Plant responses to water deficit

Elizabeth A. Bray

Plant responses to water deficit are dependent on the amount of water lost, the rate of loss and the duration of the stressed condition. The characterization of a large number of genes induced by stresses involving water deficit has significantly improved understanding of the response. There appear to be several pathways for gene induction involved, and these are being elucidated by the analysis of DNA elements, mutants and gene expression patterns. However, it has been difficult to resolve the functions of many drought-induced genes against the background of other stress-induced changes, and thus it is now important to integrate information about cellular and whole plant responses.

Plant water deficit occurs when the rate of transpiration exceeds water uptake, and is a component of several different stresses including drought, salinity and low temperature. Such water deficit is a normal component of some developmental processes, such as seed development, common to most higher plants. Cellular water deficit can result in a concentration of solutes, changes in cell volume and membrane shape, disruption of water potential gradients, loss of turgor, disruption of membrane integrity, and denaturation of protein. Complete loss of free water will result in desiccation or dehydration. The ability of the whole plant to respond and survive cellular water deficit depends on whole-plant mechanisms that can integrate the cellular responses. Responses to water deficit may occur within a few seconds (such as a change in the phosphorylation status of a protein) or within minutes and hours (such as a change in gene expression).

Resistance to water deficit occurs when a plant withstands the imposed stress, and may arise from either tolerance or a mechanism that permits avoidance of the situation. Whole-plant mechanisms can contribute to the avoidance of water deficit during the plant’s life cycle, and avoidance can also occur at the cellular level. The response depends on the species and genotype, the length and severity of water loss (Box 1), the age and stage of development, the organ and cell type, and the subcellular compartment. An example of avoidance at the cellular level is the process of osmotic adjustment, where the osmotic potential of the cell is lowered in order for the water potential gradient to favor water uptake and maintenance of turgor.

The determination of the function of an observed response is one of the most complex issues in plant stress biology. In trying to understand responses to stresses involving a water deficit component, many genes induced by periods of water deficit have been identified and characterized. Interest has centered on these differentially expressed genes, because it has been postulated that induction of new genes will permit adaptation to stresses. However, the simple accumulation of mRNA does not equate with adaptive function for any specific gene. The accumulation of mRNA during water deficit may indicate gene induction, but additional regulatory mechanisms, such as translational regulation and post-translational modification, may be required for a fully functional gene product. In an unfavorable environment, plants must withstand multiple abiotic and biotic stresses, and a response observed during one type of stress may in fact have a role in the amelioration of another condition. For example, osmotin (a gene product that is induced in response to water deficit and salinity) has antifungal properties, and its resistance to water deficit in relation to the extent and rate of water loss

The response of the plant is dependent on the extent and rate of water loss. A slow rate of loss may permit acclimation to the water deficit and limit the extent of injury, while a rapid rate of loss may preclude acclimation. The same water deficit in a sensitive species and a resistant species may not trigger the same response. The triangles indicate the extent (relative length of vertical axis) and time (relative length of horizontal axis) of each parameter.
expression improves fungal resistance in plants subjected to water deficit. Nevertheless, it is possible that osmotin is a multifunctional protein with properties also directly involved in salinity tolerance.

Many recent studies have been undertaken from a cellular viewpoint in order to understand the processes of stress perception, signal transduction and the regulation of gene expression. However, because plants are multicellular, the integration of these cellular responses throughout the organism is a critical consideration (Box 2).

**Cellular events leading to water deficit-induced gene expression**

**Cellular perception of the stress**

The first step in the regulation of the water deficit response is the recognition of the stress (Fig. 1). Loss of water from the cell is perceived, triggering a cellular signal transduction pathway. In this way, a physical stress can be converted into a biochemical response.

There are several aspects of cellular water loss that could be measured by the stress recognition mechanism: decrease or loss of turgor; change in cell volume or membrane area; loss of membrane 'stretch'; change in water activity or solute content; and alteration in cell wall–plasma membrane connections or protein–ligand interactions. There are already clues to the recognition mechanism in bacteria and yeast, where mutants with altered cellular perception of osmotic stress have been isolated and the genes responsible described. In yeast, two osmosensors have been identified: Sshlp is a transmembrane protein that is activated under conditions of high osmolarity; and the other is a 'two-component regulatory system' composed of three proteins (Slinlp, Yplp1 and Ssklp1), which is inhibited under conditions of high osmolarity. Both are involved in the regulation of the HOG pathway, a phosphorylation cascade that controls the yeast osmotic response. The three proteins that comprise the 'two-component' osmosensor perform a four step His-Asp-His-Asp 'phospho-relay'. This phosphorylation mechanism is distinct from phosphorylation cascades – it is not for amplification, but is proposed to be a stringent method of regulation providing multiple checkpoints and/or a means to integrate additional signaling pathways. Even in these model systems the component of cellular stress that triggers the signal transduction pathway is not understood. It is predicted that there are multiple receptors involved in the mechanism whereby yeast cells sense osmotic stress. Plants appear to have a similar mechanism, as a plant SLN1 homolog has recently been shown to complement a yeast sln1 mutant (K. Shinozaki, pers. commun.).

**Box 2. Adaptive responses to water deficit and gene-product functions**

Whole plant signaling mechanisms control the physiological and molecular responses to water deficit. Specific responses may occur in different organs and cell types and may be controlled by the stage of development, as well as the extent and duration of the water deficit.

**Signal transduction events**

Following cellular perception of water loss, a signaling mechanism must be activated to induce specific genes. Not all stress-induced genes are induced under the same conditions or in the same cell types, and thus there appear to be several different signaling mechanisms. One of the major signals operating during drought stress is the plant hormone abscisic acid (ABA), and it is also involved in many other abiotic stresses (Table 1). However, not all water deficit-induced genes are regulated by ABA. It is unclear whether the perception of water deficit can directly induce a number of genes and/or whether additional signaling molecules accumulate during the stressed condition (Fig. 1).

**Identification of potential members of the signal transduction pathways by DNA sequence homology**

Many components of signal transduction pathways in other organisms have also been identified in plants, and many of these genes are induced during periods of water
Perception of cellular water deficit

**Perception and signal transduction of ABA**

Signal transduction pathways must be multilayered. Just as there is a recognition mechanism for environmental stimuli, there must also be a mechanism for the recognition of ABA in the cell (Fig. 1). Currently, the location, number and type of receptors for ABA is not known, although there is evidence that ABA can be recognized both inside and outside the cell\(^7\). These results and the response of gene expression to structural analogs of ABA may indicate that there are multiple receptors for ABA.

The ABA signal transduction pathway probably comprises a protein kinase/phosphatase cascade interacting with Ca\(^{2+}\). However, not all of the components have been identified, and a clear picture of the interactions has not been established. The most concrete evidence has been obtained from an Arabidopsis mutant, abi1, with decreased responsiveness to ABA. The ABI1 gene encodes a polypeptide with a protein phosphatase 2C domain at the C-terminus and an EF hand-like calcium-binding domain at the N-terminus\(^8\) (although the protein has not been shown to bind calcium). In addition, a MAP kinase has been implicated in ABA-regulated gene expression in aleurone layers\(^1\). A newly discovered component of the ABA signal transduction pathway is a protein farnesyl transferase that is proposed to catalyse the modification of a receptor or component of the signal transduction pathway for membrane localization\(^1\).

Table 1. The involvement of water deficit and ABA in plant responses to the environment

<table>
<thead>
<tr>
<th>Type of stress</th>
<th>Water deficit involved?</th>
<th>ABA involved?</th>
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<tbody>
<tr>
<td>Drought</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Salinity</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Freezing</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Chilling</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Wounding</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Heat</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Light</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Pathogens</td>
<td>Sometimes</td>
<td>Sometimes</td>
</tr>
</tbody>
</table>

DNA elements controlling gene expression during water deficit

The most comprehensive information about the mechanism of regulation of gene expression in response to water deficit has been obtained from the investigation of DNA elements and sequence-specific DNA binding proteins. Presently, two classes of DNA elements have been identified: the ABA-response element (ABRE); and the dehydration-responsive element (DRE) (also called a C-repeat\(^2\)).
are insufficient for controlling the genes that are induced by water deficit, and new elements are being characterized.

**The ABA-response element**

The consensus for the ABRE, RYACGTGGYR, where R refers to a nucleotide with a purine base and Y refers to a nucleotide with a pyrimidine base, is defined by the core element ACCT. This element was first defined in the Em gene from wheat, and is bound by the bZIP protein EmBP-1. It has also been shown to be a functional element for ABA-regulated gene expression in maize, barley, rice, tobacco and Arabidopsis. However, this core is also found in other DNA elements, including the G box, which is involved in the regulation of gene expression by light, auxin, jasmonic acid and salicylic acid.

The ABRE has been shown to be sufficient for ABA-regulated gene expression, but in some cases it must be associated with a coupling element. In HVA22, a barley gene, the coupling element CE1 (TGCCACCGG), along with one of the ABREs, is required for ABA-induced gene expression

Two of the three consensus ABREs are functional and contribute to incremental increases in ABA gene regulation. A second coupling element, CE3 (ACCGGTGTCCTC), was discovered in another ABA-induced gene of barley, HVA1 - a group 3 lea (Ref. 15). The coupling elements CE1 and CE3 are not fully interchangeable, which indicates that they are involved in the specific control of gene expression. These results also indicate that other sequence-specific DNA-binding protein(s) are required. One of these is the transcriptional activator VP1, first shown to be involved in the regulation of C1 (a gene encoding a Myb-like DNA-binding protein involved in anthocyanin biosynthesis) in maize seeds. The presence of VP1 has a synergistic effect with ABA on HVA1 (but not HVA22) expression. Similarly, one of two ABA-dependent genes from Craterostigma plantagineum (a desiccation-tolerant plant) requires the expression of the VP1 homolog abi3 when expressed in transgenic Arabidopsis.

Although it has been suggested that it is the flanking sequences of the ACCT core that are important for the regulation of genes controlled by ABREs, the coupling elements and transcriptional activators add another layer to the control of gene expression by ABA during water deficit.

**The dehydration-responsive element**

The DNA sequence of the DRE from the rd29A gene from Arabidopsis, TACCGACAT, has been shown to be involved in the regulation of this gene by an ABA-independent pathway induced by water deficit, low temperature and salinity. The regulation of this gene is independent of ABA in the first few hours after dehydration, but becomes dependent on ABA in the later stages of expression. Interestingly, an ABRE is present in the 5'-flanking DNA sequence, but this does not appear to be required during the first few hours of water deficit. A gene encoding the transcription factor CBP1, which binds the DRE, has been isolated from Arabidopsis (M.F. Thomashow, pers. commun.).

**Additional sequence-specific DNA elements**

The expression characteristics and DNA sequences of water deficit-regulated genes dictate that there must be additional DNA elements, and several of these elements are beginning to be defined. In the Arabidopsis gene rd22, which requires protein synthesis for expression, there is a DNA element, CACATG, that is similar to the element bound by the transcription factor MYC (Ref. 18). The core DNA element AGCCC found in the CdeT27-45 gene of C. plantagineum is necessary, but not sufficient, for ABA-regulated expression.

An element identified in the C1 gene of maize is necessary and sufficient for ABA induction (CGTGTCGTC-CATGCA) (Ref. 20). This element is similar to CE3.

The presence of a known DNA element does not ensure its involvement in the regulation of a specific gene. Mechanisms that are not apparent in the linear structure of the gene, such as the chromatin structure, may allow a higher order of regulation, and play an important role in gene regulation; such mechanisms would not be predictable from DNA sequence analysis, or in the course of promoter analysis experiments. For example, it has now been shown that the binding activity of the bZIP protein, EmBP-1, to the ABRE of the wheat Em gene is dependent on the nucleosomal position.

**Box 3. Mechanisms of cellular adaptation to water deficit**

Cellular adaptation to water deficit involves several different mechanisms, and the induction of individual genes and gene products may serve to improve cellular function in multiple ways. Roles for adaptive responses and individual gene-product functions are proposed.

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**Potential cellular functions of proteins/genes induced by periods of water deficit**

There are many changes in gene expression that occur during water deficit, some of which are part of the response to the immediate water loss. However, other genes may be induced even though they are not directly concerned with water deficit, and be directed towards other integrated stresses or be a response induced by injury. Therefore, adaptive significance cannot be determined by the simple demonstration that the gene is expressed. In general, as occurs with other stresses, the response to water deficit involves mechanisms to avoid water loss, protect the cellular machinery and repair damage (Box 3).

**Mechanisms to avoid water deficit**

One response to plant water deficit is the synthesis of osmolytes: these are compatible solutes that can accumulate to high levels without disrupting protein function. Osmolytes may include amino acids (e.g. proline), sugar alcohols (e.g. pinitol), other sugars (e.g. fructans) and quaternary ammonium compounds (e.g. glycine betaine). The
enzymes involved in the synthesis of these compatible solutes permit an osmotic adjustment, or the net accumulation of solutes. This results in decreased osmotic potential, thereby maintaining the favorable water potential gradient across cell membranes and protecting the cellular turgor. However, there is increasing evidence that these compounds also have novel roles (Fig. 2).

Proline, the most widely studied compatible solute, is synthesized from L-glutamic acid via L-pyrroline-5-carboxylate synthetase (P5CS) and L-pyrroline-5-carboxylate reductase (P5CRR). The reaction is catalyzed by two enzymes, P5C synthetase (P5CS), which is encoded by the Echerichia coli gene encoding mannitol-1-phosphate dehydrogenase, resulting in the accumulation of mannitol to approximately 100 m/l in the cytosol. Plants that have a moderately increased tolerance to salinity, expression in tobacco of L-pyrroline-5-carboxylate synthetase (P5CS), closed from moth bean (Vigna aconitifolia), resulted in a twofold increase in proline content compared with the wild-type during water deficit. The plants with a greater proline content as a result of P5CS were able to grow better during water deficit and salt stress, although the mechanism for improved tolerance was not established. Increased fructan (a polyfructose) accumulation was engineered into tobacco using the gene encoding levan-sucrase (SacB) from Bacillus subtilis. Transgenic plants with bacterial fructan accumulation had significantly greater growth and dry weight accumulation in response to a water deficit than the wild-type tobacco. Trehalose accumulation has also shown to promote stress tolerance in transgenic tobacco.

In all of these cases the improvement in stress tolerance did not appear to be caused by osmotic adjustment, because the amount of osmolyte accumulated was insufficient to account for an alteration in water potential gradients at the cellular level. However, there may have been maintenance of water potential gradients at the whole-plant level because of a role of these compounds in root growth. Novel roles for osmolytes may include radical scavenging, and the use of reducing power during maturation. This protein has inspired interest because of its architectural properties. Many hydrophilic globular proteins accumulate in vegetative organs during periods of water deficit. Many groups of LEA proteins have now been defined by their sequence homologies in a range of different species. Largely as a result of their extreme hydrophilic nature, LEA proteins have been predicted to play various roles: maintenance of protein or membrane structure; sequestration of ions; binding of water; and operation as molecular chaperones.

Two of these classes of proteins have been shown to have a functional role in stress tolerance: HVA1, a group 3 LEA protein from barley; and LE25, a group 4 LEA protein from tomato. Overexpression of HVA1 improves drought and salinity resistance in transgenic rice plants. The LE25 protein was expressed in yeast (Saccharomyces cerevisiae) and found to confer improved resistance to high salinity and freezing. However, although these experiments show that the organism in which the protein is overexpressed can better withstand the stress, few additional clues to the mechanism of protein function are provided.

Mechanisms to protect the cellular machinery

Many hydrophilic globular proteins accumulate in seeds during the maturation phase when seeds are developing desiccation tolerance. These 'late embryogenesis abundant' (LEA) proteins are also expressed during water stress and salinity.

Transport proteins, ion channels and carriers also play an important role in water deficit avoidance or osmoregulation. Potassium channels control K+ uptake and may also regulate Na+ uptake, which can be an important determinant of salinity tolerance. Aquaporins, a family of membrane proteins that transport water, may be involved in controlling cellular water status in response to water deficit. The phosphorylation status of two aquaporins of spinach is influenced by the apoplastic water potential. The phosphorylation of aquaporins is thought to increase the ability of the channels to transport water, and it has therefore been proposed that decreased phosphorylation status during water deficit should slow the loss of water from individual cells.

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ubiquity, and several possible functional roles, including a chaperone-like activity\textsuperscript{35}, are being addressed.

**Mechanisms to repair damage**

Many proteins involved in damage limitation or the removal of toxic compounds are induced during water deficit. For example, ubiquitin, chaperones and proteases may all be involved in the recovery of proteins or their building blocks. Genes encoding enzymes that detoxify reactive oxygen species are also induced\textsuperscript{35}. It is difficult to ascertain whether the induction of these genes is to repair damage caused directly by reduced water content, or if they accumulate to ameliorate damage caused by a secondary stress or to restrict pathogen invasion.

The characterization of genes induced by water deficit has greatly improved our understanding of plant responses to the environment, yet there are still many unanswered questions:

- Which of the gene products provide adaptive responses to the lack of water?
- How important is an individual gene?
- Are there redundant pathways?
- What types of responses do these genes control?

To achieve a complete understanding of gene function, these questions must be addressed in the context of the whole plant as well as the cell.

**Comparison of the water deficit response in tolerant species and crop plants**

It is perhaps surprising to discover that some of the responses to water deficit are similar in tolerant species such as *Mesembryanthemum crystallinum* and *C. plantagineum*, and in crop plants such as tomato and maize or even in the model plant *Arabidopsis*. Many similar genes have now been identified in all of these species as a consequence of what appears to be a universal response to water deficit. Nevertheless, there are clear differences in survival rates. Differences between species in gene expression and genome content can be identified, and may play a role in this difference in stress tolerance. In *M. crystallinum*, pitinito accumulates and the genes encoding the biosynthetic pathway are upregulated by stress\textsuperscript{38}. In *Arabidopsis*, the gene for the first step in pitinito accumulation is present, but it is not upregulated, and the remaining genes for pitinito biosynthesis are not present in the *Arabidopsis* genome. The identification of species differences and the addition of novel pathways from tolerant plants into transformed crop species to determine whether stress resistance is improved should be fruitful areas of future research.

**The whole-plant response**

The integrated response of the whole plant to water deficit must also comprise sensing and signaling mechanisms. The plant hormone ABA is the best-known signal at the whole-plant and cellular levels; it can move throughout the plant in the vascular system, and acts as a signal for changes in stomatal conductance\textsuperscript{37} and gene expression\textsuperscript{38} in response to soil drying.

It is now clear that stress responses are dependent on the tissue, cell type and developmental stage of the plant. However, DNA elements and transcription factors that control organ-specific responses to water deficit have not been identified. Interestingly, responses during the acquisition of desiccation tolerance in seed maturation are very similar to vegetative responses to water deficit. This provides considerable support for the hypothesis that the observed responses are adaptive.

Finally, in considering the whole-plant response, it is important to provide plants with stress conditions similar to those experienced in the field. Controlled conditions can never fully mimic field conditions, because in the field plants may experience multiple abiotic and biotic stresses.

**Questions for the future**

Understanding of the plant response to water deficit has been advanced considerably by the application of molecular techniques. New responses and new interpretations of previously observed responses have been uncovered. Yet there are many more important questions that must be addressed to achieve a clear view of plant adaptation to water deficit:

- How is the stress perceived, and how does it lead to cellular and whole-plant signal transduction pathways?
- How is ABA recognized?
- Which water deficit-induced genes are required for tolerance to water loss?
- Which attributes of tolerant plants can be incorporated successfully into crop plants?

Answers to these questions will allow the engineering of the plant stress response to suit the demands of the local environment, and to ensure plant production even under adverse conditions.

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The arbuscular mycorrhizal symbiosis:
an underground association

Maria J. Harrison

Arbuscular mycorrhizas are symbiotic associations formed between zygomycetes (Order Glomales) and the roots of most terrestrial flowering plants. Existing in natural ecosystems throughout the world, the association creates an intimate link between plant roots and the soil, and plays a pivotal role in the acquisition of mineral nutrients. The ability of the association to enhance plant growth and development has stimulated research, and the recent application of biochemical, genetic and molecular approaches is providing new insight into the symbiosis.

The term mycorrhiza, which literally means 'fungus root’, was first used in 1885 (Ref. 1) to describe the mutualistic associations that occur between plant roots and fungi. Mycorrhizal associations are grouped into a number of different types, of which the arbuscular mycorrhizal (AM) symbiosis (also referred to as the vesicular-arbuscular mycorrhizal symbiosis⁵) is the most common, and has been estimated to occur in more than 80% of flowering plant species on land⁶. These associations are extremely ancient, and AM fungi have even been identified in fossils of early Devonian land plants⁷ – it could be that they assisted plants in their colonization of land⁸.

The AM association is endomycorrhizal: the obligately biotrophic fungi colonize the cells of the root in order to...