Plant Response to Water-deficit Stress

Elizabeth A Bray, University of Chicago, Chicago, Illinois, USA

When plants do not receive sufficient water they are subjected to a stress called water deficit. Water deficit in the plant disrupts many cellular and whole plant functions, having a negative impact on plant growth and reproduction. Plants have evolved many different mechanisms to deal with the occurrence of this stress as it occurs in their environments. Availability of water is the most important factor in the environment that reduces the production of our crops.

Effects of Water Deficit on Plant Growth and Development

Drought, a period of abnormally dry weather, results in soil-water deficit and subsequently plant-water deficit. The lack of water in the environment constitutes a stress when it induces an injury in the plant. Water deficit in the plant disrupts many cellular and whole plant functions, having a negative impact on plant growth and reproduction. Crop yields are reduced by 69% on average when plants are exposed to unfavourable conditions in the field (Boyer, 1982). Availability of water is the most important factor in the environment that reduces the production of our crops. As water is increasingly needed for human populations and prime agricultural lands are used for housing, the availability of water will have a greater impact on our ability to produce crops. See also: Agricultural Production

In nature, certain species are adapted to dry environments. The genotype determines the ability of the plant to survive and thrive in environments with low water availability. In addition, the duration of the water-deficit stress, the rate of stress imposition and the developmental stage of the plant at the time of stress imposition also affect plant growth and the ability of the plant to produce a crop. Whether the amount of water present in the environment is a stress is different for each species. Sensing mechanisms, yet to be identified, initiate the responses to water deficit, which occur at the molecular, metabolic, cellular, physiological and developmental levels. Many of these responses are driven by changes in gene expression. See also: Hot Deserts

Resistance can Occur through Avoidance or Tolerance of Water-deficit Stress

Resistance to water deficit may arise from the ability to tolerate water deficit or from mechanisms that allow avoidance of the water deficit (Figure 1). Some species, such as desert ephemerals, are able to escape drought by completing their life cycle when water is plentiful. Others avoid water deficit with the development of a large root system that permits improved extraction of water from the soil. Avoidance of water deficit may also be achieved by using mechanisms that save water as in succulents. Some plants may also have improved water-use efficiency, such as found in crassulacean acid metabolism plants, in which stomata are open at night and an alternative form of carbon assimilation promotes the use of less water. However, these plants do not tolerate water deficit. Other plants have biochemical and morphological mechanisms such as found in mosses and resurrection

Figure 1 Resistance to water-deficit stress can arise from mechanisms involving avoidance or tolerance of the water deficit.
plants that permit the plants to withstand dehydration. See also: Crassulacean Acid Metabolism; Plant Stress Physiology

Morphological characteristics of some species permit continued survival in arid environments, while other plants must acclimate to the stress to permit survival. Some responses may promote physiological adaptation to water deficit; other responses may indicate that an injury has occurred. It is difficult to distinguish functions of the responses to water-deficit stress – Which responses are a direct result of water deficit and are effective in preparing the cell to function in a water-deficit condition? Some mechanisms promote survival and limit injury, but may do this using a mechanism that slows crop production, and are therefore not useful strategies to utilize in crop plants. It is important to identify mechanisms that permit continued growth during periods of water deficit to promote crop production.

**Water Dynamics of the Plant Cell**

The water within the cell is defined in terms of its free energy content or ability to do work. The free energy per unit volume of water is the water potential ($\psi_w$). Water is taken into the plant if the water potential is less than that of the environment surrounding the cell (Figure 2), since water moves down a chemical gradient. If the water potential in the soil solution is higher than that of the cells, water can be transported into the cells of the root. The water potential of the cell is dependent upon two important parameters: the osmotic potential ($\psi_s$) and the turgor pressure ($\psi_p$). The content of solutes in the water of the cell ($\psi_s$) and the pressure of the cellular contents against the cell wall ($\psi_p$) decrease the water potential. An additional component, the matric potential ($\psi_m$), or the binding of water to surfaces, also reduces the cell water potential. Equation (1) is used to describe cell water potential.

$$\psi_w = \psi_s + \psi_p + \psi_m$$  \[1\]

Changes in cellular water relations trigger further events that are manifested in plant responses at the molecular, metabolic, cellular, physiological and developmental levels. See also: Plant–Water Relations

**Whole Plant and Physiological Responses**

The water of the plant can also be viewed in the context of a soil–plant–air continuum: the plant is a column of water between the soil and the air. Transpiration, release of water from the plant, will continue when water is available. Under drought conditions in the field, soil-water content drops, which does not favour water movement into the root cells. Water will be lost through transpiration and will not be fully replaced, causing a loss in turgor in the plant cells. As a defence against water loss, transpiration decreases. In the leaf, the pore of the stomatal complex closes in response to soil-water deficit. As turgor decreases in the guard cells surrounding the stomatal pore, the cells fill the pore, thus reducing the stomatal aperture; this is the main cause of reduced transpiration. An *Arabidopsis* leucine-rich repeat receptor-like kinase (ERECTA) regulates leaf transpiration efficiency through a combined effect on stomatal density and photosynthetic capacity (Masle et al., 2005). When the stomata are closed, uptake of carbon dioxide is also reduced, reducing the carbon assimilation rate of the plant. Depending upon the duration of the water deficit, this may reduce crop production and cause injury to the chloroplasts through the process of photoinhibition. There may also be an interaction with other stresses, such as heat stress, when transpiration is reduced that will also contribute to the strain on the plant. See also: Forest Ecosystems; Photosynthesis: Ecology; Plant Stress Physiology; Plant Temperature Stress

As cellular turgor approaches zero, growth will be inhibited. Cellular expansion or growth depends upon cellular turgor. The pressure of the cellular contents against the cell wall is the driving force for cell expansion and turgor is dependent upon uptake of water. The stage of development of the plant at the time of the occurrence of water deficit will alter the outcome. Growth will return if turgor is restored as the plant acclimatizes to water-deficit stress. See also: Plant Cell Growth and Elongation; Turgor Pressure

![Figure 2](image-url)
Osmotic Adjustment Permits Water Uptake and Turgor Recovery

In a process called osmotic adjustment, metabolism may be altered to maintain cellular water content through an increase in the concentration of solutes. In these cells, the osmotic potential is lowered and thus the water potential of the cell is lowered, permitting water uptake to be maintained (Figure 2). Turgor will fully or partially recover depending upon the external water potential. The cells will avoid a loss of water, yet they must be able to withstand low cellular water potential. The solutes that accumulate, called osmolytes, include sugars, proline and quaternary ammonium compounds such as glycine betaine. They are generally thought to be neutral to metabolic processes, and therefore do not disrupt plant function. The ability to adjust osmotically is dependent upon the genotype and is a more successful defense in resistant genotypes. Interestingly, osmolytes may have additional functions in stress resistance including the ability to stabilize proteins and ameliorate oxidative stress that may arise when plants are subjected to water deficit. Plants engineered to produce more glycine betaine have improved photosynthetic capacity when grown under water-deficit conditions. Engineering to increase accumulation of the osmolyte mannitol has not resulted in a level of accumulation that is sufficient to alter osmotic adjustment, but plant performance in response to stress is improved nonetheless. These overexpression studies may indicate that the alternative roles, rather than the first studied role in osmotic adjustment, may be the more important role for osmolytes. See also: Ozone and Reactive Oxygen Species

Membrane permeability to water and ions is also involved in the control of cellular water potential and turgor. The water channels, called aquaporins, are proteins that form a channel in the membrane that specifically facilitates transport of water across the membrane. These channels facilitate water transport using osmotic or hydraulic driving force. There are four different classes of these proteins. Plasma membrane intrinsic (PIP) and tonoplast intrinsic (TIP) are water channels in the plasma membrane and tonoplast, respectively. NOD26-like intrinsic proteins have glycerol transport activity and the function of the final class is uncharacterized. All but 4 of the 35 aquaporin-like genes in the Arabidopsis genome are downregulated at the RNA level by water-deficit conditions. This decrease in transcript abundance may limit loss of water from the cells. Plants engineered to reduce the expression of aquaporins have implicated them in root hydraulic conductivity, water-deficit stress resistance and in the ability of plants to recover from water-deficit stress. Other transport proteins including K⁺ channels are also likely to be involved in the response to water deficit. See also: Plant Ion Transport; Water Channels: Aquaporins

The Role of the Signalling Molecule Abscisic Acid

Loss of water is a physical stress in the environment that initiates biochemical events. The mechanism to sense the stress and the signal transduction events that follow are not understood. However, it is certain that the cell must have a mechanism to recognize a decrease in water content, which is probably related to turgor pressure. One intermediary in the signalling pathway is the plant hormone abscisic acid (ABA). The ABA content of the plant increases in response to water deficit by alterations in ABA biosynthesis and catabolism. ABA accumulates in all of the plant organs and this response is important for physiological and molecular responses to water deficit, with the most studied response being stomatal closure.

The pathway of ABA biosynthesis is complex and required a number of dedicated scientists using genetic, biochemical and molecular approaches to unravel it. The breakdown of carotenoids, rather than synthesis from a smaller carbon backbone, is the pathway taken. During periods of water deficit, the enzyme, 9-cis-epoxy-carotenoid dioxygenase (encoded by a family of genes named NCED), completes this oxidative cleavage of cis-xanthophylls to xanthoxin (Figure 3). The Arabidopsis gene family contains nine members, and five are likely involved in ABA biosynthesis, with the one member NCED3, playing a major role during stress. There are two steps remaining in the synthesis of ABA with ABA-aldehyde as the immediate precursor to abscisic acid. The gene ABA2 from Arabidopsis encodes a short-chain alcohol dehydrogenase that converts xanthoxin into abscisic aldehyde. Abscisic aldehyde oxidase 3, AAO3, one of four such oxidases in Arabidopsis, oxidizes abscisic aldehyde to ABA. This enzyme requires a sulphurylated form of molybdenum cofactor (MoCo), and thus the Arabidopsis gene ABA3, a MoCo sulfurbate is also required for ABA biosynthesis. This requirement was originally identified in the tomato mutant flacca which also has a mutated MoCo sulfurbate. In response to water-deficit conditions, NCED3, AAO3 and ABA3 are all induced.

Since the plant hormone is rapidly degraded, the breakdown of the molecule also has an important role in controlling the concentration of ABA in the plant. ABA is catabolized by hydroxylation and conjugation (Figure 3). The hydroxylation pathway results in oxidation of the 7', 8' or 9'-carbon of the ring structure. The major pathway of catabolism begins with 8'-hydroxylation by the cytochrome P450 monoxygenase CYP707A, followed by spontaneous conversion to phaseic acid. Dihydrophaseic acid (DPA) and DPA glucoside are further catabolites. ABA and its hydroxylated catabolites may also be conjugated with glucose.

The accumulation of ABA in turn initiates a series of events (Figure 3), many of which promote plant adaptation to the conditions of water deficit. First, ABA must be recognized in the cell. One ABA receptor, encoded by a gene
controlling flowering time in Arabidopsis, FCA, has recently been identified (Razem et al., 2006), although, this ABA receptor is unlikely to be the only receptor involved in water-deficit responses since this receptor does not function in the control of stomatal conductance. FCA is an RNA-binding protein that promotes transition to flowering. ABA recognition initiates a signal transduction pathway that is composed of such signalling components as kinase/phosphatase cascades, RNA-processing proteins and calcium. Many mutants in ABA signalling pathways are being used to sort out the complex interactions that control ABA responses. Finally, signal transduction fulfils the action of ABA by activating gene expression. See also: Floral Meristems

Genes Regulated by Water Deficit Affect Water-deficit Resistance

The information contained within the genome of each species dictates the plant response. The genome controls the regulation of the response to water deficit as well as the effectiveness of the response. Microarrays, largely done using the model plant Arabidopsis thaliana, have been used to catalogue the many genes that are induced and repressed in response to conditions that may lead to cellular water-deficit stress (e.g. Seki et al., 2002). Induced genes are candidates for those that function in the regulation of the plant response or in the adaptation of the plant to the stress. Different research groups, using different methods of exposing Arabidopsis plants to cellular water-deficit stress, have identified more than 800 induced genes (Bray, 2004). These genes can be placed in at least four different functional groups: signal transduction, transcriptional regulation, cellular metabolism and transport and protection of cellular structures (Figure 4). There also remain a substantial group of genes without a known or predicted function. The many different gene products are predicted to function in all of the organelles throughout the cell. See also: Functional Genomics in Plants; Gene Expression in Plants

Figure 3  ABA biosynthesis beginning with a carotenoid and proceeding through the major pathway for catabolism. Both synthesis and breakdown contribute to the level of ABA in a particular organ of the plant in response to water deficit. The numbers in boxes represent enzymes that have been cloned in Arabidopsis. (1) 9-cis-epoxy-carotenoids dioxygenase 3 (NCED3) catalyses the cleavage of cis-xanthophylls during water-deficit stress. (2) The product xanthoxin is converted into ABA aldehyde by a short-chain alcohol dehydrogenase, ABA2. (3) Abscisic aldehyde oxidase AAO3, an enzyme that requires a sulfurylated form of MoCo (synthesised by (4) MoCo sulfursase (ABA3)), completes the final step of ABA biosynthesis. (5) The key step of ABA catabolism is the hydroxylation of the 8’ methyl group to yield 8’ hydroxy ABA by a cytochrome P450 monoxygenase CYP707A3. Phaseic acid is then formed spontaneously. The increased concentration of ABA initiates a signal transduction pathway through an unknown sensing mechanism. This leads to induction of specific genes.
Many proteins that function in signal transduction pathways are induced by stress. Multiple kinase/phosphatase cascades function to regulate the stress response, including a subunit of G proteins and type 2C protein phosphatases (e.g. ABI1) that are induced under multiple laboratory conditions. There are at least six different classes of transcription factors that participate in gene induction or repression in response to water deficit. Homeobox domain and NAC domain containing transcription factors are induced by multiple experimental treatments that mimic water-deficit stress. Accumulation of proteins encoded by genes that have metabolic or structural functions are thought to promote adaptation to the stress. One class of genes that may play a role in protection is called the late embryogenesis abundant (LEA) genes. The LEA genes are also developmentally programmed for expression in desiccating seeds. These genes encode small hydrophilic proteins that are predicted to protect proteins and membranes through chaperone-like functions. Plant genomes encode many of these genes that fall into several different classes based on their amino acid structure and several of them are commonly induced by water-deficit treatments. One of these classes is known as dehydrin. Metabolism may also be altered by cellular water deficit with genes encoding enzymes involved in osmotic adjustment and repair or degradation of damaged cellular contents being induced. Many other enzymes are also induced, but their potential functions are not as readily deduced. Genes that are repressed by water deficit include those involved in photosynthesis and growth, although these genes are not commonly repressed under all water stress conditions. See also: Regulatory Genes in Plant Development: Homeobox; Seeds

The microarray experiments also highlight that the expression of sets of genes are timed differently. The expression profiles indicate that some genes are induced early and transiently while others are gradually increased. Genes induced early largely encode transcription factors and components of signalling cascades. Those in the later category may be those involved in the adaptation of the plant to prolonged water deficit.

### Mechanisms of Gene Regulation

The expression of genes in response to water deficit can be regulated at the transcriptional, post-transcriptional and translational levels. The majority of research has been done to explore the mechanisms of transcriptional regulation. In response to water-deficit stress, there are two major transcriptional pathways of gene expression defined by the
involvement of ABA. The ABA-independent pathway is controlled largely by a family of transcription factors called drought response element binding protein (DREB), which contains a DNA binding motif originally identified in a flower patterning protein called APETALA2, AP2 (Figure 4). The consensus promoter element TACCGACAT was originally identified in cold response genes and called the C repeat. There are two main families of DREB transcription factors, DREB1 (3 genes) and DREB2 (8 genes). In Arabidopsis, DREB2A and B and DREB1D are induced by water deficit and salinity stress, but not by low temperature stress. DREB2A and B proteins require post-translational modification for activation by a mechanism that is not understood.

ABA-dependent gene induction during water deficit is controlled by at least five different classes of transcription factors. The ABA response element (ABRE) with the consensus ACCTGG/TC is bound by bZIP-type transcription factors (Figure 4). Three Arabidopsis bZIP transcription factors (AREB1/ABF2, AREB2/ABF4, ABF3) are expressed in response to water-deficit stress and ABA treatment. Activation of the transcription factors requires ABA accumulation and the induction of an ABA-responsive protein kinase which activates the transcription factor through phosphorylation.

Other transcription factors are also involved in ABA regulation of gene expression during cellular water deficit. Three genes encoding a class of transcription factors that is unique to plants, the NAC domain proteins ANAC019, ANAC055, and ANAC072, are induced by water deficit and ABA treatment. The NAC domain is a 60 bp DNA binding domain that is predicted to form a helix-turn-helix motif. MYB, MYC and homeodomain transcription factors, and a family of transcriptional repressors (Cys2/His2-type zinc-finger proteins) are also involved in the ABA response to water deficit.

**Deduction of Gene Function through Overexpression in Transgenic Plants**

The function of an individual gene can be tested by overexpressing or knocking out the expression of that gene and exposing the plant to stress conditions. The performance of the transgenic plants can then be used to deduce the function of specific genes. However, the conclusions are only as good as the stress test used to evaluate the performance of the plants.

A number of different types of proteins are likely to function to improve stress resistance. Genes encoding the enzymes of osmolyte biosynthesis permit the synthesis of these osmotic compounds in response to stress. Over-expression of genes promoting the synthesis of many potential osmolytes has resulted in transgenic plants with improved survivability or growth compared to the wild type. The same result has been obtained for a number of LEA proteins as well as scavengers for reactive oxygen species. Thus these individual genes have been shown to function as determinants of the water-deficit response to a defined laboratory stress. Regulatory genes, such as signalling molecules and transcription factors, control a set of genes, and overexpression studies can also be used to determine if individuals in this class of genes are functional determinants of stress resistance. Overexpression of genes encoding components of signal transduction pathways involving protein kinases, phosphoinositides and calcium have also improved survivability. In addition, five different classes of transcription factors have been shown to have this attribute. Since some of the transcription factors require posttranslational modification that occurs only in cells that are subjected to water deficit, these genes must be modified prior to construction of the transgenic plants to produce protein that do not require activation.

**Bringing our Knowledge to Crop Plants in the Field**

Biotechnological approaches utilizing our basic knowledge of plant stress response may provide a means to develop crops that respond to stress in a manner that improves tolerance or avoidance of water deficit. In the process of characterizing the function of individual genes, many genes have been identified that improve the response to water-deficit stress in the laboratory situation. However, the ultimate test, the response of a transgenic crop plant to drought in the field, has rarely been reported, with the majority of this research being completed in the private sector. Strategies involve altering the expression of single genes, which have the potential to alter a particular aspect of cellular metabolism or to alter the expression of a large set of genes (Figure 5). Given our current state of knowledge, only
trial-and-error can determine if a particular gene set will promote plant adaptation to drought conditions in the field. See also: Plant Breeding and Crop Improvement; Transgenic Plants

The largest set of genes would be induced if the initial sensor of cellular water deficit were activated inappropriately. Currently, this is not feasible since the initial sensor(s) of the cellular changes in water content has not been identified. Yet, overexpression of signalling molecules that are likely to be downstream of the initial sensor has resulted in improved response of plants to laboratory-imposed stress. Since ABA is one of the most important signalling molecules acting after the stress has been initiated, methods that alter ABA accumulation or ABA sensitivity are promising means to increase crop adaptation to stress. Plant sensitivity to ABA is decreased by the activity of farnesyl transferase. Transgenic *Brassica napus*, in which downregulation of farnesyltransferase is driven by a drought-inducible promoter, have greater seed yield than the parental line when grown in the field (Wang *et al.*, 2005). There was no yield penalty reported under nonstress conditions. Increased expression of one of the many transcription factors that act during water-deficit stress causes a subset of the genes that are responsive to water deficit to be induced. The DREB genes are thought to be a promising source of stress resistance and are being transferred to crop plants for field testing.

Future studies optimizing promoters and protein accumulation characteristics can be used to further the utility of this approach for the farmer.

**References**


**Further Reading**


