

# **Managing Drought Stress in California Agricultural Systems**

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## Abstract

California is currently facing a historic drought, and this has led many farmers in the state to severely cut back on irrigation. Optimal use of water for irrigation requires a comprehensive understanding of how plants respond physiologically to water stress (Chapter 1). By monitoring water requirements in crops and managing irrigation to meet those requirements, growers can significantly reduce water use (Chapter 2). This can be done through improving application efficiency of irrigation technology as well as increasing the water use efficiency of the crops themselves. Deficit irrigation practices can be used to manipulate the physiology of water use in plants and increase crop tolerance to drought stress. Imposing minor stress on plants induces chemical signaling within the plant that decreases stomatal aperture, increases root to shoot ratio and manipulates root architecture to optimize water gain and reduce loss. Though these practices have reduced yield compared to conventional irrigation, these reductions are minimal in most cases and can be considered better than severely reduced yields due to poor irrigation planning. Ultimately, deficit irrigation practices increase the yield obtained per unit of water applied. Additional benefits have also been reported with the use of deficit irrigation, such as improved yield quality and reduced shoot vigor.

Monitoring irrigation is an essential first step to optimal irrigation management, and it is an intrinsic part of integrated pest management. Drought stress affects the dynamics of certain plant pathogens and arthropod pests in both

positive and negative ways, and this is important to consider when utilizing deficit irrigation practices. Often, avoiding plant stress is crucial, but this is not simply achieved by watering to avoid drought stress. It is important to create the best environment for healthy plant growth, and this may often mean reducing irrigation when necessary to improve a crops tolerance to drought stress and/or pest pressure (Chapter 3).

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## Preface

For the past two years, I have been interning in the western United States, mostly working in the area of plant diagnostics for diseases and pests and agricultural research. From May to September 2014, I worked for Oregon State University in their plant pathology diagnostic lab in the Columbian Basin on the arid side of the Cascade Mountains. We saw mostly cases involving potato pathogens, including fungal, bacterial, and viral diseases, but we also encountered samples of various vegetables and some horticultural specimens. I also took part in testing potato psyllids for infection with the Zebra Chip bacterium (Cand. *Liberibacter solanacearum*).

From May to July of 2015, I worked in the Central Valley near Fresno with California Agricultural Research, a company that mainly dealt with GLP (Good Laboratory Practices) Regulatory trials involving Pesticide Residue where my main duties included monitoring pest and disease problems and advising on how to manage them. Starting in September 2015, I took a position at Pacific Ag Research Group in San Luis Obispo, California. I was responsible for starting up and maintaining their newly built pathogen lab, where I maintain a collection of mostly fungal pathogens and help design, prepare and implement disease and other trials.

Having lived in Nebraska for the duration of my doctoral degree work, I had learned about the current drought in California and how severe it had become. However, the summers of 2014 and 2015 allowed me to witness firsthand the consequences of this historic drought on agriculture in one of the worst hit areas of California. Beginning in 2014, when I was driving from southern California up to

eastern Oregon, I began to notice an amazing number of dead orchards and fallow fields, especially toward the center of the state, alongside signs that read, “No Water, No Jobs” and “Congress Made Drought.” These signs were in reference to the severe rationing of water not only in response to the drought, but also the allocation of water to be released in the delta to protect endangered fish species. The drought had compounded the issue of water rationing and many farmers in the Central Valley were forced to rely on wells that were quickly drying up or forced to scale back production.

In the Columbian Basin of Oregon, the issue was quite different. The Columbia River that flows from the Northern Rockies is very large by the time it reaches the Oregon-Washington border. As a result, there is little restriction on water access in the Columbia Basin agricultural area, even though it is considered very arid. Growers tended to over irrigate in this valley, using mostly sprinkler and pivot irrigation, and I saw many cases of disease problems that arose from poor irrigation both here and in California.

Previously I had read some very interesting research on irrigation strategies that were reported to improve both water use efficiency and crop quality by actually maintaining a degree of drought stress on crops, and this is what inspired me to write my doctoral paper. Since irrigation is a fundamental part of growing crops in semi arid regions, I wanted to explore possible strategies for coping with water scarcity and exactly what is the best way to irrigate. Much of the research that I read pointed to the idea that water should not just be applied to



increase yield, for this eventually leads to diminishing returns, but that optimal water use may actually require irrigating less than conventionally thought.

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## **Chapter 1: Physiology of Drought Stress**

### **Introduction**

California's average annual precipitation ranges from 5 inches or less in the Imperial Valley and desert southeast to up to 200 inches in the Northwest of the state (Figure 1.1) (USGS, 2005). In the prime agricultural regions of the state, the average is between zero and twenty five inches per year. Further, the region is dominated by a Mediterranean climate in which most of the precipitation falls between November and February and is stored as either snowpack or in groundwater basins. Consequently, most crop production is irrigated and depends on stored water, especially during the summer months when precipitation amounts to less than one inch.

Despite the arid nature of the agricultural regions of California, they are among the most important production areas in the United States and, for some commodities, the world. In 2013, California produced the most cash receipts (\$46.4 billion) of all states in the US (USDA, 2015). California produces 80% of the world's almonds, 40% of the world's Pistachios, and 90% of grapes grown in the US (USDA, 2015).

In 2011, California began one of the worst droughts in modern history when winter precipitation fell below average. By 2014, California reached the third driest year on record in recent history (Figure 1.2), preceded only by 1924 (during the Los Angeles-Owens valley water wars) and 1977 (Miller, 2014). By June 2015, water cutbacks for cities were mandated of up to 25% reduction of potable urban water use (Kostyrko, 2015). Prior to this mandatory reduction, farmers in the Central

Valley were already denied their full, contracted allocation of surface water from the Sacramento River Delta according to a 2007 ruling (NATURAL RESOURCES DEFENSE COUNCIL, et al. v. DIRK KEMPTHORNE et al., 2007). The ruling was in response to the NRDC lawsuit against the EPA to reduce pumping from the southern end of the Sacramento San Joaquin Delta in an effort to reduce salinization and protect the endangered Delta Smelt and reduced water allocation by around 50% (Fresno County Farm Bureau, 2007).

The winter of 2015 and 2016 has been characterized by a strong El Niño weather pattern with increased precipitation, but it is unclear whether there will be sufficient precipitation to declare an end to the current drought or when and how severe the next drought will be. With a growing population, demand for fresh fruits and vegetables, increasing pressure to protect endangered species and increasingly restricted access to fresh water, growers in California have to produce crops with less water or face going out of business. Novel irrigation technology and techniques are allowing growers to reduce water use up to 50% while maintaining yields, improving crop quality in certain cases and even reducing the severity of some pests and pathogens. This document summarizes the effects that drought stress has on crops and how technology and crop management practices are changing to meet these challenges.

Water is essential for plant growth and reproduction as a medium for biochemical reactions and cellular integrity. The chemical characteristics of water give it properties that best support life. Water molecules have a dipolar structure with two free pairs of electrons and the slightly positive charge from two hydrogen

atoms. This polarity makes it highly self-cohesive and a good solvent. The hydrogen bonds formed between water molecules also impart a high specific heat and latent heat of vaporization, both of which enable plants to regulate internal temperature when properly hydrated. A high specific heat means water must absorb a large quantity of energy to increase in temperature, and conversely, must lose the same to decrease in temperature ( $4.19 \text{ J g}^{-1} \text{ }^{\circ}\text{C}^{-1}$  @  $20^{\circ} \text{ C}$ ). A high latent heat of vaporization ( $2454 \text{ J g}^{-1}$ ) allows water to remove energy from leaf tissue as it evaporates from within the leaf because of the energy needed to completely break hydrogen bonds. This evaporative cooling buffers against high ambient temperatures and internal leaf temperature increases resulting from the capture and processing of light in photosynthetic reactions in leaf cells.

### **Water transport in plants**

Water transport from roots to shoots is driven by evaporation from leaf mesophyll cells and diffusion through stomata. As water evaporates from curved surfaces in cell wall matrices, a negative tension is created that pulls water from xylem vessels. Through actions of cohesion and adhesion, primarily due to hydrogen bonding, xylem vessels act like a wick that draws water from moist soil. However, water transport and evaporative cooling require a great deal of water and plants tend to lose around 90% of the water they take up from the soil through transpiration (Davenport, Hagan, & Uriu, 1977).

There are two points of resistance to water diffusion out of the leaf: stomatal resistance and boundary layer resistance. A small amount of water can escape through the cuticle, but this is negligible compared to water diffusion through

stomata. Gas exchange only takes place through stomata, causing water loss and carbon dioxide fixation to be tightly linked. When stomata are open, roughly 50 to 400 molecules of water are lost for every molecule of carbon dioxide that is fixed, depending on the efficiency of photosynthesis in the plant (Taiz & Zeiger, 2010). In regulating water loss through stomata aperture, carbon sequestration suffers due to reduced diffusion of carbon dioxide into the leaf.

Plants regulate water loss and carbon dioxide intake by regulating stomata pore aperture. In plants not experiencing drought stress, stomata open in response to light on a diurnal pattern and aperture widens or narrows in response to CO<sub>2</sub> concentrations and ambient water vapor (Taiz & Zeiger, 2010; Mansfield & Meidner, 1966). However, drought stress has a strong influence on stomata closure to reduce water loss, as will be discussed in more detail.

Some plants are able to compensate for reduced CO<sub>2</sub> diffusion through variations in leaf anatomy and carbon metabolism. As apposed to C<sub>3</sub> plants which have the site of RUBISCO activity (the main enzyme involved in carbon sequestration) in close proximity to internal leaf cavities where CO<sub>2</sub> diffuses to, C<sub>4</sub> plants physically separate the site where CO<sub>2</sub> is sourced in the leaf and where carbon is fixed in bundle sheath cells. New carbon atoms are chemically fixed to phosphoenol pyruvate to eventually form the four-carbon molecule malate. Carbon dioxide is then released into the cytoplasm of bundle sheath cells effectively increasing CO<sub>2</sub> at the site of carbon sequestration. This allows C<sub>4</sub> plants to reduce stomatal aperture and prevent water loss while being able to cope with resulting lowered CO<sub>2</sub> concentrations in leaf air space (Taiz & Zeiger, 2010). Most crops

grown in large quantities in California are C3 plants and very few C4 crops, that include grasses such as corn wheat and barley, are grown in the state.

### **Factors that influence water loss and gain**

Water loss is influenced by vapor pressure deficit, temperature and wind. Vapor pressure deficit is the difference between internal leaf saturation vapor pressure and ambient air vapor pressure or a measure of how dry the air is compared to internal leaf humidity. Thus, it depends on relative humidity and temperature. Relative humidity is a measure of the water vapor quantity in the air

$rh = \frac{e_{air}}{e_{s_{air}}} \times 100\%$ , where  $e_{air}$  is the saturated vapor pressure of air. Drier air has a much lower water vapor pressure than internal leaf spaces. Under such conditions, plants loose more water if stomata remain open. Drought conditions in semi-arid regions are defined by weather systems dominated by dry air, with a shorter or more infrequent wet season, increasing the length of time during the year where vapor pressure deficit can be elevated.

Temperature has a two-fold impact on evapotranspiration. Besides affecting the relative humidity of ambient air, temperature contributes to the heat energy input into the liquid phase of water within the leaf, as well as the saturation vapor pressure of water vapor within internal leaf spaces. Combined, these effects act to increase transpiration demand.

Wind exacerbates water loss due to its effect on the boundary layer that forms around leaves. The boundary layer is a layer of still air at the surface of the leaf that contributes to the total resistance of water vapor diffusion out of the leaf.

Increased wind speeds disturb this layer of still air, effectively reducing its thickness and the resistance to water vapor diffusion out of the leaf. Daudet et al. (1999) found the following relationship between wind speed ( $U$  in  $\text{m}\cdot\text{s}^{-1}$ ) and boundary layer conductance  $g_b^H$  ( $\text{mm}\cdot\text{s}^{-1}$ ) as:  $g_b^H = 10U + 7.1$ . This equation demonstrates a ten fold increase in conductance (the inverse of resistance) with each additional  $\text{m}$  per second increase in wind velocity.

Water supply to the plant is determined by available soil water content. This depends on characteristics of the soil, such as texture, organic matter content, and soil structure. Sandy soils tend to have less water holding capacity and a smaller range of volumetric water content available to plants. Loam soils (soils containing a mixture of soil particle sizes) tend to have greater water holding capacity and available soil water content (ASW) than sandy soils, with silt loam usually containing the largest range of ASW. Available soil water content is reduced in clay soils, even though they tend to have the highest water holding capacities, due to a greater ratio of micropores to macropores (Bronick & Lal, 2005). Water drains more easily from macropores and is held tightly in micropores by cohesive and adhesive forces. Water availability to plants is dependent on a proper range between the two pore classes, when the difference between field capacity (all but the largest of pores are filled such that no excess water drains) and wilting point (soil water tension is stronger than a plant's ability to withdraw it) is the greatest (Bronick & Lal, 2005). Soil organic matter can improve the structure of soils so pore size range can be maintained through microbial activity, producing microaggregates containing micropores and surrounded by macropores (see review by Bronick & Lal, 2005 for

more information). Also, organic matter itself has a large water holding capacity (Hudson B. D., 1994)

### **Effect of water stress on cell growth**

The greatest impact of water stress is seen on cell growth (Hsiao, Acevedo, Fereres, & Henderson, 1976). Cell growth and division in plants is dependent on irreversible cell expansion due to turgor pressure ( $\psi_p$ ) and cell wall extensibility ( $m$ ) (Hsiao & Xu, 2000). This relationship, first proposed by J. A. Lockhart in 1965, has been found to have a threshold potential ( $Y$ ) below which no cell expansion can take place and above which the relationship is linear (Matthews, Volkenburgh, & Boyer, 1984). The slope of cell wall extensibility depends on cell wall extension response (Green, et al., 1971; Nonami & Boyer 1990).

$$\begin{aligned} &\text{for } \psi_p > Y \\ &G = m(\psi_p - Y) \\ &\text{for } \psi_p \leq Y \\ &G = 0 \end{aligned} \tag{1}$$

The rigidity of a plant cell wall is controlled by inherent properties of the cell wall and new cell wall production. Plant cell walls are a combination of cellulosic fibers that have a high tensile modulus ( $10^{11} \text{ N m}^{-2}$  (Cosgrove, 2000)) surrounded by a hemicellulose matrix that includes glycans that connect the cellulose fibers. The tautness of these connecting glycans is controlled by proteins called expansins that are believed to loosen the polysaccharide bonds between cellulose fibers through the “turgor-driven polymer creep” model first proposed by McQueen-Mason and Cosgrove (1994).



McQueen-Mason, Durachko, & Cosgrove (1992) discovered these proteins in a groundbreaking experiment where they added plant proteins to denatured cell walls under pH of 4.5 in an extensionometer and found that cell wall expansion resumed. Expansin activity is stimulated by a decrease in extracellular pH induced by auxin activated proton pumps (Cosgrove, 2000). At an intercellular pH of 7 no cell wall relaxation is observed. In response to increased auxin production in the growing region, proton pumps can decrease external pH to around 4.5, increasing expansin activity. Generally, the cell wall becomes more rigid in drought hardened plant cells. The threshold potential goes up, requiring more turgor for growth, and cell wall extensibility goes down, becoming more rigid and increasing growth effective turgor ( $\psi_p - Y$ ) (Matthews, Volkenburgh, & Boyer, 1984). This results in greater sensitivity of shoot growth to additional drought stress, a mechanism that prevents excessive vigor and water loss.

Water uptake ( $g$  in  $s^{-1}$ ) depends on the growth-induced water potential gradient ( $\psi_o - \psi_w$ , where  $\psi_o$  is the external water potential and  $\psi_w$  is the internal water potential) across the cell wall/membrane and the volumetric hydraulic conductance ( $L$ ), which is controlled by aquaporin permeability.

$$g = L (\psi_o - \psi_w) \quad (2)$$

When combined, equations 1 and 2 give a growth rate of:

$$G = (mL/(m + L))(\psi_o - \psi_s - Y) \quad (3)$$

where  $\psi_s = \psi_w - \psi_p$  and is the internal osmotic potential of the cell (Nonami & Boyer, 1990). This equation demonstrates that growth rate is directly related to the difference between osmotic potential gradient across the cell membrane ( $\psi_o - \psi_s$ )

and the threshold potential ( $Y$ ), indicating that threshold potential and osmotic potential are important to growth rate. It also indicates that the product of cell wall extensibility and hydraulic conductance, and their sum, form a ratio and the relationship between growth rate and either of these parameters is more complicated than osmotic potential ( $\psi_s$ ) and threshold potential.

By measuring these parameters in soybean (*Glycine max*) seedlings transplanted from moist soil to water deficient vermiculite, Nonami & Boyer (1990) found that, while the growth induced water potential gradient ( $\psi_o - \psi_w$ ) increased after subjection to growth media with a lower water potential, the hydraulic conductance ( $L$ ), cell wall extensibility ( $m$ ) and the growth effective turgor pressure ( $\psi_p - Y$ ) were slow to recover, reaching 50% or less of their original values after three days. This indicates that actively growing plant cells are conditioned to reduce cell growth in response to drought events.

### **Root sensing mechanisms to drying soil**

Roots sense changes in water potential of drying soil through hyperosmolality sensing proteins, and also possibly through changes in root cell water status, mechanical changes in root cell volume or both. The response is a signal cascade, mainly regulated by the stress hormone abscisic acid that affects both roots and shoots. Recently, Robbins & Dinneny (2015) wrote a detailed review of the research on hydrotropism, or the active growth of roots towards areas of higher water potential or water content, and water stress sensing and response summarized here. Yuan et al. (2014) demonstrated that the *A. thaliana* gene OSCA1 (Reduced Hyperosmolality-Induced Calcium Ion Increase 1) is involved in cytosolic

$\text{Ca}^{2+}$  increases characteristic of early drought stress response (Knight et al. 1997). Mutants lacking the gene did not show increased root growth or exhibit stomatal closure in response to sorbitol treatments to reduce water potential. Other genes implicated have been Arabidopsis Histidine Kinase1 (AHK1) (Urao, et al., 1999), Mechanosensitive Channel Of Small Conductance-Like (MSL) (Kloda & Martinac, 2002) and Feronia (FER) (Shih, et al., 2014). AHK1 proteins are osmolality-sensing proteins in high concentration on stomata of *A. thaliana*. However, mutants lacking this gene have shown no change in osmotic adjustment due to drought stress (Kumar, Jane, & Verslues, 2013). MSL proteins one and two are similar to bacterial osmolality-sensing proteins and are found on organelles, regulating their internal water status in relation to cytosolic water potential. FER proteins are receptor-like kinases involved in mechanoreception, especially in hypoosmotic stress, and may be involved in hydrotropism.

### **Role of abscisic acid in root to shoot signaling of drying soil conditions**

In response to drought stress, roots produce abscisic acid (ABA), a plant stress hormone that has three crucial roles in drought response: stomata closure in leaves to regulate water loss, inhibit shoot growth, and recovered root growth. ABA is transported through the xylem to aerial organs (Davies, et al., 2000). These responses lead to greater water uptake and decreased water loss by increasing root to shoot ratio and preventing excessive water loss through open stomata. In some plants, such as tomato (Holbrook, Shashidhar, James, & Munns, 2002) and sunflower (Fambrini et al. 1995), evidence suggests that stomata respond to ABA produced in leaves as well.

Xylem sap has also been demonstrated to become more alkaline in response to drought stress and has been proposed to act synergistically with ABA to influence stomata closure (Schachtman and Goodger 2008). Absciscic acid is a weak acid ( $pK_a=4.7$ ) and is absorbed passively by mesophyll cells in non-ionic form. Kaiser and Hartung (1981) found an exponential decrease in ABA absorption in mesophyll cells with increasing apoplastic pH. Alkaline pH is believed to lead to an increase in apoplastic ABA concentrations and ABA receptor binding to plasma membrane ABA G protein coupled receptor (GCR2 in *Arabidopsis thaliana* (Liu, et al., 2007)) in guard cells. However, Schachtan and Goodger (2008) point out that stomatal response to increased apoplastic pH varies between species and some plants such as soybeans close stomata under drought conditions long before their xylem sap pH increases.

Absciscic acid binding to guard cell receptors stimulates cytosolic calcium ion levels via reactive oxygen species formation, primarily nitric oxide and hydrogen peroxide. The internal increase in calcium ion concentration affects a number of signals that lead to stomatal closure. Primarily the increase in  $Ca^{2+}$  causes an efflux of anions, mostly  $Cl^-$ , through slow- (S-gated) (Linder & Raschke, 1992) and rapid-gated (R-gated) ion channels (Hedrich, Busch, & Raschke, 1990), and membrane depolarization triggering a potassium ion efflux by opening outward potassium ion channels (Schroeder, Raschke, & Neher, 1987). Conversely, elevated  $Ca^{2+}$  concentrations in the cytosol inhibit inward potassium cation channels and outward proton pumps (Lemtiri-Chlieh & MacRobbie, 1994; Schroeder & Hagiwara, 1989). The latter can lead to a significant pH increase of the apoplast immediately surrounding the guard cells that also inhibits inward potassium cation channels.

Calcium influx also induces the release of calcium, chlorine and potassium ions from the vacuole (Schroeder, et al., 2001). ABA stimulates nitric oxygen, cADPR and IP3 production that stimulate vacuolar calcium efflux as well. The net effect is an increase in osmotic potential of stomatal guard cells and loss of turgor pressure, and this deformation relaxes guard cells, reducing stomatal aperture (Schroeder, Allen, Hugouvieux, Kwak, & Waner, 2001).

### **Effect of ABA on root and shoot growth**

In response to ABA and other drought stress signals, root cells rapidly adjust osmotically in order to reduce internal water potential lower than external water potential and modify cell wall chemistry in the meristem, allowing them to maintain growth under low water potentials (Hsiao & Xu, 2000; Westgate & Boyer, 1985). Absciscic acid has the opposite effect in shoot meristems in that endogenous ABA treatment inhibits shoot growth, possibly due to its effect on stomatal aperture and consequently photosynthesis (Blum, 2011) and the fact that cell walls in shoot meristems become more rigid and apoplastic pH tends to be high. Further, the osmotic adjustment that shoots experience to maintain turgor is much slower than that in root tips (Hsiao & Xu, 2000).

Research is still being conducted on the exact nature of signaling pathways for osmotic adjustment in root meristems. While evidence has suggested ABA regulates the increase of some solutes involved in osmotic adjustment of root cells, some researchers have reported ABA independent signals for solute accumulation. For instance, ABA signals have been shown to directly regulate accumulation of the amino acid proline (Yamaguchi & Sharp, 2010) and possibly indirect K<sup>+</sup> ion

transport (Osakabe, et al., 2013). Proline accumulation is considered a major contributor to osmotic adjustment during drought stress, accounting for up to 45% of solute contributions to decreased osmotic potential in maize primary roots (Voetberg & Sharp, 1991). Osakabe et al. (2013) demonstrated that a series of potassium pumps in the K<sup>+</sup> uptake transporter (KUP) family and guard cell outward rectifying K<sup>+</sup> channel (GORK; a potassium efflux transporter) are indirectly regulated by ABA signaling and expressed in root growing tips during drought stress in *A. thaliana*. However, Verslues & Bray (2006) found that osmotic adjustment was independent of ABA signaling in *A. thaliana*. They found that proline accumulation, which may contribute to osmotic potential and has roles in reactive oxygen species scavenging and other protective roles, increases in response to ABA signaling.

More clearly understood is the effect of ABA on cell wall extensibility in roots. Absciscic acid promotes auxin accumulation in root tips, likely via isoflavanoid and flavonoid production, leading to acidification of the cell wall necessary for expansin activity (Yamaguchi & Sharp, 2010). Xu et al. (2013) found that, in addition to increased ABA accumulation in roots, auxin transport to root tips also increases. Through experiments with *Arabidopsis* and rice, primary root tips exposed to 5% polyethylene glycol (PEG) to reduce water potential to -0.48 MPa, or exogenous ABA at 0.1  $\mu$ M, they found a significant increase in root elongation and proton efflux in the growing region between the two treatments and the control, but no difference between treatments. *Aba3-1*, which is responsible for conversion of ABA-aldehyde to ABA, the final step of absciscic acid (ABA) biosynthesis (TAIR, The Arabidopsis

Information Resource, 2006), mutants subjected to PEG showed no difference in root elongation rate or proton-ATPase activity, but exogenous ABA treatment allowed for the recovery of both.

In another experiment, Xu et al. (2013) found that 24 hour exposure to either PEG or ABA treatments caused increased primary root elongation rate, plasma membrane H<sup>+</sup>-ATPase activity, proton extrusion, and root hair density. When plants exposed to fluoridine, an ABA synthesis inhibitor, all parameters were strongly inhibited. Concurrently, auxin levels in the root tip were significantly elevated in the PEG and exogenous ABA treatments. Inhibitors of auxin influx reduced all root growth parameters in the control plants and impeded root growth parameters under low osmotic potential (with PEG) or when exposed to exogenous ABA.

ABA also affects a number of other hormones involved with root growth inhibition, such as ethylene (Sharp, 2002), and plays an important role in reactive oxygen species (ROS) protection by regulating the expression of several ROS response genes. These include metal chelating proteins like metallothioneins and ferritins, proteinase inhibitors, proline accumulation and flavonoid production pathways in the growing region of roots (Yamaguchi & Sharp, 2010).

Metallothioneins chelate heavy metal ions, such as iron and copper, and ferritins chelate free iron, metals which can react with hydrogen peroxide to form hydroxyl radicals. Proteinase inhibitors are thought to be important to prevent the degradation of oxidized proteins, allowing for recovery from oxidative stress that may inhibit root growth. Proline and isoflavonoids act as antioxidants as well;

however, proline and isoflavanoids have significant roles in the maintenance of root growth under low soil water potential.

### **Effect of drying soils on root architecture: role of cytokinins and auxin in hydrotropism**

In addition to inducing recovered and maintained root growth under moderate drought, drying soils also affect the architecture of root zones in many plants. Exposure to periodic drought stress causes roots to grow deeper into the soil profile and access soil horizons that retain plant available water longer. In grain crops such as sorghum and wheat, drying topsoils have been shown to induce “compensatory growth” in deeper roots resulting in prolonged exposure to available water (Blum, 2011).

Roots sense gradients in water potential and exhibit a phenomenon known as hydrotropism in which root tips bend in the direction of greatest water potential. Hydrotropism is still not completely understood on the cellular level; however, studies with *Arabidopsis* mutants have shown several key components. Abscissic acid stimulates amyloplast shrinkage in columella cells so that gravitropism can be overridden (Cassab, Eapen, & Campos, 2013). The NHR1 and AHR1 genes, both directly regulated by ABA, are involved in reduced amyloplast size, because mutants lacking both retain normal sized amyloplasts during hydrotropic stimulation and ABA treatment (Cassab, Eapen, & Campos, 2013). Amyloplasts are degraded in water stressed roots as well as in response to hydrotropic stimulation (Cassab, Eapen, & Campos, 2013). ABA and cytokinin are currently thought to modulate auxin-controlled gravitropism.