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Plant cellular responses to water deficit

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Abstract

Water availability and drought limit crop yields worldwide. The responses of plants to drought vary greatly depending on species and stress severity. These responses include changes in plant growth, accumulation of solutes, changes in carbon and nitrogen metabolism, and alterations in gene expression. In this article, we review cellular and molecular responses to water deficit, and their influence on plant dehydration tolerance.

Introduction

A large percentage of the world's crops are exposed to chronic or sporadic periods of drought (Boyer 1982). Even in the absence of drought, the timing and amount of rainfall are primary determinants of crop selection and yield (Boyer 1982; Ludlow and Muchow 1990). One approach to improve crop performance in water limited environments is to select for genotypes that have improved yield in this environment. This approach has proved partially successful, but difficult due to the variability of rainfall and the polygenic nature of drought avoidance and tolerance. A complementary approach to improve plant performance in water limited environments involves the identification and selection of traits that contribute to drought avoidance, drought tolerance or water use efficiency (Ludlow and Muchow 1990). The list of potentially important traits is extensive because water deficit negatively affects nearly all stages of the plant's life cycle. A partial list of potentially important traits includes: matching plant phenology to the available water supply, seedling desiccation tolerance, water extraction efficiency, water use efficiency (stomatal and cuticle characteristics), osmotic adjustment, and modulation of partitioning to maintain reproductive structures (Ludlow and Muchow 1990). Most of these traits are complex and our understanding of their control and molecular basis is limited.

Plant responses to water deficit can be analyzed by systematically identifying traits that relate to drought tolerance followed by analysis of the physiological, cellular, biochemical and molecular basis of the trait. In addition, genes that show modified expression in response to a defined water limitation can be cloned and analyzed in order to obtain insight into mechanisms that plant cells use to respond to water limitation (i.e., Guerrero et al. 1990; Covarrubias et al. 1995; Bartels et al. 1993; Bray et al. 1993). Cellular responses to water limitation vary depending on the degree of water deficit, the duration of the stress and plant species. Furthermore, specific cellular responses vary depending on the organ, cell type and developmental stage of the plant/cell. Responses need to be sorted into those that involve adaptations that allow plant cells to continue growth and development, those that allow plant survival and those that are a response to lethal disruption of function.

Table 1 provides a partial list of cellular responses to water deficit. These cellular responses are arranged and will be discussed as a function of severity of water deficit. Overall, the responses are divided into three categories; water deficit induced inhibition of growth processes, cellular changes resulting from the metabolic impact of stomatal closure, reduced transpiration and photosynthesis and cellular changes that enhance dehydration tolerance.

Table 1. Cellular responses to water deficit

Decreasing Water Content ⟶

Growth inhibition (shoot > root)
- polysome reduction
- osmotic adjustment
- altered cell wall extensibility
- altered carbon/nitrogen utilization

Photosynthesis decreases
- ABA levels increase
- photodamage increases
- transpiration decreases
- nitrate levels decline
- compatible solute synthesis

Dehydration tolerance
- dehydrin synthesis
- disaccharide accumulation

Growth is very sensitive to water status

Growth requires cellular pressure that exceeds the wall's yield threshold and simultaneously, a water potential gradient from soil/xylem to the growing zone (Nonami and Boyer 1990a; Nonami and Boyer 1990b). Normally, the difference between the pressure potential and wall's yield threshold is small (0.1–0.2 MPa). Similarly, the water potential gradient allowing water movement into growing zones is not large. Therefore, a small change in the water potential of the soil or a change in the rate of water loss from leaf surfaces can lead to growth inhibition.

The cellular response to water deficit induced growth inhibition depends on the developmental stage of the plant, and the organ/tissue examined. In soybean seedlings, growth of hypocotyls was much more sensitive to water limitation than roots (Creelman et al. 1990). This difference was found to be caused, in part, by differential sensitivity of the two growing regions to ABA which accumulates in response to water deficit (Creelman et al. 1990). In *Z. mays*, stem cell wall extensibility decreased whereas root cell wall extensibility increased in response to water deficit (Hsaio and Jing 1987). This differential response must contribute to the difference in root and shoot growth responses in water limiting conditions. In well watered plants, the polysome content of the soybean stem growing zone is high (Mason et al. 1988). However, shortly after seedlings are transferred to soil of lower water potential, growth and polysome content decreased (Mason et al. 1988). The decrease in polysome content was ascribed to an inhibition of translation initiation because of a shift from large to small polysomes without large changes in mRNA content (Mason et al. 1988). In roots, where growth was only transiently inhibited, only small changes in polysome content were observed.

The inhibition of stem growth in water limited soybean seedlings did not cause irreversible changes in cell size (Mullet 1990). When plants were rewatered, cells in the zone of cell elongation expanded and reached full size. Therefore, the primary effect of water deficit was to change the rate of growth of stem cells. Low growth rate in stems was due in part to the accumulation of ABA. In addition, numerous changes in gene expression, most not modulated by ABA, were observed in growing zones. For example, genes encoding B-tubulin, actin and an auxin inducible gene showed decreased expression in the growing zones of plants transplanted to low water potential soil (Creelman and Mullet 1991). Decreased expression of tubulin and actin in the growing zone of water deficient plants seems reasonable because the demand for these gene products is reduced in parallel with growth inhibition. Interestingly, the expression of genes encoding cell wall proteins was altered by water deficit. RNA levels of genes encoding glycine rich proteins, and HRGPs (extensions) decreased in the soybean stem growing zone, whereas the abundance of mRNA of a gene encoding a proline rich protein increased. Changes in the expression of these genes, and the corresponding proteins, could contribute to alterations in cell wall extensibility.

In soybean seedlings, root growth is less sensitive to decreases in soil water potential than stem growth (Creelman et al. 1990). This leads to an increase in root/stem ratio that is commonly observed in plants exposed to water deficit. The difference in growth inhibition in the two organs can be traced to the fact that root growth is less sensitive to ABA than stem growth. Furthermore, ABA is required for continued root growth in water limiting conditions, most likely due to ABA induced accumulation of proline in roots (Voetberg and Sharp 1991). Proline accumulation helps maintain turgor in the root tip and promotes continued growth in low water potential soils.

Osmotic adjustment and compatible solute accumulation

One important cellular response to water deficit is the accumulation of osmotically active compounds that

allow cells to reestablish turgor and to extract additional water from the soil (Jones and Turner 1978; Morgan, 1983. Osmotic adjustment can occur either through uptake of solutes or the breakdown of osmotically inactive compounds. In the case of soybean seedlings described above, the stem growing zone accumulates sugars in response to mild water deficit (Creelman et al. 1990). In contrast, non-elongating portions of the stem do not accumulate sugars, and have reduced turgor relative to the growing zone.

Seedlings growing in sufficient water utilize carbon and nitrogen containing compounds from cotyledonary reserves, for growth. These compounds can be temporarily stored in the form of proteins and starch, and later broken down during cell growth. Seedlings exposed to low water potential soils show inhibition of stem growth and, therefore, stem cells in the growing zone are not utilizing solutes for growth. The sugars and other compounds that accumulate in response to water deficit are most likely derived from continued transport from cotyledons. In addition, it is possible that stored reserves in the growing cells could be depolymerized to support osmotic adjustment. Carbon compounds for osmotic adjustment in leaves can be derived from photosynthesis, stored carbon (starch) or amino acid (storage proteins) reserves.

In addition to the ability to retain, take up or mobilize the cellular sugars and amino acids found in well watered plants, most plants also have the ability to induce the synthesis of new compounds that contribute to osmotic adjustment. Unlike sodium and other ions, these compounds are compatible with the function of cellular enzymes at high concentrations. For example, plants will accumulate glycine betaine (and related quaternary ammonium compounds), and polyols in response to water deficit or excess salt. Several water deficit inducible genes are known to encode enzymes in pathways that lead to compatible solutes. For example, genes encoding enzymes for betaine biosynthesis are activated in response to water deficit. In pea, leaf dehydration caused the induction of a gene that encodes an aldehyde reductase that may be involved in osmotic adjustment (Guerrero et al. 1990). Similarly, aldose reductase is induced during seed development in barley (Bartels et al. 1993).

Mammals, bacteria and yeast are able to modulate cellular activity to adjust to changes in osmolarity. Renal cells in mammals can modulate the level of organic osmolytes in response to urinary osmolarity. These cells accumulate myo-inositol, sorbitol, betaine and glycerolphosphocholine (Nakanishi et al. 1989). Single cell organisms can rely more on the uptake of solutes to adjust cell turgor. Exposure of *E. coli* to high external osmoticum results in altered K^+ pump activity, changes in expression of the *kdp* operon and synthesis of a high affinity transport system. *E. coli* and *Salmonella* also accumulate proline and betaine in response to increases in medium osmolarity. Two additional systems that permit osmoregulation of the bacterial cell matrix have been reported. The first involves compensatory synthesis of two porin proteins (encoded by *ompF* and *ompC*) located in the outer bacterial membrane. Furthermore, some bacteria accumulate membrane derived oligosaccharides in the periplasm when cells are shifted to high osmolarity media (Miller et al. 1976).

Water channels

The dynamics of water transport in plants, especially under water limiting conditions, has received new attention due to the discovery of water channel proteins in plants (rev. Chrispeels and Maurel 1994; Verkman 1992). The water channels are related to the superfamily of membrane intrinsic proteins (MIP) that were first characterized in *E. coli*. These proteins have 6 membrane spanning domains with strongly conserved amino acid stretches located in the loops between transmembrane segments 2/3 and 5/6 (Reizer et al. 1993). Some of the proteins in this superfamily are glycosylated, and several are phosphorylated, which may regulate channel activity. In plants, one member of this family, *Trg31* (pea), was initially identified because expression of the gene was induced when leaves were partially dehydrated (Guerrero et al. 1990). A similar dehydration inducible gene was isolated from *Arabidopsis thaliana* (Yamaguchi-Shinozaki et al. 1992).

The functions ascribed to the different MIP channels vary. *GlpF*, one of the best characterized genes in this superfamily, is inducible by glycerol (or glycerol-3-phosphate) and facilitates diffusion of glycerol into *E. coli* (Sweet et al. 1990). Glycerol is subsequently trapped in the cells through the action of glycerol kinase (*glp*K). Other small compounds can also utilize the *glp*F channel including urea, xylitol, and ribitol. MIP26, a major membrane protein of the mammalian lens, has also been characterized extensively (Reizer et al. 1993). This protein forms tetragonal complexes in the plasma membrane, which are, for the most part, not associated with gap junctions. The channels

are large and facilitate diffusion of molecules up to 1,100 daltons. Facilitated diffusion through the channels is voltage dependent and protein phosphorylation may regulate channel activity. Interestingly, it has been speculated that MIP26 is required to remove water and small molecules from the extracellular space between lens cells in order to improve lens clarity. Hereditary cartaract problems may be caused by defects in the gene encoding MIP26 and proteolysis of this lens protein during aging is correlated with cartaract formation. Proliferation of MIP26 occurs in the lens fiber cells which lose ribosomes during maturation. It is interesting to note that *Trg*31-GUS fusions are highly expressed in plant vascular tissue. Sieve elements in vascular tissue also lose nuclei and protein synthetic capacity when mature.

Other non-plant MIP proteins have been identified in *Drosophila* and yeast (Reizer et al. 1993). In *Drosophila*, mutations in a MIP protein (*bib*) lead to altered cell development suggesting that the membrane channel is needed to transport molecules providing information for cell development. In yeast, a protein in this family was identified by the finding that the *fps1* gene (which encodes a MIP protein) could complement a defect in utilization of non-fermentable carbon sources. Finally, an additional member of the MIP family has been isolated from renal and red blood cells (Preston et al. 1993). This protein, designated CHIP, has been expressed in *Xenopus* and shown to facilitate water diffusion into cells.

Several MIP related proteins have been identified in plants including *Trg31* (and a similar drought inducible gene in *Arabidopsis*) (Guerrero et al. 1990; Yamaguchi-Shinozaki et al. 1992). The first plant MIP protein to be characterized was NOD26 (Miao et al. 1992). This protein is abundant in the peribacteroid membrane in nodules and is thought to facilitate the transport of compounds between plant cells and the compartment containing bacteria involved in nitrogen fixation. More recently, several MIP-like tonoplast intrinsic proteins (TIPs) and their corresponding genes have been characterized by Chrispeels and coworkers (Chrispeels and Maurel 1994). One member of the *Tip* gene family is expressed during seed formation, whereas a second member of this gene family is expressed during other stages of plant development, especially in zones of cell elongation. The compounds transported through the TIP channels have not been identified but one TIP protein was found to form water channels when expressed in *Xenopus*.

Water deficit – carbon and nitrogen metabolism

Hanson and Hitz (1982) reviewed the area of metabolic limitations caused by drought and adjustments that benefit plants under these conditions. Water deficit often leads to decreases in stomatal aperture which in turn limits CO_2 fixation. Simultaneously, stomatal closure reduces transpiration and limits the flow of water from roots to leaves. This in turn reduces the movement of nitrate and other compounds from roots to leaves and thus the reduction of nitrogen and synthesis of amino acids can be inhibited in water limited environments. The reduction of carbon fixation creates another problem for chloroplast containing cells. In the absence of a sink for energy (carbon reduction), the chloroplast must deal with the dissipation of excess photon energy in a non-damaging manner. Chloroplasts have several energy dissipating mechanisms including the xanthophyll cycle (Demmig et al. 1988) which creates energy sinks in the antennae beds, chlorophyll protein phosphorylation that alters the distribution of excitation energy flow between the photosystems, and protective mechanisms including carotenoids for quenching chlorophyll triplet states, and ascorbate and glutathione to help remove toxic oxygen compounds. In addition, over a longer time scale, water deficit and high light conditions lead to a reduction in the light harvesting antennae which decreases the energy load on the chloroplast.

Some of the metabolic changes induced by water deficit are the result of diminished growth of young leaves and, therefore, decreased utilization of carbon and nitrogen produced in older leaves. This change in the ratio of production to utilization may require the induction of nitrogen and carbon storage mechanisms that buffer these transient changes during mild water deficit conditions. Consistent with this idea, the expression of the genes encoding the soybean vegetative storage proteins (*Vsp*) is increased in young leaves when water is withheld from plants (Mason and Mullet 1990). Older leaves do not show increased expression of these genes under these conditions even though they are capable of expressing *Vsp* if leaves are wounded. Expression of the *Vsp* is activated by sugars and jasmonate, and is inhibited by phosphate (Mason et al. 1992). Influx of sugar exported from older photosynthetic leaves and uptake into the growing leaves could lead to an accumulation of sugar phosphates. These conditions induce *Vsp* expression and result in the accumulation of storage proteins and utilization of the excess carbon in the young leaves.

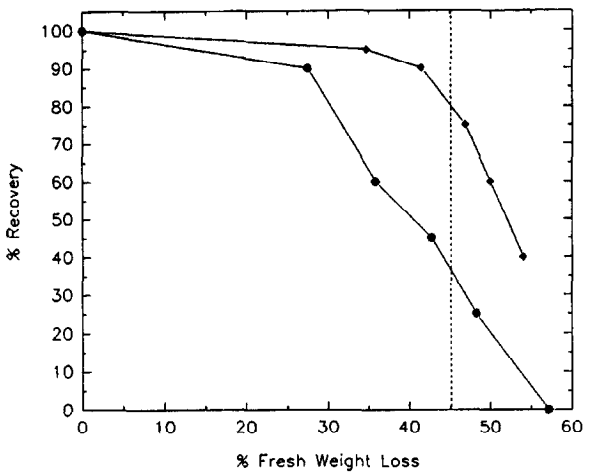

Figure 1. Time course of fresh weight loss and seedling recovery in control plants (circles) and in plants that had been previously exposed to a non-lethal water deficit for 24 h prior to seedling desiccation (triangles). Soybean seedlings were grown 48 h post germination prior to the start of the experiment. A subset of the seedlings were dehydrated for 8 h until approximately 30% fresh weight loss had occurred. These seedlings were then kept at 100% relative humidity for an additional 16 h prior to further dehydration. For dehydration survival testing, control and pretreated seedlings were transferred to dry vermiculite and placed at 89% relative humidity. At designated times seedlings were removed from the dry vermiculite, weighed and planted in well-watered vermiculite. Seedling growth and recovery were measured 24 h later. Data points are the means for 3 groups of 20 plants.

Dehydration tolerance

Mild water deficits that cause a reduction of plant turgor, or losses of 10–15% of a plants water content result in large changes in growth and metabolism but rarely cause plant death unless these conditions persist for long periods of time. However, as shown in Figure 1, soybean seedlings dehydrated so that they lose more than 30% relative water content, show reduced survival. Some stages of the plants life cycle are less susceptible to large reductions in water content. In particular, during seed development, a programmed reduction in water content can occur without loss in viability. In addition, prior to the establishment of a large root system, seedlings may often need to survive large reductions in water content. Furthermore, some mosses and the resurrection plants can tolerate extreme dehydration during the vegetative stage of the life cycle (Oliver 1991; Piatkowski et al. 1990). At present, it is not clear why most crop plants lose dehydration tolerance in the vegetative state and whether this adaptation would be of some benefit for production.

Soybean seedlings are able to increase their dehydration tolerance in response to water deficit. This result is shown in Figure 1 as an increase in survival of seedlings exposed to a non-lethal water deficit prior to dehydration and survival testing. Similar observations have been made with barley seedlings (Chandler et al. 1993). Protection against lethal damage in seeds is correlated with the accumulation of sugars and proteins (i.e., dehydrins) (Blackman et al. 1992; Close et al. 1993). In general, the presence of disaccharides such as trehalose or sucrose have been found to help stabilize membranes during drying (Crowe et al. 1993). It is thought that these compounds can interact with polar groups on membranes and effectively replace hydrogen bonding that is normally contributed by water. The proteins that accumulate in dehydrating cells are generally highly hydrophilic and have only limited secondary structure (primarily random coil) (Dure 1993). A subset of the proteins that accumulate late in embryogenesis (LEA proteins) fit this description (D19, D113, D11, D7 families). These proteins have been proposed to bind water, to act as reverse chaperones, to preserve membrane structure, and to bind phosphate and other ions (Dure 1993).

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