced glutathione (GSH) as reductant in a reaction catalyzed by DHA reductase (DHAR). Oxidized glutathione (GSSG) is regenerated by glutathione reductase (GR) using NADPH as reductant.

with K_M in the μM rather than mM range, it is therefore considered to play an essential role in removal of this compound during stress. Higher plants have a number of genes encoding enzymes of the AsA-GSH cycle with nine genes encoding APX, five encoding MDHAR, four encoding DHAR, and two encoding GR in *Arabidopsis*. Many of the transcripts include organellar-targeting sequences and proteins are distributed among the cytosol, chloroplasts, mitochondria, and peroxisomes.

In addition to the antioxidant systems that operate in the soluble fraction of cells, a number of systems are also present for the control or removal of oxidants in membranes. Tocopherols, present primarily in the plastids, have the capacity to quench and scavenge $^1O_2$ where in the former reaction, it has been estimated that a single molecule of α-tocopherol can deactivate up to 120 molecules of $^1O_2$ prior to being degraded, whereas in the scavenging reaction, tocopherols are irreversibly capacity to scavenge lipid peroxyl radicals resulting in the generation of tocopheryl radicals, which can be reduced back to tocopherols by reaction with ascorbate. Carotenoids can also quench $^1O_2$ as well as excited triplet chlorophyll that generates $^1O_2$ by transfer of excitation energy from the pigment to oxygen. As with the quenching reaction of tocopherols, energy is lost from the carotenoid by thermal dissipation meaning that a single carotenoid molecule has the capacity to quench many excited chlorophyll or oxygen molecules. Like the tocopherols, carotenoids can additionally undertake a chemical scavenging reaction in which they can reduce $^1O_2$ and also lipid peroxyl radicals.

The Concepts of Oxidative Stress and Redox Signaling

Under normal physiological conditions, the products of plant electron transport chains (NADPH, ATP) are utilized for carbon assimilation and other key biosynthetic pathways allowing the components of the electron transport chains to maintain an appropriate redox balance. Under such conditions, ROS production is balanced by the capacity of antioxidant systems to remove them and a condition of redox homeostasis is maintained. However, under adverse environmental conditions, over-reduction of electron transport chains and other metabolic impairments can lead to elevated ROS production. For example, drought or pathogen stress result in stomatal closure, reduced mesophyll CO_2 concentrations, enhanced photorespiration, and reduced CO_2 fixation. Subsequent NADPH accumulation limits the availability of NADP to act as a terminal electron acceptor in the PET chain resulting in over-reduction, the univalent transfer of electrons to O_2 and excessive ROS production. Oxidative stress occurs when antioxidant systems are overwhelmed resulting in the accumulation of ROS to levels sufficient to oxidize cellular components at a rate exceeding those beyond which they can be repaired or replaced, ultimately leading to cell death.

In reality, plants are rarely exposed to conditions of extreme oxidative damage sufficient to cause uncontrolled cell death, and it has now been demonstrated that cell death is usually a genetically programmed event responding to specific signals. For example, singlet oxygen–induced cell death is dependent on chloroplast localized EXECUTER proteins and proceeds via signaling pathways involving the phytohormones ethylene, jasmonate, and salicylate. It is now well established that many of the responses of plants to suboptimal environments are the result of genetically predetermined outcomes that respond to specific signaling pathways. Hence, it is perhaps more enlightening to consider plant responses to environmental stress within the concept of redox signaling rather than simply in terms of oxidative stress.

As sessile organisms, plants have a requirement to sense their external environment and adapt their physiology and metabolism to prevailing conditions in order to optimize opportunities for growth and reproduction. Key environmental variables include light quality and intensity, temperature, mineral, and water availability, as well as the presence of beneficial or pathogenic organisms. In order to be successful, plants require sensory systems that allow monitoring of the external environment as well as mechanisms that allow integration of all of the information and signal transduction systems to produce a tailored and dynamic response appropriate to all external variables at any given time. The capacity to sense ROS and cellular redox balance provides plants with a sensory mechanism allowing the direct integration of energy generating and utilizing systems with the prevailing environment. These signaling functions of ROS and redox mean that the outcomes of genetic modification approaches to oxidative stress tolerance cannot always be predicted, and it has been found that modifications that might initially be expected to enhance stress tolerance can frequently have the opposite effect.

The Application of Genetic Modification to Oxidative Stress Tolerance

Several strategies have been developed for the genetic modification of oxidative stress tolerance in both model and crop plants. These have included the manipulation of electron transport chains to reduce ROS production, manipulation of biosynthetic enzymes of low molecular weight antioxidants to enhance their accumulation, the manipulation of enzymes involved in the recycling and regeneration of low molecular weight antioxidants, and the manipulation of antioxidant enzymes required for ROS removal. The generation of

transgenic plants has contributed significantly to our basic understanding of ROS and redox signaling. Similarly the use of genetic engineering techniques has revealed insights into oxidative stress resistance in short-term laboratory studies under controlled stresses. However, very little published research is available concerning longer term field studies on crop plants engineered for enhanced oxidative stress resistance, and significant additional research is required to demonstrate the capacity of engineered plants to contribute to the problems of food security under changeable and adverse environments.

Reducing ROS Production in Electron Transport Chains

Plants have evolved a number of inducible mechanisms for the minimization of ROS production in electron transport chains under stress, and several laboratories have sought to exploit these mechanisms to engineer plants with enhanced tolerance to oxidative stress–inducing conditions.

The RET chain has both a cytochrome c pathway that links electron transport to transmembrane proton movement and ATP synthesis as well as an inducible alternative oxidase (AOX) pathway that is proposed to function in minimizing ROS accumulation under stressful environmental conditions. Several experiments have been conducted to examine the impact of AOX manipulation in plants in response to environmental stress. Paradoxically, tobacco plants that constitutively overexpressed AOX exhibited enhanced sensitivity to ozone or low temperature. Transgenic plants exhibited reduced activity of key antioxidant enzymes such as SOD, APX, and CAT suggesting that RET-derived ROS are required to prime the antioxidant system in tobacco. On the contrary, rice plants engineered to overexpress AOX did exhibit enhanced tolerance to low and high temperatures as defined by growth phenotype. Temperature stress tolerance was associated with reduced lipid peroxidation and cellular ion leakage suggesting that the growth phenotype was directly related to a reduced oxidative load.

The PET chain has also been a target for genetic manipulation to reduce ROS production. Ferredoxin represents the terminal electron acceptor from photosystem I (PSI) and acts as a cosubstrate for ferredoxin-NADP reductase (FNR) that transfers electrons to NADP, generating reducing power to drive photosynthetic carbon assimilation. The reaction catalyzed by FNR is considered to be rate limiting for photosynthesis under high light leading to over-reduction of the PET chain with the subsequent generation of ROS. Transgenic tobacco plants overexpressing FNR exhibited reduced membrane and photosystem II (PSII) damage following exposure to photooxidative stress, demonstrating that increased FNR activity ameliorated ROS accumulation.

While FNR represents a significant sink for photosynthetic electrons, ferredoxin can also donate electrons to a broad range of enzymes involved in key developmental and metabolic processes, therefore increasing the ferredoxin pool could create a larger electron sink. However, ferredoxin levels decrease post-translationally under conditions of environmental stress, and hence this approach has limited application. In Cyanobacteria, the decline in ferredoxin levels is compensated by the accumulation of a flavodoxin, which acts as an alternative electron acceptor. Expression of the *Anabaena* flavodoxin in tobacco induced tolerance to a wide range of abiotic stresses including high light, high temperature, drought stress, UV treatment, and ROS-inducing methyl viologen (MV). Transgenic plants had lower levels of $O_2^{\cdot -}$ and H_2O_2 in stressed leaves as well as reduced lipid peroxidation. Similarly, the redox state of ascorbate and glutathione pools was more reduced under stress in transgenic plants although no changes were observed in key antioxidant enzyme activities.

Enhancing Capacity of Antioxidant Enzymes to Enhance ROS Scavenging

Plants engineered to have elevated SOD activity were first reported over two decades ago where tobacco plants expressing a pea Cu/Zn-SOD in chloroplasts had enhanced resistance to high light and the superoxide inducing herbicide MV. Subsequently a large number of reports of transgenic plants have been published in which various forms of SOD have been expressed in cytosolic, mitochondrial, and plastidic compartments in a range of model and crop species, conferring resistance to a broad range of abiotic stresses. Plastid-targeted Cu/Zn-SODs were expressed in the model plant tobacco (*Nicotiana tabacum*) as well as crop plants including cotton (*Gossypium hirsutum*), potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*), and Chinese cabbage (*Brassica campestris*). Enhanced tolerance to a range of abiotic stresses including MV, high light, UV light, heat, cold, drought, salt, and the atmospheric pollutant SO_2 was observed. Plastid-targeted expression of MnSOD isoforms enhanced tolerance to a similar range of stresses in *Arabidopsis*, maize (*Zea mays*); and drought in rice (*Oryza sativa*), alfalfa (*Medicago sativa*), and cotton. Similarly, maize overexpressing *Arabidopsis* FeSOD targeted to chloroplasts exhibited greater tolerance to MV and additionally exhibited increased growth rates under optimal or low temperatures. Tobacco plants expressing the same construct exhibited protection against MV, however, they were not protected against salt stress. This was accompanied by an inability of the transgenic plants to activate cytosolic and chloroplastic Cu/ZnSOD activity, revealing the significance of the broader ROS signaling network.

Few studies have reported the impact of manipulating the antioxidant capacity of crops on field performance; however, transgenic alfalfa (*M. sativa*) plants expressing a mitochondrial MnSOD exhibited improved winter survival and yield, despite having only marginally improved freezing tolerance as determined by short-term electrolyte leakage measurements. Similarly rape seed plants (*Brassica napus*) overexpressing a mitochondrial MnSOD exhibited greater tolerance to freezing, heat shock, and drought in laboratory tests and also exhibited greater seedling emergence and vigor in field experiments.

Although the presence of superoxide in the cytosol is likely to be limited, targeting SODs to this cellular compartment can also enhance stress tolerance in transgenic plants. For example, overexpression of cytosolic Cu/Zn-SOD enhanced tolerance to MV in potato, rice, and cassava. MV treatment results in the generation of $O_2^{\cdot -}$ in the plastids, and this charged radical is

unlikely to diffuse out of this organelle; it is therefore not clear why enhancement of cytosolic SOD activity would improve resistance to this stressor. However, transgenic tobacco plants expressing a cytosolic Cu/Zn-SOD had higher activity of a range of other antioxidant enzymes under both optimal and stressful environmental conditions suggesting that the impact of the transgene may have indirect consequences.

The product of the SOD reaction is another ROS, H_2O_2, and several studies have reported improved stress tolerance following the expression of SOD in conjunction with genes encoding enzymes for H_2O_2 metabolism. For example, cotton or Chinese cabbage plants simultaneously expressing SOD and catalase are more resistant to salt stress than plants expressing either gene alone. On the contrary, overexpression of SOD and APX that uses ascorbate to metabolize H_2O_2 did not provide greater resistance than the expression of either gene alone in cotton. Tobacco plants expressing a combination of chloroplastic Cu/ZnSOD, APX, and DHAR exhibited enhanced salt stress tolerance relative to plants expressing only SOD and APX suggesting that ascorbate regeneration was a limiting factor in stress tolerance.

Catalase does not require any cofactors, and several reports have provided evidence for enhanced oxidative stress tolerance in plants overexpressing catalase genes either from bacterial or other plant sources. For example, expression of the *Escherichia coli* catalase–encoding gene *katE* in tobacco or tomato chloroplasts resulted in a threefold increase in catalase activity. This was associated with an enhanced capacity for plants to withstand photooxidative stress induced by MV or high light in combination with environmental conditions limiting carbon assimilation. The protective mechanisms of catalase expression were apparently multifactorial, not only maintaining the activity of thiol-modulated stromal enzymes of the Calvin cycle as well as antioxidant enzymes such as cytosolic APX and GR, but also protecting chloroplast translational capacity thereby maintaining the capacity to replace photodamaged D1 protein, an essential component of PSII.

Transgenic expression of the KatE protein targeted to the cytosol has been shown to enhance salt tolerance in the crop plants potato and rice. Similarly, rice plants were protected from salt stress when transformed with both a catalase and glutathione S-transferase from the salt-tolerant plant *Suaeda salsa*. Protection was also observed against stress induced by cadmium and high temperatures where expression of the transgenes maintained leaf chlorophyll and CO_2 fixation and had profound impacts on antioxidant parameters following stress imposition. Under single or combined stress, not only were catalase and GST activities significantly higher in the transgenic plants, but also activities of SOD, APX, MDHAR, DHAR, and GR resulting in improved redox status of both the ascorbate and glutathione pools and again highlighting connections between different components of plant antioxidant systems.

Compared with catalase, APX and the downstream enzymes of the AsA-GSH cycle represent a high affinity H_2O_2 removal mechanism. Transgenic plants overexpressing genes encoding all of the enzymes of the cycle have been generated and assessed for their capacity to withstand oxidative stress. Several groups have focused on developing transgenic plants that overexpress gene products targeted to the chloroplasts to enhance photooxidative stress tolerance induced by a range of external stimuli. In the model plants *Arabidopsis* and tobacco, expression of APX targeted either to the chloroplast stroma or to the thylakoid membrane increased resistance to MV as estimated using a range of parameters such as chlorophyll bleaching, cellular electrolyte leakage, photosynthetic carbon fixation, and photochemical efficiency. Similarly, APX overexpressing model and crop plants were more resistant to a range of environmental stresses such as salt, drought, and low or high temperature. The significance of ROS/redox signaling in plant response to biotic stress was highlighted in *Arabidopsis* plants engineered to have increased thylakoidal APX activity. Under optimal growing conditions, these plants had significantly lower levels of transcripts encoding two pathogenesis-related (PR) proteins suggesting that crop plants engineered to resist oxidative stress induced by abiotic factors may exhibit reduced pest and disease resistance in the field.

A role for cytosolic APX activity has also been demonstrated in stress tolerance. For example, *Arabidopsis* plants engineered to express APX from *B. campestris* exhibited enhanced tolerance to an acute heat stress (40 °C, 5 h) as estimated by reduced H_2O_2 accumulation and retarded chlorophyll degradation. In rice plants, transgenic expression of cytosolic APX protected against cold stress where the maturing spikelets of transgenic plants exhibited reduced H_2O_2 accumulation, reduced lipid peroxide accumulation, and enhanced fertility relative to wild-type plants following several days exposure to temperatures of 12 °C.

The observation that overexpression of APX alone is sufficient to enhance oxidative stress tolerance in a range of crop species suggests that increased rates of ascorbate oxidation in these plants are compensated either by ascorbate biosynthesis or ascorbate recycling. However, as observed in plants overexpressing SOD and CAT, manipulation of the level of one antioxidant enzyme can affect activities and transcript levels of other antioxidant enzymes. For example, overexpression of genes encoding MDHAR has been shown to increase the extractable activity of not only MDHAR, but also APX, SOD, DHAR, and GR in both optimal and stressful growing environments in both model and crop plants. In *Arabidopsis*, these changes conferred tolerance to freezing as estimated by the capacity of plants to maintain photosynthetic pigments and fresh weight accumulation as well as the size and redox status of the ascorbate and glutathione pools. In the vast majority of transgenic plants examined, MDHAR overexpression resulted in increased levels of AsA and a more reduced AsA redox status under both optimal and stressful conditions.

As with MDHAR, overexpression of DHAR has been widely observed to confer oxidative stress tolerance, and a primary effect appears to be to increase the cellular AsA pool and maintain it in a more reduced state. Tomato and potato plants overexpressing either cytosolic or chloroplastic DHAR exhibited increased levels of ascorbate in both leaves and sink tissues (fruits and tubers). Plants were more tolerant of MV, drought, or salt stress as estimated by parameters such as H_2O_2 accumulation, electrolyte leakage, and chlorophyll content, which were able to maintain higher net photosynthetic rates and exhibited improved seed germination and growth. Salt tolerance as estimated by the capacity to maintain fresh weight gain was also

observed in transgenic rice overexpressing cytosolic DHAR, and the trait was associated with not only enhanced activities of DHAR, but also APX, MDHAR, and GR as well as improved AsA/DHA ratios. In another rare example of data concerning field performance, these plants exhibited an approximately 20% increase in seed yield in two separate seasons when grown under paddy conditions. However, benefits may be limited to well-watered agricultural conditions as guard cell redox status has an important role in the control of stomatal aperture. Tobacco plants with enhanced DHAR activity had a more reduced AsA pool and increased transpiration rates. Under well-watered conditions, this led to enhanced stomatal conductance and net CO_2 assimilation; however, under conditions of water limitation, plants lost water more rapidly than control plants; and CO_2 assimilation was rapidly inhibited suggesting any benefits in yield may be offset by lower water use efficiency.

Relatively few studies have reported the impact of manipulating GR activity on stress tolerance. Transgenic expression of GR in the cytosol, chloroplasts, or mitochondria in tobacco significantly raised cellular glutathione levels; however, there was little relationship between GR activity, cellular glutathione, and resistance to ozone or MV in different lines transformed with the same construct. As discussed below, cellular GSH content and redox status appears to play a key role in maintaining appropriate redox signaling, and it is likely that manipulation of GR activity had negative impacts on maintenance of other antioxidant defenses.

A further enzymatic mechanism for the removal of reactive H_2O_2 is represented by the peroxiredoxins, glutathione peroxidases, and glutaredoxins, and transgenic expression of transcripts encoding these enzymes has been reported to protect against oxidative stress. For example, 2-cysteine peroxiredoxins have well-characterized activities in protection of chloroplasts from oxidative stress, and the overexpression of these enzymes in both dicotyledonous and monocotyledonous crop plants protected photosynthetic efficiency and prevented lipid peroxidation following MV treatment or heat shock. Similar results were obtained in tomato following expression of *Arabidopsis GRXS17* encoding a glutaredoxin. In this case, protection did not appear to be directly related to the scavenging of plastid-generated ROS as the protein was primarily located in the cytoplasm at moderate growing temperatures. However, following heat shock, the protein migrated to the nucleus, and plants expressing the transgene exhibited accumulation of transcripts encoding not only heat shock factors, but also heat shock proteins targeted to a range of organelles. Indirect protection was also observed following expression of a GPX in tobacco plants which exhibited higher growth rates than wild-type plants under chilling or salt stress. Enhanced growth was associated with a greater degree of oxidation of the glutathione pool and the growth phenotype could be replicated by supplying wild-type plants with oxidized glutathione suggesting that the GSH redox state rather than any specific antioxidant activity of the enzyme was the driving factor for enhanced growth under stress. The issue of the impact of transgene expression on cross talk between biotic and abiotic signaling pathways was highlighted in transgenic tomatoes expressing a mouse glutathione peroxidase. Transgenic plants exhibited reduced resistance to both a necrotrophic and biotrophic pathogen compared with untransformed control plants highlighting potential issues concerning the deployment of GM technologies to combat oxidative stress induced by abiotic environmental conditions.

Genetic Modification to Increase the Concentration of Cellular Antioxidants

Ascorbic Acid

Four potential biosynthetic routes have been described for AsA in plants and the expression of transgenes encoding enzymes from at least three of these pathways has been utilized to enhance plant AsA content (Figure 4). Evidence suggests that the Smirnoff–Wheeler pathway is the most significant for AsA biosynthesis in plants and null mutations in genes encoding specific enzymes of the pathway are embryo lethal in *Arabidopsis*. Transgenic plants expressing genes encoding all of the enzymes specific to the pathway (GGP, GPP, GDH, and GLDH) as well as several enzymes shared with other pathways of sugar metabolism (GME, GMP, and PMI) have been generated and assessed for their resistance to oxidative stress. Transgenic plants exhibit ascorbate content approximately twice those of untransformed plants under nonstressed conditions irrespective of the specific gene expressed and were resistant to a range of abiotic stresses including salt, osmotic stress, MV, cold, and heat.

Enhanced AsA content has additionally been achieved by overexpressing genes encoding enzymes required for the *myo*-inositol and galacturonic acid pathways, the significance of which remain to be resolved *in planta*. Transgenic potatoes overexpressing either GAR or GLO had ascorbate levels elevated up to threefold in both the leaves and tubers and exhibited enhanced resistance to MV, salt, and osmotic stress. Stress resistance was manifested as reduced lipid peroxidation and maintenance of chlorophyll under stress conditions leading to maintained root and shoot growth as well as enhanced mini-tuber yield *in vitro*. Similar results were obtained in *Arabidopsis* expressing a gene encoding MIO or GLO which in addition to exhibiting enhanced biomass production under cold or salt stress produced more biomass under optimal growth conditions.

Biochemical phenotyping of plants engineered to contain high ascorbate has revealed a number of potential protective mechanisms. As well as having enhanced ascorbate, transgenic plants also exhibit enhanced activity of a range of antioxidant enzymes including SOD, CAT, APX, GPX, DHAR, and GR. Changes in enzyme activities have additionally been associated not only with a more reduced status of the AsA pool, but also absolute increases in levels of reduced GSH. Again these data highlight the significance of cross talk between different elements of plant antioxidant systems.

Glutathione

Plants engineered to accumulate glutathione have exhibited mixed responses to oxidative stress highlighting the significance of the glutathione pool for redox sensing and signaling. In early work, tobacco plants were modified to express genes encoding either or both of the GSH biosynthetic enzymes,

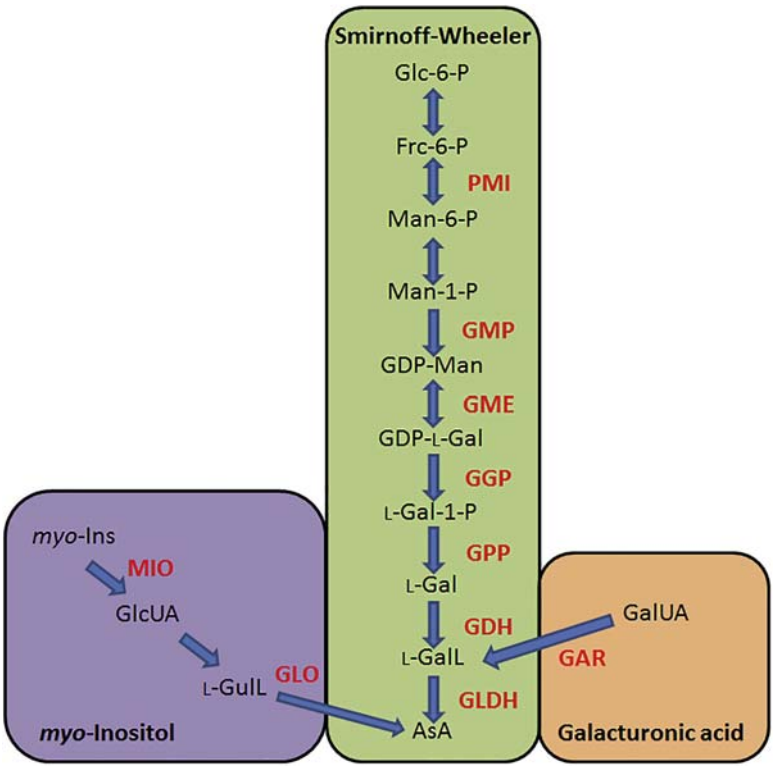

Figure 4 Genetic modification of ascorbate synthesis. Three separate pathways for AsA biosynthesis have been subject to genetic modification in plants. The Smirnoff–Wheeler pathway synthesizes AsA from sugars via the intermediate L-galactose, and many of the genes encoding enzymes of both the core AsA biosynthetic pathway and those shared with other metabolic pathways have been manipulated. The galacturonic acid pathway was first described in strawberry fruit, and it utilizes the enzyme galacturonic acid reductase to convert D-galacturonate to L-galactono-1,4-lactone. The *myo*-inositol pathway uses *myo*-inositol as a substrate and proceeds via L-gulono-1,4-lactone, a key intermediate in the animal biosynthetic pathway. Enzymes that have had their activity manipulated are highlighted in red; PMI, phosphomannose isomerase; GMP, GDP-mannose pyrophosphorylase; GME, GDP-mannose epimerase; GGP, GDP-L-galactose phosphorylase; GPP, L-galactose-1-phosphate phosphatase; GDH, L-galactose dehydrogenase; GLDH, L-galactono-1,4-lactone dehydrogenase; MIO, *myo*-inositol oxygenase; GLO, L-gulono-1,4-lactone oxidase; GAR, galacturonic acid reductase.

γ-glutamyl cysteine synthase (γ-ECS), and glutathione synthase (GS) in chloroplasts. Paradoxically, despite having up to fivefold foliar GSH content transformed plants exhibited photooxidative stress symptoms even when grown under relatively low light intensity of 250 μmol m^{-2} s^{-1}. While the γ-ECS plants exhibited a more oxidized GSH pool, this was not the case for plants expressing both γ-ECS and GS suggesting that the elevated GSH levels per se were inducing the oxidative stress possibly by interfering with redox signals required for optimizing photosynthesis. Similarly, in transgenic poplar stress resistance was related to levels of transgene expression and foliar GSH levels with lines exhibiting the largest increases in foliar GSH levels exhibiting diminished photosynthetic capacity and signs of necrosis in older leaves. In both tobacco and poplar, transgenes were under the control of a constitutive promoter; however, rice plants expressing a gene encoding γ-ECS under the control of a stress-inducible promoter did exhibit enhanced tolerance to both MV and salt stress. Furthermore, when grown under paddy field conditions plants exhibited improved grain yields in two consecutive seasons. These data suggest that enhancing accumulation of GSH only under conditions of stress may overcome some of the problems associated with inappropriate redox signaling under non-stress conditions.

Lipophilic Antioxidants

Several reports have described oxidative stress tolerance of transgenic plants engineered to contain higher levels of specific lipophilic antioxidant tocopherols and carotenoids. These reports have illustrated the potential for improving stress tolerance by manipulating levels of lipophilic antioxidants and have demonstrated that changes in levels of specific lipophilic antioxidants can have significant impacts on stress tolerance even in the absence of a change in the total lipophilic antioxidant pool. Furthermore, studies have additionally highlighted links between hydrophilic and lipophilic antioxidant pools.

A key role for tocopherols is the removal of lipid peroxides and termination of peroxide propagation reactions. α, β, γ, and δ-Tocopherols have different *in vivo* antioxidant capacities being capable of the protection of 220, 120, 100, and 30 polyunsaturated fatty acid molecules, respectively. Furthermore, α-tocopherol is the most biologically active form of vitamin E, hence a number of laboratories have focused on genetic modification to enhance α-tocopherol content.

The *Arabidopsis VTE4* gene–encoding γ-tocopherol methyl transferase that catalyzes the final step in the synthesis of α-tocopherol was expressed in *Brassica juncea* plants. Although this modification had no impact on total tocopherol content of transgenic plants, there was a significant increase in α-tocopherol content compared to wild-type plants under conditions of salt, heavy metal, or osmotic stress. Transgenic plants exhibited improved seed germination under stress, and seedlings exhibited lower electrolyte leakage, lipid peroxidation, H_2O_2, and $O_2^{\cdot -}$ content following stress treatment. Transgenic plants additionally exhibited higher enzyme activities and transcripts for SOD, CAT, APX, and GR as well as lower AsA and GSH contents under stress highlighting cross talk between lipophilic and soluble antioxidants. Similar results were observed in transgenic *Arabidopsis* expressing *VTE4* in combination with *VTE2* encoding homogenistate prenyl transferase catalyzing an early step in tocopherol biosynthesis. Under optimal growth conditions, transgenic plants had approximately 4.5-fold higher levels of both α- and total tocopherols that were associated with lower levels of AsA and GSH as well as increased abundance of transcripts encoding APX, DHAR, and MDHAR.

Like tocopherols, carotenoids can quench and scavenge peroxides in cellular membranes. In addition, the xanthophylls zeaxanthin and violaxanthin play a key role in dissipation of excess light energy within the chloroplasts through their function in nonphotochemical quenching (NPQ). Xanthophyll synthesis from β-carotene is catalyzed by β-carotene hydroxylase, and transgenic plants engineered to express genes encoding the enzyme had mildly elevated total carotenoid content as well as up to threefold increases in violaxanthin and zeaxanthin compared with wild-type plants. Transgenic plants were resistant to a range of stresses including high light combined with high temperature, UV light, and drought; however, there was no evidence for increased NPQ. Transgenic plants did exhibit fewer markers of oxidative stress such as lipid peroxides, and as was observed with transgenic plants engineered with altered tocopherol profiles, activities of antioxidant enzymes were significantly enhanced suggesting that the primary protective effect was via oxidative stress protection rather than increased activity of the xanthophyll cycle. This finding is further supported by the observation that tobacco plants overexpressing carotenoid ε-hydroxylase leading to the accumulation of lutein, a xanthophyll that is not required for NPQ similarly exhibited enhanced photooxidative stress resistance and reduced oxidative stress markers. Indeed, transformation of carrot plants with an algal β-carotene ketolase inducing the formation of ketocarotenoids that are normally absent from this species similarly enhanced tolerance and reduced markers of oxidative stress following exposure to UV light, MV, or H_2O_2.

Concluding Remarks

Many reports are now available regarding the utility of a broad range of GM strategies to alleviate short-term oxidative stress in plants under laboratory conditions. A small number of studies have additionally indicated that such strategies may have utility under field conditions. Laboratory studies have additionally demonstrated significant impacts of targeted modifications on the broader antioxidant signaling and defense network revealing that under some circumstances, enhancing specific components of the antioxidant systems can paradoxically have negative impacts on overall resistance to oxidative stress. Similarly, enhancing antioxidant capacity has been shown to have unintended impacts on biotic interactions reducing resistance to a range of microbial and insect pathogens. These results suggest that under field conditions, enhancing oxidative stress resistance may have unintended consequences and point to an urgent need to conduct further field trials in order to develop an armory of techniques that can be deployed to maintain food production under uncertain environmental futures.

See also: **Abiotic Stress:** Cold Stress; Free Radicals, Oxidative Stress and Antioxidants; Salt Stress. **Bioethics:** Food Security. **Photosynthesis:** Photoinhibition; Photorespiration; Photosynthesis. **Plants and the Environment:** Global Warming Effects; Plants and Atmospheric Pollution.

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Plant Responses to Waterlogging

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Nomenclature

Anoxia Absence of oxygen
Hypoxia Deficiency of oxygen
Aerenchyma Tissue containing gas spaces formed or present in plants often as a result of oxygen deficiency; aerenchyma may be lysigenous – where spaces are formed by cell death – or schizogenous – where spaces are formed by the separation of cell walls

Introduction

The chief consequence of waterlogging for higher plants is the development of oxygen deficiency (hypoxia or anoxia), particularly in the root environment. This deficiency results from the fact that oxygen diffuses ca. 1000 times more slowly in water compared to air. In freely drained soils, roots grow into gas filled spaces between soil particles. These pores in the soil structure connect to the surface, and therefore the atmosphere, where the oxygen partial pressure is 20.8 kPa. Oxygen concentrations similar to that of the atmosphere can occur to a depth of more than 1 m in the soil. Waterlogging results in the filling of these interstitial spaces within the soil and prevents oxygen diffusion resulting in hypoxia (partial oxygen deficiency) or anoxia (the almost total absence of oxygen). In addition to lack of availability of oxygen, tissue may be damaged by the accumulation of gaseous products, such as carbon dioxide, that would otherwise freely diffuse from the root. In agriculture, waterlogging may result in reduced yield or the death of the crop; this is particularly noticeable early in the growing season where flooded areas of a field may result in failure of seeds to germinate or death of newly germinated seedlings.

Root growth is affected by even small decreases in available oxygen. The critical oxygen pressure (COP – the oxygen concentration at which growth or metabolism are inhibited) for corn (*Zea mays*; maize) at 25 °C is 20 kPa – only just below the oxygen concentration of air. As aerobic metabolism generates a much higher ATP yield than anaerobic metabolism (fermentation), tissue relying on anaerobic metabolism rapidly becomes depleted of ATP and of energy storage reserves. When oxygen concentrations decrease further, tissue damage will occur. Most plant tissues will die rapidly in anoxic conditions, with seedlings being particularly vulnerable. However, plant adaptations exist that permit some plants to survive hypoxia and in some instances anoxia in the soil environment.

Low oxygen concentrations detected by the tissue cause a rapid change in the free calcium concentration of the cytoplasm. This is central to the initiation of responses to low oxygen (see below) and almost certainly results from calcium release from mitochondria. Production of the gaseous hormone ethylene (ethene) and its precursor aminocyclopropane-1-carboxylic acid (ACC) is also of key importance. Ethylene induces changes in root structure and activity and ACC transport to the shoot induces further damage, like defoliation, often observed in waterlogging. Experiments using garden pea have shown that flooding can result in a threefold increase in the rate of ethylene production by roots within an hour of a flooding event. Furthermore, it has been suggested that waterlogging restricts the diffusion of the ethylene they synthesize out of the root, so internal concentrations of ethylene are probably significantly higher than in unflooded roots even without the additional ethylene synthesis. A rapid increase in internal ethylene concentration may well be the initial signal to the roots that waterlogging has occurred, though it is also the case that direct measurements of oxygen in the proximity of suddenly flooded seedling root tips shows oxygen is reduced to less than one fourth of atmospheric partial pressure within an hour which suggests that reduced oxygen may also be acting as a trigger. The evidence implicating ethylene comes from a number of experiments in various species that reveal that exogenously applied ethylene gas at 1–10 ppm triggers several responses typical of flooded roots, such as aerenchyma formation, despite normal oxygen levels. Likewise, experiments with maize and pea indicate that exposing root systems to hypoxic conditions in the absence of flooding will also induce aerenchyma formation.

Two major classes of adaptations to hypoxia and anoxia exist: anatomical and biochemical. In the former case, the structure of the tissue is modified, to create spaces for gas diffusion and to minimize oxygen demand. Biochemical adaptations include the induction of pathways of anaerobic metabolism and enzymes for the removal of toxic waste.

Anatomical Adaptations to Waterlogging

In some species of plants, waterlogging causes an increase in the size of intercellular spaces. This increases the root's porosity slightly. This is also thought to be a result of increased exposure to ethylene, which causes measurable radial expansion of cortical cells. But the chief anatomical adaptation to waterlogging shown by plants is the formation of aerenchyma – tissue containing long gas spaces significantly larger than intercellular spaces. It is formed in the roots of wetland species, and in some dryland species in various adverse conditions. In some species, aerenchyma is formed constitutively (i.e., is always present) while in others it is a result of the abiotic stress, commonly

hypoxia resulting from waterlogging. There are two types of aerenchyma: lysigenous and schizogenous. Lysigenous aerenchyma is formed when previously formed cells die within a tissue (e.g., the root cortex or the center of the vascular cylinder) to create a gas-conducting space. Lysigenous aerenchyma is found in rice (*Oryza sativa*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), corn (*Zea mays*), tomato (*Solanum lycopersicum*), sunflower (*Helianthus annuus*), garden pea (*Pisum sativum*), fava bean (*Vicia faba*), and some cultivars of soybean (*Glycine max*), to name a few. Schizogenous aerenchyma is formed when intercellular gas spaces form within a tissue as it develops and without cell death taking place. Spaces are formed by the differential growth of adjacent cells with cells separating from one another often followed by some cell division. Wetland species like dock (*Rumex*) show characteristic schizogenous aerenchyma, while some species (like *Sagittaria lancifolia*) show both schizogenous and lysigenous aerenchyma in leaves and roots, respectively.

As mentioned above, lysigenous aerenchyma formation is initiated by the gaseous plant hormone ethylene formed in hypoxic conditions. Lysigeny is the result of the activation of a programmed cell death pathway. The first point of aerenchyma formation in many species, such as corn and rice, is the death of cells in the mid-cortex of the root, a short distance behind the growing tip. Cell death in corn then progresses into surrounding cells (Figure 1). In cool-season leguminous plants, cell death begins in the center of the vascular cylinder, likewise near the root tip, and results in a vascular cavity (Figure 2). Studies of cell death in corn, pea, and soybean

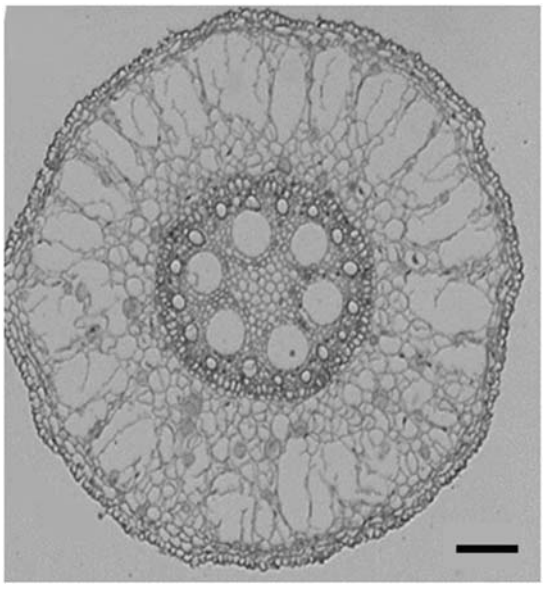

Figure 1 Cortical aerenchyma in a cereal grass seedling root. Light micrograph of a transverse freehand section of a root from a corn seedling grown in aquaculture under hypoxic conditions (3% oxygen) at 18 °C. Aerenchyma spaces form in a radial pattern reminiscent of spaces between the spokes of a wheel. Scale bar = 100 μm. Reproduced with permission from Gunawardena, A., Pearce, D.M., Jackson, M.B., Hawes, C.R., Evans, D.E., 2001. Characterization of programmed cell death during aerenchyma formation induced by ethylene or hypoxia in roots of maize (*Zea mays* L.). Planta 212, 205–214.

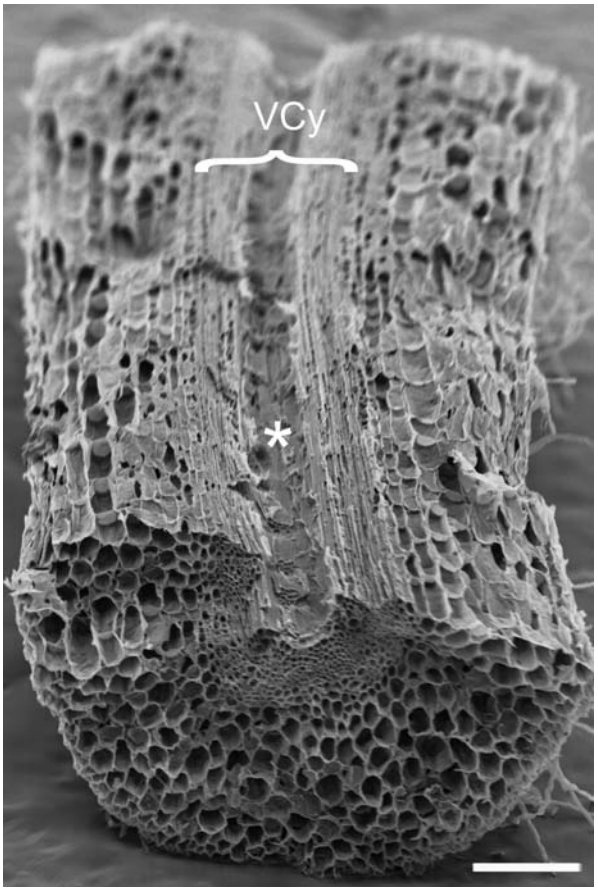

Figure 2 Vascular aerenchyma in a legume seedling root. Scanning electron micrograph transverse and longitudinal view of a segment of a pea primary root showing a vascular aerenchyma cavity (*). The seedling was grown in saturated vermiculite at 25 °C. Scale bar = 250 μm, VCy = vascular cylinder. Reproduced with permission from Sarkar, P., Niki, T., Gladish, D.K., 2008. Changes in cell wall ultrastructure induced by sudden flooding at 25 °C in *Pisum sativum* (Fabaceae) primary roots. Am. J. Bot. 95, 782–792.

suggest that the pathway initiated resembles apoptosis in animal cells in a number of ways. For example, typically there is condensation of chromatin, lobing sometimes subdivision of the nucleus, and a systematic and regular fragmentation of the nuclear DNA. Other organelles may swell and become deformed. These events involve a complex cascading biochemical transduction pathway. In some species, such as pea and soybean, it has been shown that reactive oxygen molecule species (ROS), such as hydrogen peroxide, quickly accumulate in the target cells, and soon (within 2 h of flooding) cytochrome *c* is released from mitochondria and begins to accumulate in the nucleus, possibly to serve as a transcription factor. It is also possibly an activator of specific categories of proteases (protein-digesting enzymes). These are similar to the animal caspases that work during apoptosis and that are released from their inactive forms to trigger further 'cascades' of destructive processes. While protease cascades have not been specifically demonstrated in plant PCD systems, proteins with caspase-like activity (sometimes called metacaspases) have been shown to significantly increase their activities with in

just a few hours of a PCD-inducing stimulus. More typical of plant than animal cells is the rupture of the membrane of the central vacuole, which lowers cytosolic pH and releases hydrolytic enzymes, such as vacuolar processing enzyme (VPE), a process referred to as 'mega-autophagy' or 'vacuolar cell death,' though, as described above, many processes other than vacuolar rupture are typically involved. One major difference from animal apoptosis, however, is the need for the cell walls of the dying cells to be removed, and this is achieved by the induction and release of cell wall–degrading enzymes. At the end of the process, gas spaces are created behind the root tip for varying distances along the root that have been shown by measurement with oxygen microprobes to convey oxygen to the growing tissues. Removal of cells also reduces the demand for oxygen in that zone.

Biochemical Adaptations to Waterlogging

Anaerobic conditions created by waterlogging cause rapid changes in gene expression and epigenetically induced biochemical pathways. Initially, normal protein synthesis stops and genes encoding polypeptides known as 'transition polypeptides' are induced. Subsequently, a range of new proteins (anaerobic proteins, ANPs) are transcribed. In corn, for instance, this numbers at least 20 proteins. Most of these are metabolic enzymes involved in establishing anaerobic metabolism because, in the absence of oxygen, the citric acid cycle and oxidative phosphorylation cannot function. This shifting of primary energy metabolism to anaerobic fermentation pathways may allow minimal cell maintenance for a time period long enough for flooded soils to drain somewhat. Three pathways of anaerobic metabolism (fermentation) have been described in plants. Induction of pyruvate decarboxylase and alcohol dehydrogenase results in alcoholic fermentation, with the production of ethanol and carbon dioxide. Very big increases in the expression levels of genes for these two enzymes have been demonstrated in soybean as a result of waterlogging. Induction of lactate dehydrogenase results in lactic acid fermentation, with the production of lactate. The third pathway involves the production of alanine from glutamate and pyruvate. In corn, waterlogged roots first produce lactic acid and subsequently carry out alcoholic fermentation. The transition from lactate to alcoholic fermentation occurs as the cytosolic pH of the tissue decreases (cytoplasmic acidosis – one of the consequences of metabolism in low oxygen), favoring the activity of the latter pathway. Alanine fermentation is common in some (e.g., barley) but not all (e.g., corn) roots in waterlogging, and alanine aminotransferase is induced by hypoxia in these species.

Of 20 genes observed to be induced by anaerobiosis in corn, many are directly involved in carbohydrate metabolism (e.g., alcohol dehydrogenase, aldolase, glyceraldehyde-3-phosphate dehydrogenase, phosphohexose isomerase, pyruvate decarboxylase, and sucrose synthase). The importance of the induction of these enzymes in waterlogging is illustrated by the fact that corn mutants lacking alcohol dehydrogenase can only survive a few hours of submergence at 27 °C when most corn genotypes will survive for 3 days. Other enzymes also induced by hypoxia are likely to be involved in the anatomical changes described above. These include enzymes of cell wall loosening and degradation like xyloglucan endotransglycosylase. In pea the composition and amount of cell wall pectins (gluelike molecules) change and diminish. Studies of changes in gene expression during flooding of soybean roots show that mRNA for polygalacturonase, which hydrolyzes pectins, and matrix metaloproteinase2, a cell wall protein–degrading enzyme, are increased to very high levels. Flooding triggers an increase in gene expression of fivefold or greater in over 1000 soybean genes within 6 h in tissues of soybean roots where vascular aerenchyma is forming in response to flooding.

While the initiation of anaerobic fermentation results in survival until waterlogging ends and normal oxygen pressures are restored, anatomical changes provide a mechanism for long-term supply of oxygen to submerged tissues. In the extreme case of deepwater rice, which is exposed to flooding for more than a month during the growing season, a further characteristic permits survival. In this species, flooding induces shoot elongation at the rate of up to 250 mm per day, so that it is never submerged. Here, the combination of anatomical changes, modified metabolism, and modified growth permit survival in extreme conditions.

Crop losses due to waterlogging worldwide are large; for instance, in Western Australia 2 million ha of land used for wheat is susceptible to waterlogging, with yield losses ranging from 15% to 50%. Estimated losses of wheat alone in Australia were Aus$300 million per annum in 2000. In the United States, crop losses due to flooding, more than half of which was corn (maize) and soybean, were estimated to exceed $3 billion in 2011. Alterations in rainfall patterns leading to increased flooding as a result of global environment change are likely to significantly increase these losses. Detailed investigations of these responses to flooding-induced hypoxia may lead eventually to increased waterlogging tolerance in plants by allowing the use of a combination of molecular genetic and traditional plant breeding approaches to directly enhance crop tolerance. This currently is an important goal of anaerobiosis research.

See also: **Physiology:** Basic Water Relations; Uptake, Loss and Control. **Plant Nutrition:** Growth and Function of Root Systems.

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Salt Stress

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Glossary

Backcross Crossing of the offspring with one of the parents to obtain offspring that have the genetic composition that is more similar to one of the parents.

Compatible osmolytes Compounds such as sugars or amino acids (nontoxic to the plant) used to counterbalance additional charges present in the plant during ionic stress.

Genetic engineering of a plant Addition of new DNA to an organism with the aim to provide the plant with a new, beneficial trait, also known as genetic modification of a plant.

Genetic marker A DNA sequence with a known location on a chromosome or in close proximity to a gene of interest.

Ion exclusion tolerance Preventing Na^+ from reaching the shoot; mechanisms include transporting Na^+ toward the outer parts of the root (and potentially back into the soil) and retrieval of Na^+ from the transpiration stream.

Marker-assisted selection Using genetic markers to identify individuals containing the gene of interest and selecting these for further breeding/analyses.

Quantitative trait A measurable phenotype that depends on multiple genes and the environment; for instance, Na^+ content in the shoot, plant height, and yield are quantitative traits.

Quantitative trait locus (QTL) Regions of DNA linked to, or containing, the gene(s) that underlies a quantitative trait.

Salt glands and salt bladders Specialized compartments into which Na^+ can be sequestered; only found in some species.

Shoot ion-independent tolerance Preventing the immediate decrease in shoot growth that occurs upon salinity stress (before Na^+ accumulates in the shoot); possible mechanisms involve sensing and signaling.

Tissue tolerance Maintaining low cytosolic Na^+ concentrations by compartmentalizing Na^+; at the cellular level Na^+ can be compartmentalized into organelles, such as the vacuole, and at the tissue level Na^+ can be transported to older leaves to protect the young, photosynthetically active leaves from high Na^+ concentrations.

Tonoplast Membrane around the vacuole.

Salinity Stress: A Threat for Global Agricultural Productivity

The world population is increasing and is expected to surpass 9 billion people by the year 2050. Feeding this growing population will require an estimated 70% increase in world food production from current levels. However, during this same time frame, arable land – land which is used for agriculture – is expected to increase only slightly. Therefore, the required increase in food production must come by increasing output of land currently in production or by expanding production into marginal lands that are not currently suitable for agriculture. While many factors limit plant growth in arable and marginal lands, one of the most important is soil salinity.

Soil salinity refers to the accumulation of salts in the soil, and can occur through natural processes – such as through weathering of rocks or through deposition from the ocean by wind, rain, and floods – or through artificial processes – such as through irrigation. Soils are classified as saline when electrical conductivity (ECe) of the soil reaches 4 dS per meter (dS m^{-1}). Worldwide, more than 800 million hectares of land (more than 6% of the world's land area) are considered saline; of land used for agriculture, 2% of non-irrigated land and 20% of irrigated land is affected by salinity. Each year, about 1% of irrigated land is abandoned due to excessive soil salinity. Although several types of salt can accumulate in soils, sodium chloride (NaCl) poses the greatest threat because it is the most common salt in soils and because it is highly soluble, resulting in a high concentration of Na^+ and Cl^- present in the soil. Some plant species show more sensitivity to Cl^- than to Na^+, but in most species, including cereal crops, Na^+ is the more toxic ion; hence, here the focus will be on Na^+.

Great variability in salt tolerance exists among crop species, although most are relatively sensitive. Rice, a staple food source for billions of people, is one of the most salt-sensitive cereals. Wheat is somewhat more tolerant, while barley is one of the more salt-tolerant cereals. Among non-cereals, beans and potatoes are very sensitive, tomatoes are sensitive, and sugar beet and cotton are more tolerant. It is estimated that the annual cost of worldwide losses in crop yield due to soil salinity is US $27.3 billion. Several land management strategies have been developed in an attempt to ameliorate soil salinity and reduce associated crop losses. These practices include physical removal of salts from the soil, increased field drainage, and purification of irrigation water; however, these practices are costly and rarely effective. Thus, much focus has been placed on understanding how plants respond to soil salinity in order to enhance tolerance of important crop species.

Na^+ Entry and Movement within Plants

By lowering the osmotic potential of the soil, dissolved salts in the soil solution can inhibit plant growth even without

entering the plant; however, the greatest inhibitory effect of salt – and particularly of Na^+ – comes after entry into plant cells. Na^+ enters plant cells by moving passively across the plasma membrane. Once inside cells, Na^+ can remain in the cytosol, be sequestered within organelles such as the vacuole, or be effluxed back out of the cell. Na^+ efflux from cells can result in Na^+ being extruded from the plant (if occurring in epidermal roots cells) or accumulating in the apoplast (if occurring in internal cell layers such as the cortex). Na^+ in the apoplast can re-enter cells or move radially through the root until reaching the Casparian strip, at which point it must be transported into endodermal cells in order to reach the stele. Na^+ remaining in the cytosol of root cells can move radially to the root stele and be exported from stelar cells into the xylem apoplast, where it is taken up in the xylem transpiration stream and moved up the plant into the shoot. Na^+ can be retrieved from the xylem, but most often, Na^+ entering the xylem eventually accumulates in leaves. Some particularly salt-tolerant species are able to extrude Na^+ from leaves via specialized salt glands or bladders, but such adaptations are rare in crops.

Mechanisms of Salinity Tolerance

Salinity affects plant growth in many ways (Figure 1). At the cellular level, a reduction of cell expansion in roots and young leaves can be observed within minutes to hours of a plant being exposed to salinity. This rapid response is independent of the accumulation of Na^+ in the shoot, and has therefore been referred to as the shoot ion-independent phase. At the whole plant level, this shoot ion-independent response can result in smaller leaves that emerge more slowly, early flowering, and closing of stomata. Although part of this growth reduction may be due to osmotic stress resulting from salt in the soil, there still appears to be a Na^+-specific component of the growth reduction. It is unclear how Na^+ inhibits growth even before accumulating in the shoots, but the response may be mediated by rapidly moving signals in the plant, such as waves of calcium ions (Ca^{2+}) or reactive oxygen species (ROS).

Salinity also affects plant growth after Na^+ has entered cells and accumulated in the shoots; indeed, shoots are the site of greatest Na^+ toxicity. Na^+ is toxic to plant cells primarily because it interferes with potassium (K^+)-based metabolism. K^+ is a required cofactor for many enzymes, but because Na^+ is similar in structure to K^+, it is able to interfere with K^+-stimulated enzymes and prevent their activation. At the cellular level, this can cause reductions in both cell division and cell expansion. In leaves, Na^+ can reduce the rate of photosynthesis, leading to smaller leaves, smaller roots, and ultimately plant death. To reduce the toxic effects of Na^+ in leaves, plants have evolved mechanisms to either exclude Na^+ from leaves or tolerate the Na^+ that enters them.

Preventing Na^+ from reaching leaves involves reducing influx from the soil, increasing efflux out of the root, reducing

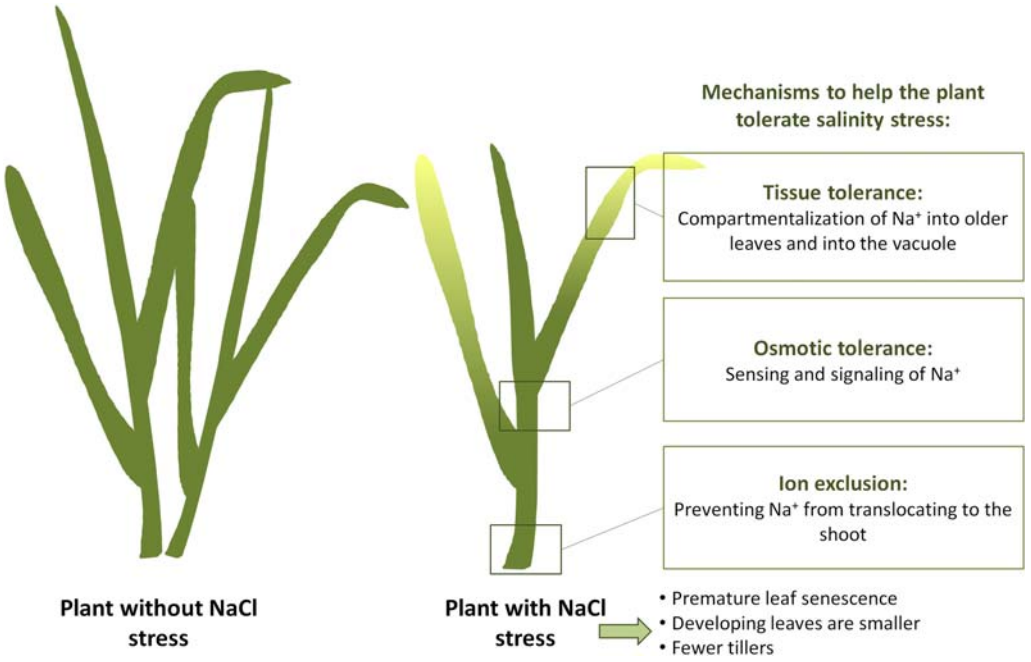

Figure 1 Visible effects of salt stress on the plant phenotype and the three main salinity tolerance mechanisms in a crop plant. Plants subject to salinity stress often show symptoms of yellowing of the leaf (which can be attributed to premature leaf senescence), slower development of new leaves, smaller leaves, and fewer tillers/reduced branching. Tissue tolerance refers to processes that reduce the amount of Na^+ in the cytosol of photosynthetically active leaves, at the tissue level by translocation of Na^+ into older leaves, and at the cellular level by compartmentalization of Na^+, for instance, into vacuoles. Osmotic tolerance refers to processes that minimize the effects of the shoot ion-independent component of salinity stress; these may involve yet unknown mechanisms such as sensing and signaling. Ion exclusion refers to processes that occur predominantly in the root, where the translocation of Na^+ to the shoot is limited, therefore reducing the accumulation of Na^+ in the shoot. These mechanisms include, for instance, retrieval of Na^+ from the xylem, compartmentalization of Na^+ into cortical cells, and the efflux of Na^+ out of the root (into the soil).

xylem loading, and increasing retrieval from the xylem. The mechanisms regulating Na^+ influx are not fully understood, but may involve nonselective cation channels and other Na^+ transporters. Variation in Na^+ influx rates have been measured in plant species with contrasting levels of salinity tolerance, and in some cases, this has been attributed to higher selectivity of nonselective cation channels for K^+ over Na^+. Na^+ efflux is likely mediated in part by SOS1, a Na^+/H^+ antiporter localized to the plasma membrane. SOS1 has also been implicated in playing a role in loading Na^+ into and retrieving Na^+ from the xylem, although it is thermodynamically unclear how the protein could perform both functions. A more clear role for Na^+ retrieval from the xylem has been assigned to Na^+ transporters belonging to the HKT family.

Despite these mechanisms to limit the amount of Na^+ reaching the shoot, plants growing in the continued presence of high salt concentrations will eventually begin to accumulate Na^+ in the shoot. Plant mechanisms to tolerate this shoot Na^+ include the preferential deposition of Na^+ into older, less photosynthetically active leaves or the sequestration of Na^+ in intracellular compartments. Sequestration of Na^+ within organelles decreases the amount of toxic Na^+ in the cytosol, and has been attributed to members of the NHX family of Na^+/H^+ antiporters; however, mounting evidence indicates that these proteins may preferentially transport K^+, suggesting that other proteins may transport Na^+ into organelles. The compartmentalization of Na^+ in organelles – especially large organelles such as the vacuole – can help plants maintain turgor pressure but also requires a corresponding increase in compatible osmolytes (such as proline, sucrose, and glycine betaine) in the cytosol.

The mechanisms of salinity tolerance are complex and are effective on many levels (such as the molecular, cellular, or plant level). Understanding these mechanisms and knowing the key proteins that contribute to salinity tolerance can inform research and the biotechnological approaches used to improve the salinity tolerance of crop plants. There are two main approaches currently used to develop crop plants with improved salinity tolerance: conventional breeding and the generation of genetically modified plants using transgenes. These approaches are outlined below. A third approach is the domestication of halophytes and involves the breeding of halophytes to obtain extremely salt-tolerant plants with desirable agronomic traits. However, very few halophytes have been domesticated to date; hence, this approach will not be further discussed here.

Breeding of Crops with Improved Salinity Tolerance

One approach to improve a plant's salinity tolerance is to introduce genes that will help the plant to maintain productivity despite being exposed to salinity stress. These genes can be introduced into a commercial variety (a variety that has the desired productivity and quality in non-stressed conditions) by conventional breeding with a salt-tolerant relative. This relative, which has been selected for its superior salinity tolerance traits, could, for instance, be a wild species or a landrace. Wild species usually cannot be used as commercial crops as they have many detrimental agronomic traits (such as low-yield and poor-quality traits). Landraces are often local varieties that are only being used in some remote/rural locations, but are predominantly not commercially viable because they also have many detrimental agronomic traits. For the conventional breeding process, a commercial variety is crossed with the variety that contains the tolerance trait(s) of interest (e.g., a wild variety with superior salinity tolerance). The generated offspring will have half of the genome from the commercial variety and half of the genome of the wild relative. To obtain offspring that do not carry the detrimental traits of the wild relative, many backcrosses to the commercial variety are necessary, so that the offspring's genetic composition will be predominantly derived from the commercially viable parent and only contains the genetic region(s) determining salinity tolerance from the wild/landrace parent. This process takes many generations and often requires extensive phenotypic characterizations in the field. Recent advances, such as the use of marker-assisted selection (MAS), improve the screening process. For this technique, the genetic region contributing to salinity tolerance in the wild/landrace parent has to be identified, and a genetic marker has to be developed that allows determining the presence or absence of the genomic region linked to salinity tolerance. It is assumed that the presence of the genetic marker is linked to the presence of the desired phenotypic trait, that is, salinity tolerance. Consequently, the presence of the genetic marker (and, hence, the salinity tolerance trait) is assessed on DNA derived from the offspring generations. Only offspring with the desired genomic region will be used for breeding the subsequent generations. The major benefit of this technology is that DNA can be obtained from very young offspring individuals, and the very laborious and time-consuming phenotypic characterization over many plant life cycles in the field can be omitted in the first generations.

One example where conventional breeding combined with MAS was successful in producing a crop with improved salinity tolerance is the introgression of a genetic region from a wild wheat relative, *Triticum monococcum*, into a commercial wheat variety, Tamaroi. In a field study carried out over a number of years, the improved Tamaroi variety showed a yield increase of 24% compared to the Tamaroi control in soil with high salinity. Importantly, the improved variety produced similar yields in soil with low salinity, relative to the Tamaroi control; a yield penalty under non- or low-saline conditions would be detrimental for commercial viability. In this study, the genomic region conferring improved salinity tolerance was identified using quantitative trait loci (QTL) analysis, and the genomic region was named *Nax2*. The QTL approach is an important tool to link genomic regions to quantifiable traits, such as salinity tolerance. The likely candidate gene underlying *Nax2* belongs to the HKT family, and was named *TmHKT1;5-A*. The naming indicates that the gene originates from the wild wheat relative *T. monococcum*. It has been shown that the *TmHKT1;5-A* gene product is a transporter involved in the increased removal of Na^+ from the xylem in the roots, resulting in reduced Na^+ content in the leaves. As discussed previously, maintaining reduced Na^+ content in leaves is one tolerance mechanism to enable the plant to maintain low cytoplasmic Na^+ concentrations in photosynthetically active tissues, which enables the plant to maintain normal cellular and metabolic functions under saline conditions.

Genetic Engineering of Crops with Improved Salinity Tolerance

The genetic engineering approach is based on the introduction of one or multiple genes from an organism into the plant of interest. The donor organism does not have to be related to the plant, and can be, for instance, a different plant species, an alga, or a bacterium. Salinity tolerance research utilizing genetically modified plants is well established, particularly using model organisms such as *Arabidopsis thaliana* (*Arabidopsis*) or *Nicotiana benthamiana* (a close relative of tobacco). Research with these model plants has led to the discovery of genes conferring salt tolerance, and these are currently being used in the development of genetically engineered crops. For instance, a recent study used QTL analysis of shoot Na^+ accumulation to identify a locus contributing to salinity tolerance in *Arabidopsis*. The protein kinase *AtCIPK16* was identified as the candidate gene mediating reduced shoot Na^+ concentration, thereby contributing to salinity tolerance. Using a transgenic approach, this *AtCIPK16* gene was subsequently expressed in barley. It was shown that hydroponically grown transgenic lines had a reduced shoot Na^+ content and enhanced salinity tolerance based on the shoot fresh weight. So far, these *AtCIPK16*-transgenic barley lines were only tested in a controlled environment in the greenhouse. Ultimately, it is important that the engineered plants have improved salinity tolerance in the field. A recent study demonstrates that the use of a different *Arabidopsis* gene, *AtAVP1*, improves the salinity tolerance of transgenic barley not only in the greenhouse, but also in the field. Higher yields were observed in soil with low salinity and also with high salinity stress compared to the control plants that did not express the *AtAVP1* gene. In *Arabidopsis*, *AtAVP1* encodes a pyrophosphatase that is localized to the tonoplast membrane and facilitates the hydrolysis of pyrophosphate (PP_i) to two inorganic phosphates (P_i), which provides energy for the translocation of a proton (H^+) across the membrane into the vacuole. The process by which AtAVP1 confers salinity tolerance is not yet fully understood. It is hypothesized that the translocation of H^+ from the cytosol into the vacuole provides the electrochemical force for Na^+/H^+ antiporters, hence mediating the compartmentalization of Na^+ into the vacuole. Evidence also supports a different hypothesis, where the reduction of cytosolic [PP_i] by hydrolysis optimizes metabolic processes, such as gluconeogenesis, improving heterotrophic growth and therefore improving plant performance overall, not only during salinity stress.

In most countries, the scientific testing and commercial use of genetically modified crops is currently subject to strict regulations, and engineered crops are not widely accepted by the public. However, research in the past decade has demonstrated the potential of these crops to overcome increasing agricultural constraints such as soil salinity.

See also: **Abiotic Stress:** Free Radicals, Oxidative Stress and Antioxidants. **Arable Crops:** The Domestication of Crop Plants. **Crop Diseases and Pests:** Genomic Selection in Crop Plants. **Plant Breeding and Genetics:** Marker-Assisted Selection; Molecular Markers; Plant Breeding, Practice; Plant Breeding, Principles; Plant Genomes; Transformation and Transgene Expression. **Plant Nutrition:** Ion Transport.

Further Reading

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PHOTOSYNTHESIS

Contents

C_3 Plants

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Nomenclature

Carbon assimilation Conversion of inorganic carbon dioxide into organic carbon compounds, enriched with energy, such as sugars
Chloroplast stroma Soluble compartment of chloroplasts, enclosed by the envelope membranes, and contains enzymes, metabolites, nucleic acids, and lipid granules
Metabolic flux Rates of turnover of biochemical intermediates through metabolic pathways
Metabolomics The systematic study of the unique chemical fingerprints, in the form of small metabolite profiles, that are left by specific cellular reactions
Posttranslational modification Modification of a protein (such as folding and cutting) which occurs after translation and may involve processes, such as phosphorylation, carbamylation, and proteolytic cleavage
Proteomics The analysis of the expression, localization, functions, and interactions of the proteins occurring in living organisms

Abbreviations

BPGA 1,3-bisphosphoglycerate
CA1P 2-carboxy-D-arabinitol 1-phosphate
DHAP Dihydroxyacetone phosphate
E4P Erythrose 4-phosphate
F6P Fructose 6-phosphate
FBP Fructose-1,6-bisphosphate
FBPase Fructose-1,6-bisphosphatase
GAPDH Glyceraldehyde 3-phosphate dehydrogenase
PGA 3-Phosphoglycerate
PRK Phosphoribulo kinase
R5P Ribose 5-phosphate
ROS Reacvtive oxygen species
Rubisco Ribulose-1,5-bisphosphate carboxylase/oxygenase
RuBP Ribulose-1,5-bisphosphate
S7P Sedoheptulose 7-phosphate
SBP Sedoheptulose-1,7-bisphosphate
SBPase Sedoheptulose-1,7-bisphosphatase
TPT Triose phosphate transporter
Xu5P Xylulose 5-phosphate

Introduction and Scope of this Article

Photosynthetic carbon assimilation by plants is an important event in the global carbon cycle. Plants fix carbon primarily into 3-phosphoglycerate (PGA, a 3-carbon compound) and hence the process is named the C_3 photosynthesis or C_3 pathway or Calvin cycle. The other two variants of photosynthetic carbon assimilation are C_4 photosynthesis (or C_4 pathway) and crassulacean acid metabolism (CAM). However, the carbon from C_4 acids formed initially during these two pathways has to be refixed ultimately through the C_3 or Calvin cycle. Thus, the C_3 photosynthesis is the basic route of carbon assimilation while the C_4 pathway and CAM function as carbon-concentrating mechanisms and form adjuncts of the Calvin cycle. Plants possessing only the Benson–Calvin cycle are called C_3 plants, while the other two categories include C_4 plants and CAM plants.

The present article focuses primarily on the photosynthetic carbon assimilation through C_3 pathway. We present an updated view on the basics of carbon assimilation through C_3 pathway. The topics of C_4 pathway, CAM, and assimilate partitioning are all described in other articles of this book.

Biochemistry/Reactions

The combined use of two-dimensional paper chromatography and radio isotopic carbon (^{14}C) unravelled the route of C_3 pathway. C_3 photosynthesis has three principal phases: carboxylation, reduction, and regeneration. During the first phase of carboxylation, carbon dioxide is accepted by ribulose-1,5-bisphosphate (RuBP) to give two molecules of PGA. During the next phase of reduction, PGA is reduced to triose phosphate (triose-P), by using assimilatory force or power (generated in light reactions) of ATP and NADPH. The last phase is the regeneration of the primary acceptor of CO_2, RuBP, from triose phosphate through a series of reactions. For each molecule of CO_2 fixed, three ATP and two NADPH molecules are required.

The first step of C_3 photosynthesis is catalyzed by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which prefers CO_2 as the substrate. The Km values of Rubisco for CO_2 of C_3 plants are in the range of 15–25 μM compared to the concentration of dissolved CO_2 within the plant cell (of <10 μM). As a result, the efficiency of Rubisco is low and limited at the atmospheric CO_2. However, this phenomenon is compensated by the presence of large amounts of Rubisco protein, which could be up to 50% of leaf protein. Rubisco is a bifunctional enzyme, besides being a carboxylase Rubisco; it also catalyzes oxygenase activity, where O_2 reacts with RuBP to form phosphoglycolate, in addition to PGA. The affinity of Rubisco for CO_2 and O_2, at typical *in vivo* concentrations, is quite similar, and as a result the carboxylase and oxygenase reactions are unavoidable in the present atmospheric levels of O_2 and CO_2.

The next phase of reduction of PGA to triose-P involves three steps: (1) activation of PGA to 1,3-bisphosphoglycerate or BPGA (catalyzed by PGA kinase); (2) reduction of BPGA to glyceraldehyde 3-phosphate (GAP) using NADPH as a reductant (catalyzed by GAP dehydrogenase, GAPDH); and (3) interconversion of GAP and dihydroxyacetone phosphate (DHAP) by the enzyme triose-P isomerase (Figure 1). Both GAP and DHAP are triose phosphates.

During the third phase, RuBP (the CO_2 acceptor) is regenerated from triose-P through a series of reactions involving condensation and rearrangement. The triose phosphates, GAP and DHAP, are condensed by aldolase to form fructose-1,6-bisphosphate (FBP). This six-carbon sugar is then irreversibly hydrolyzed to fructose 6-phosphate (F6P) by fructose-1,6-bisphosphatase (FBPase). A two-carbon entity is transferred from F6P to GAP by the enzyme transketolase to form xylulose 5-phosphate (Xu5P) and erythrose 4-phosphate (E4P). Then, E4P is combined with DHAP to form sedoheptulose-1,7-bisphosphate (SBP), by the enzyme aldolase. This seven-carbon product, SBP, is hydrolyzed by sedoheptulose-1,7-bisphosphatase (SBPase) to yield sedoheptulose 7-phosphate (S7P). Two carbons from S7P are transferred to GAP by transketolase producing Xu5P and ribose 5-phosphate (R5P). The resulting R5P is converted to ribulose 5-P (Ru5P) by phosphopentose isomerase. The two molecules of Xu5P are converted into Ru5P by phosphopentose epimerase. The final step of the regeneration phase is the irreversible conversion (using ATP) of Ru5P to RuBP by phosphoribulokinase (PRK).

Products

The exportable products from the Calvin cycle are triose-P (GAP/DHAP) and fructose 6-P. The triose-P is used for two major products of photosynthesis: (1) starch (a glucose polymer), formed via fructose-P and accumulates during the day inside the chloroplast; and (2) sucrose, which is formed in the cytosol. Triose phosphate is exported from the chloroplast via the phosphate translocator on the inner chloroplast envelope membrane and is further metabolized to sucrose in the cytosol. Sucrose is largely exported from the leaf to different sink tissues, e.g., roots, developing leaves, reproductive organs, and other heterotrophic tissues. Sucrose may also accumulate in the vacuole during the day. Some of the intermediates of Calvin cycle are used also for the biosynthesis of amino acids, lipids, and other secondary metabolites, such as terpenes and shikimate derivatives.

Regulation

C_3 photosynthesis is regulated in multiple ways, but is primarily initiated by illumination and becomes autocatalytic. One of the most important phenomena in the pathway is the light activation of key enzymes, through either thioredoxin-mediated dithiol reduction of cysteines on protein or through changes in the microenvironment (e.g., magnesium or pH levels) of the stroma. After a short delay, the intermediates build up and achieve steady-state rates of photosynthetic conversion of CO_2 to the appropriate levels. Then, changes in substrate availability and metabolite flux rapidly set in motion an efficient autocatalysis of the Calvin cycle. In a long-term mode, the levels and turnover of Rubisco protein also

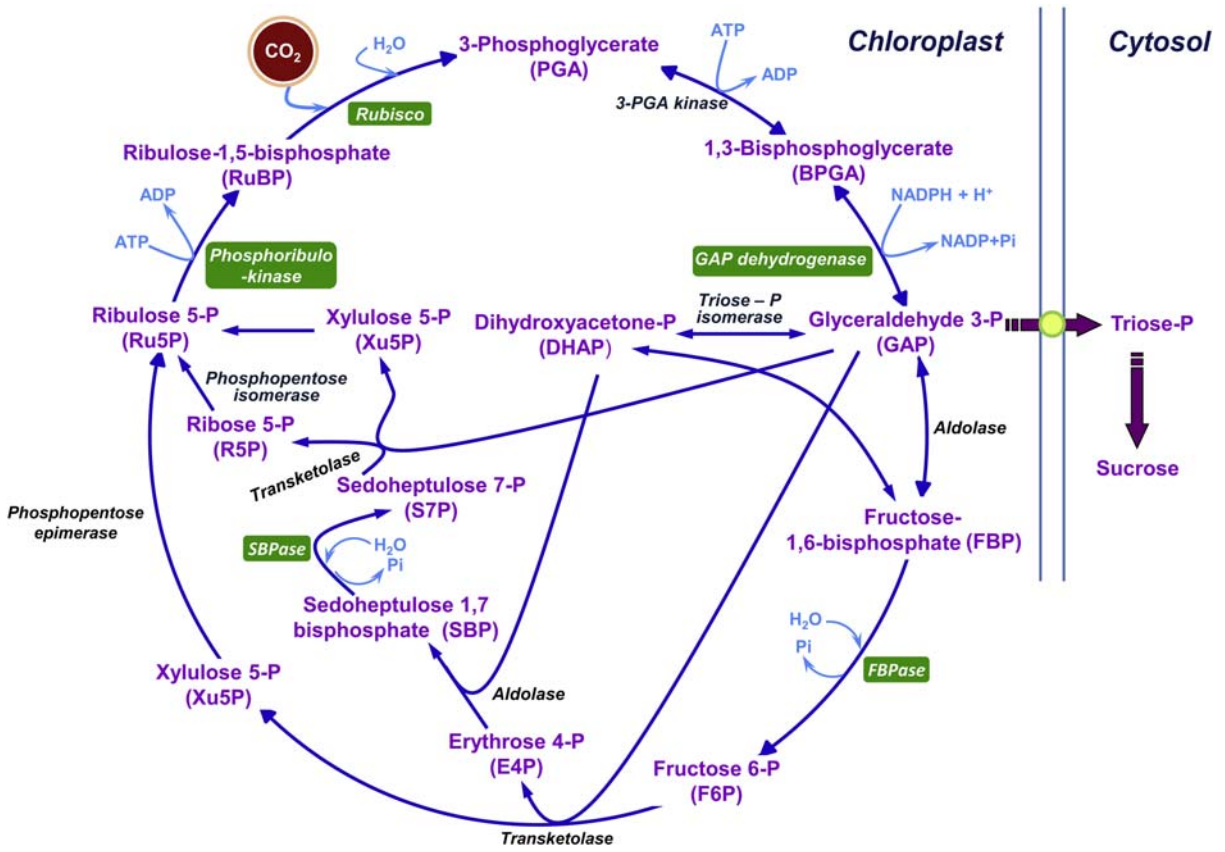

Figure 1 Schematic representation of the photosynthetic metabolism (Calvin cycle) in the chloroplasts of C_3 plants. The Calvin cycle can be divided into three phases: carboxylation, reduction, and regeneration. In the first step, CO_2 is fixed by Rubisco to form 3-phosphoglycerate from RuBP. In the second step, PGA is reduced to glyceraldehyde 3-phosphate, using ATP and NADPH. In the final stage, RuBP is regenerated. The major product of the cycle is GAP, which is either exported out of chloroplast for sucrose synthesis or used for the autocatalysis of the cycle. The enzymes which are known to have rate-limiting control over Calvin cycle activity are highlighted.

modulate the capacity of carbon fixation in the Calvin cycle. For example, limitation of nitrogen availability decreases the levels of Rubisco protein and restricts the photosynthetic carbon assimilation in the leaves.

Rubisco

Rubisco is a unique and interesting enzyme, mediating the first and key reaction of photosynthetic CO_2 assimilation: conversion of one molecule of RuBP and one of CO_2 into two molecules of PGA. Besides the carboxylation reaction, Rubisco reacts with oxygen to form one molecule of 2-phosphoglycolate and one of PGA. The plants recover part of the carbon diverted into phosphoglycolate through the process of photorespiration and lose part of carbon as CO_2. Photorespiration also helps in the recycling of nitrogen and dissipation of excess energy and reductants (ATP and NADPH) in the chloroplasts. A major drawback of Rubisco is its very slow catalytic capacity. The turnover number (K_{cat}) of the enzyme is in the range of 3–10 mol s^{-1}, while most of the enzymes have >1000 mol s^{-1} (for e.g., carbonic anhydrase can be as high as 500 000 mol s^{-1}). As a consequence large amounts of Rubisco (up to 50% of the total soluble protein) are present in chloroplasts to ensure the carbon flux.

The activity of Rubisco is highly regulated. Rubisco is inactive in the dark and is converted to an active form on illumination, which catalyzes fixation of CO_2. Activation of Rubisco is the result of carbamylation, which involves the binding of CO_2 and Mg^{2+} to a lysine residue near the catalytic site (Figure 2). Rubisco is active only when lysine-201 reacts with CO_2 near the catalytic site to form a carbamate and allows the binding of the Mg^{2+}. Carbamylation changes the conformation of the large subunit and activates the enzyme, while the active conformation is stabilized by the formation of a complex with Mg^{2+}. Carbamylation is essential for Rubisco activation, as the noncarbamylated Rubisco binds RuBP too tightly to allow catalysis.

Another protein, Rubisco activase, is also involved in mediating the light activation of Rubisco. On illumination, Rubisco activase releases the inhibitor compounds, such as 2-carboxyarabinitol 1-phosphate (CA1P), which are bound to the active site of Rubisco; otherwise, for example, in darkness, these inhibitors prevent activation (carbamylation) of the enzyme. Rubisco activase itself is activated in light by utilizing ATP produced from photosynthetic electron transport. Rubisco activase and Rubisco activation provide another mechanism of strong regulation by light of carbon assimilating reactions of photosynthesis.

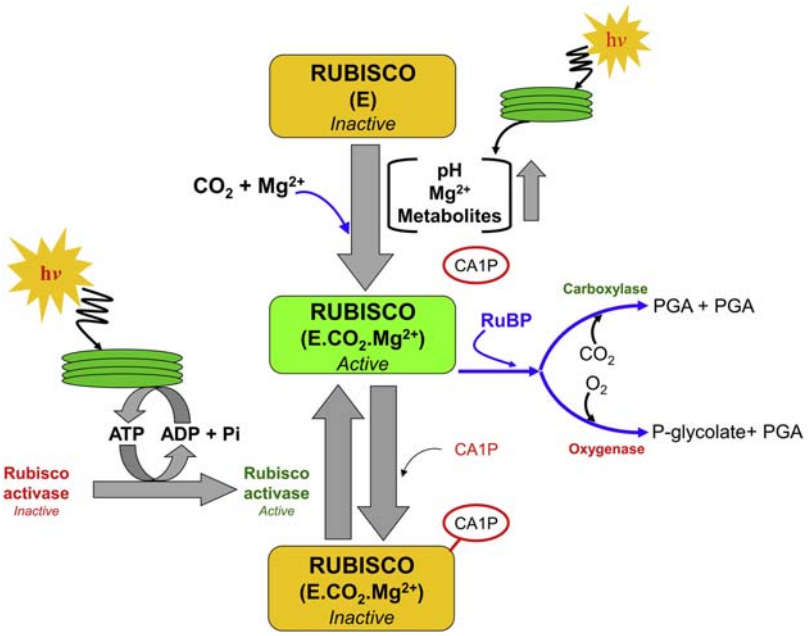

Figure 2 The reactions and regulation of Rubisco. The inactive Rubisco (E) reacts with CO_2 and forms the carbamate, gets stabilized by Mg^{2+}, and becomes active ($E.CO_2.Mg^{2+}$). The active enzyme functions as both carboxylase and oxygenase. The product of the carboxylase reaction, PGA, is metabolized in the Calvin cycle. One of the products of oxygenase, P-glycolate, is metabolized through photorespiration. However, analogues of RuBP in the stroma, can bind to the Rubisco, blocking the active site on the enzyme. For example, 2-carboxy-D-arabinitol 1-phosphate (CA1P) binds tightly to Rubisco and makes it inactive. Thus, Rubisco is activated in light in two process: (1) changes in the stromal microenvironment (increase in pH, Mg^{2+} levels, and Calvin cycle metabolites); and (2) by Rubisco activase, which itself is activated in light through ATP.

CA1P, which occurs naturally in the leaves of several plants, is a strong inhibitor of Rubisco. The affinity of Rubisco for CA1P is much stronger than that for RuBP, the substrate. As a result, CA1P, which accumulates in leaves during the night, inactivates Rubisco by blocking the binding sites. During the day (or on illumination), the bound CA1P is released from Rubisco, and this process is further accelerated by Rubisco activase. However, the physiological role of CA1P is still debated, as it is not found in all plant species.

Light Activation of Enzymes

Besides the modulation of Rubisco, light activates four enzymes of the Calvin cycle, namely GAPDH, FBPase, SBPase, and PRK, by the reduction of dithiols on the enzymes. Interestingly, these four enzymes can catalyze irreversible reactions. Although the dark activities of these enzymes may vary, the Calvin cycle is essentially inactive until illumination.

The Calvin cycle enzymes are activated as a result of the reduction of dithiols on the protein, mediated by a ferredoxin–thioredoxin system (Table 1). On illumination, ferredoxin is reduced by the thylakoids, which in turn reduces thioredoxin. Reduced thioredoxin can reduce the dithiols at or near the active site of these enzymes of the Calvin cycle. The thioredoxin-ferredoxin system (reduced in light) also activates two other chloroplastic enzymes, F_1-ATP synthase and NADP-malate dehydrogenase. Light not only activates several enzymes, but also inactivates the key enzymes of the oxidative pentose phosphate pathway, again through thioredoxin-mediated reduction of enzymes. Glucose-6-phosphate dehydrogenase is one such enzyme, which is inactivated on illumination to ensure that the oxidative pentose phosphate pathway does not operate

Table 1 Summary of Calvin cycle enzymes with the mechanisms involved in their redox regulation

Target enzyme/complex	*Mediating components*	*Posttranslational modulation of protein*
Rubisco activase	Thioredoxin	Dithiol-disulphide interchange
Glyceraldehyde dehydrogenase (GAPDH)	Glutaredoxin	Glutathionylation
Fructose-1,6-bisphosphate aldolase	2-Cys Peroxiredoxin	Nitrosylation
Fructose-1,6-bisphosphatase	NADP-thioredoxin reductase C	Reduction of disulphides
Sedoheptulose-1,7-bisphosphate	Ferredoxin–thioredoxin system	Association–dissociation of supracomplex
Ribose 5-phosphate isomerase		
Phosphoribulokinase (PRK)		
GAPDH-PRK-CP12 complex		

simultaneously with the reductive pentose phosphate pathway, thus avoiding a futile cycle.

Light causes a marked change in the microenvironment of the chloroplast stroma. The photosynthetic electron transport chain in the thylakoid membrane decreases the proton concentration, raises the pH, and increases the Mg^{2+} concentration in the stroma. Rubisco, FBPase, and SBPase respond to such changes in pH and Mg^{2+} concentrations. The enzymes are also under metabolite control. The stromal enzymes FBPase and SBPase are activated by their substrates and allosterically inhibited by their products. High levels of FBP or SBP activate these enzymes, while F6P or S7P inhibit the activity of the corresponding enzyme, to avoid excessive product accumulation and sequestration of phosphate. Similarly, PRK is inhibited by 3-PGA and ADP. As both these metabolites inhibit PRK, phosphorylation by PGA kinase can proceed even under limiting ATP supply, thus preventing the accumulation of PGA.

Multiprotein Complexes

The occurrence and operation of multienzyme complexes involving Calvin cycle enzymes have been reported. Such complexes could be an important mode of regulation. For example, GAPDH and PRK interact with a small nuclear encoded chloroplast protein, CP12, and form a complex, which may be an additional mechanism for light regulation of PRK *in vivo*. Complexes involving different combinations of C_3-enzymes have been found, and these may facilitate rapid channeling of intermediates between enzymes, improving the efficiency of the cycle. Multienzyme complexes of phosphoribose isomerase, PRK, GAPDH, and SBPase have been located on the thylakoid membrane by immunoelectron microscopy.

Flux Control and Carbon Partitioning

Flux control is an important concept, and the relative importance of individual enzymes in controlling the flux of carbon fixation through the Calvin cycle has been examined. Studies of transgenic plants with altered levels of individual enzymes in the C_3 cycle have revealed that no single enzyme has complete control of carbon fixation. Control is shared among a number of enzymes, with Rubisco, SBPase, and aldolase having the most prominent roles. Enzymes with highly regulated activity, such as PRK, do not seem to have strong control on carbon fixation, whereas aldolase, which catalyzes a reversible reaction, strongly influences the rate of carbon fixation.

The partitioning of carbon into the principal end products of Calvin cycle, namely sucrose and starch is quite important. The biosynthesis of sucrose from triose-P in cytoplasm, and export out of leaf tissue, ensures high rates of carbon assimilation. As the triose-P are transported out of the chloroplast in exchange for cytoplasmic Pi (inorganic phosphate), by triose phosphate transporter (TPT) system, the TPT can limit the rate of photosynthesis as well as sucrose biosynthesis. Similarly, an increase in the TPT transport activity might result in an increased crop yield for plants kept in elevated CO_2.

Role of Mitochondria

Besides the intraorganelle events within the chloroplasts, the interaction of photosynthesis with mitochondrial respiration and nitrogen metabolism is quite important for optimization of photosynthesis as well as protection against photoinhibition. The interplay of these three metabolic pathways involves recycling of carbon, nitrogen, reducing equivalents, and ATP, between the compartments of chloroplast, mitochondria, cytoplasm, and peroxisomes. The dependence of photosynthesis on mitochondrial respiration has been linked to both cytochrome and alternative (AOX) pathways, besides uncoupling proteins (UCP). Several mitochondrial functions which help to optimize photosynthesis include the following: (1) providing ATP for sucrose biosynthesis; (2) dissipation of excess reducing equivalents generated in chloroplasts, (3) maintenance of photorespiratory pathway, and even (4) providing intracellular CO_2 for refixation through C_3 pathway.

Mitochondrial oxidative phosphorylation is an important source of energy (in the form of ATP) for sucrose biosynthesis as well as other reactions. During high light, photochemical reactions generate excess reducing equivalents (in the form of NADPH) causing overreduction of photosynthetic electron transport and generation of reactive oxygen species (ROS), leading to photoinhibition. The excess NADPH generated in chloroplasts can be dissipated in cytosol and mitochondria, through respiratory electron transport chain. Similarly, even under moderate light conditions, overreduction of PSII acceptor side can occur, leading to photoinhibition, due to limitation of the linear electron transport chain at the cytochrome b6/f complex. The increasing reduction of the stromal acceptor pool, on the acceptor side of PSI would activate export of malate and possibly cyclic electron transport. The reductant exported via malate could be oxidized in mitochondria. The mitochondrial AOX pathway not only helps in oxidizing NADH but also minimizes the ROS production. Mitochondrial uncoupling protein (UCP-1) helps in efficient oxidation of glycine produced via photorespiration and sustains C_3 photosynthesis. Recent work suggests that there could be additional factors, such as ascorbate, ROS, and nitric oxide, which mediate interorganelle interactions.

Prospects for Improvement of C_3 Plants

Most of the crops (particularly cereals, legumes, and oilseed crops) are of the C_3-type. Thorough understanding of not only biochemistry but also improvement of photosynthetic performance in C_3 plants is therefore of global importance. The CO_2/O_2 concentrations and the *in vivo* activity of Rubisco are rate-limiting factors for carbon fixation. To overcome this limitation, increasing the Rubisco content in leaves is a possibility. However, plants with increased Rubisco contents did not improve the photosynthesis. An alternative strategy for Rubisco improvement is to increase its specificity for CO_2 relative to O_2 by direct manipulation of the enzyme, but has not yet been successful.

Despite the efforts to evolve varieties with reduced photorespiration or high photosynthetic rates, single features were not able to improve the productivity of plants. Mutants

deficient in enzymes of photorespiration were unable to grow at atmospheric levels of CO_2, indicating that the process of photorespiration (as well as Rubisco oxygenase activity) was an adaptation to the present atmospheric levels of CO_2/O_2. Another approach was to introduce a set of C_4 traits into C_3 plants, but hybridization of C_3 and C_4 species of *Atriplex* resulted in only C_3-type plants. However, a well-known factor that can improve the photosynthetic performance and productivity of C_3 plants is elevated CO_2.

The expression of C_3 photosynthesis genes in leaves is modulated by environmental signals such as light, nutrition (particularly phosphorus and nitrogen), and coordination with other organelles (e.g., mitochondria). High levels of glucose or sucrose reduce levels of a number of Calvin cycle mRNAs, including those encoding the small subunit of Rubisco, SBPase, and FBPase. Thus, attempts to modulate the interaction between carbohydrate and nutrient status may be a long-term strategy to improve carbon metabolism.

Genetic Manipulation

Remarkable improvement of photosynthetic performance has been achieved by agronomic practices. Yet increases in rates of photosynthetic carbon assimilation may not all translate into improved yield. Further, genetic modification of photosynthetic metabolism appears to be a complex task, as these processes involve manipulating suites of genes located in different tissues. Several enzymes of the Calvin cycle have been genetically engineered in plants by either overexpression or suppression of the enzyme levels (Table 2). A reduction in the levels of Rubisco, Rubisco activase, and NADP-GAPDH resulted in a significant decrease in photosynthesis, particularly at high light intensity. The manipulation of stromal FBPase or triose-P/Pi translocator affected the growth pattern only marginally, but significantly changed the pattern of assimilate translocation.

The enzymes FBPase and SBPase are usually limited by feedback inhibition by their products. The overexpression of a bifunctional cyanobacterial FBP/SBPase or plant SBPase in tobacco/rice plants increased their photosynthesis and biomass production. When the levels of SBPase activity were suppressed in tobacco (*Nicotiana tabacum*), the plants showed decreased rates of photosynthetic carbon fixation and altered carbohydrate levels in mature source leaves. These effects need to be investigated and the scope needs to be expanded. It may also be necessary to explore additional sources of Calvin cycle from suitable bacteria and their compatibility with higher plants. The photosynthetic performance under stress conditions can be improved by overexpressing genes, which can make plants tolerant.

Table 2 Genetic manipulation of some of the Calvin cycle and closely related enzymes and their effects on photosynthesis

Target enzyme/protein	*Manipulation*	*Test plant*	*Consequence*
Rubisco	Overexpression	Rice	Enhanced Rubisco content but no change in photosynthesis
	Suppression	Tobacco, rice	Decrease in photosynthesis and substantial accumulation of RuBP
Rubisco activase	Overexpression	Rice, *Arabidopsis*	Enhanced thermostability of Rubisco activase and improved photosynthesis
	Suppression	Tobacco	Decreased Rubisco activation state and decreased rate of CO_2 assimilation
Sedoheptulose-1,7-bisphosphatase	Overexpression	Rice, tobacco	Increased photosynthetic carbon assimilation; higher levels of sucrose and starch
	Suppression	Tobacco, rice	Decreased photosynthetic carbon assimilation, low starch, and diminished growth
Fructose-1,6-bisphosphatase	Overexpression	Tobacco	Increased photosynthetic carbon fixation and starch levels
	Suppression	Rice, *Arabidopsis*	Decreased photosynthetic rates and sucrose synthesis
FBP-aldolase	Overexpression	Tobacco	Enhanced photosynthesis, growth and biomass under high CO_2 due to stimulation of RuBP regeneration
	Suppression	Potato	Inhibition of photosynthesis with decreased RuBP content and growth
Glyceraldehyde 3-phosphate dehydrogenase	Suppression	Tobacco	Reduced CO_2 assimilation due to reduction in RuBP regeneration
Transketolase	Overexpression	Cucumber	Increased net photosynthetic rate, carboxylation efficiency, and activities of other Calvin cycle enzymes
	Suppression	Tobacco	Marked decrease in photosynthesis, RuBP regeneration, and growth
Triosephosphate isomerase (TPI)	Suppression	Potato	Increased carbon metabolism in roots, catabolism of sucrose while no change in plastidal TPI
Triose-P translocator	Overexpression	Tobacco	Enhanced photosynthetic carbon assimilation and growth during light
	Suppression	Tobacco, *Arabidopsis*	Decreased rates of photosynthesis, growth, and increased starch mobilization under high light but no effect under normal conditions

C_3 to C_4 Photosynthesis

Since C_4 plants have a proven ability to exhibit higher photosynthetic performance and productivity, than those of C_3 plants, an immediately attractive approach is to transform C_3 plants into C_4 type. Initial attempts were simple and aimed at transforming rice with genes encoding C_4 enzymes, such as PEPC, PPDK, and the NADP-ME. But these attempts so far did not achieve an overall efficiency of CO_2 fixation typical of C_4 plants. Introduction of an intracellular CO_2 pump can improve the efficiency of C_3 photosynthesis by a substantial suppression of photorespiration. In this direction, the information on single-cell C_4 plants and unicellular photosynthetic organisms, including cyanobacteria and green algae, is indeed a boost to the efforts of engineering rice plants to perform C_4 photosynthesis without having to introduce a dual Kranz cell system.

In order to meet the increasing needs of food for the global community, the yields of rice and other basic crop plants must be increased by at least 50% over the next 40 years to prevent malnutrition. This can be achieved by improving photosynthetic efficiency of the plants. An alternative approach has been to identify the morphological and physiological features of rice to optimize photosynthesis, by optimizing light-use efficiency of individual plants as well as in a crop stand as in 'super rice.' Though the introduction of C_4 photosynthetic genes, PEPC and PPDK genes, to the rice (C_3 plant) was successful, the increase in photosynthetic capacity and the efficiency of CO_2 assimilation were not consistent. Even the yield of rice per growing unit area and grain bearing rate decreased. Enhanced photosynthesis under high-temperature conditions was observed in genetically engineered indica rice expressing PEPC gene cloned from maize.

Concluding Remarks

The performance of C_3 plants improves considerably when the atmospheric CO_2 level is elevated. Thus, under conditions of global warming with its elevated CO_2 levels, the C_3 plants have much greater potential than the C_4 plants. However, the C_3 plants may require additional nutrients, particularly P and N, under high CO_2. This aspect needs to be studied further so that the C_3 plants can be exploited effectively under elevated CO_2. Another possibility of improving the performance of C_3 plants is to supplement them with CO_2-concentrating mechanisms, as in C_4 or CAM plants.

Despite detailed studies, the functional mechanism of Rubisco continues to be a marvel and a mystery. An increase in the ratio of carboxylase to oxygenase of Rubisco would be promising to increase the photosynthetic efficiency. Some of the red algae, such as *Galdieria* species were found to possess 'super-Rubisco' with a much higher carboxylase to oxygenase ratio than that of higher plants, but still has only a low turnover. Attempts are being made to incorporate such 'super-Rubisco' into higher plants. Further work on model C_3 organisms is essential to progress toward manipulation and improvement of C_3 pathway. Plant species such as *Arabidopsis thaliana* and rice, besides the algal species including *Synechocystis* and *Chlamydomonas*, would be useful in this regard.

A consolidated effort by plant biologists of various expertise include physiology, biochemistry, molecular biology, and agronomy would be required to achieve the objectives of making C_3 plants into C_4 type. In this context, 'Systems Biology' approach may be appropriate to understand the complex interactions in cells, organs, and entire organisms. It may be also necessary to evolve out-of-the-box approaches. Detailed analyses of proteomics and metabolomics of leaves of typical C_3 species may reveal the novel proteins and patterns of metabolite fluxes. The large-scale data ('omics') analysis involves computational and mathematical tools. These models can be used to study the adaptive significance of molecular changes in photosynthetic apparatus to photosynthetic carbon assimilation.

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See also: **Photosynthesis:** C_4 Plants; CAM Plants; Photoinhibition; Photorespiration; Photosynthesis.

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