CHAPTER 2

REGULATION OF ROOT GROWTH RESPONSES TO WATER DEFICIT

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Abstract: The growth and function of roots are essential for crop productivity under water-limiting conditions, but direct improvement of roots by plant breeding has been slow. One difficulty is the observation and quantitative measurement of root systems under conditions that are relevant to field environments. Another challenge is the identification of and selection for specific loci that could improve the acquisition of water from the soil profile. However, advances are being made in the understanding of root growth regulation and development. We review the evidence for the maintenance of root growth by ABA during water deficit, and the interactions with ethylene and other hormones. A biophysical model of cell expansion serves to focus discussion of topics relating to regulation of growth and development. The power of kinematic growth analysis is demonstrated by highlighting changes in growth regulatory processes and associated patterns of gene expression and protein composition that occur specifically in regions of the root where cell expansion is maintained under water deficit conditions. Growth is a complex process; new information adds further insight and further complexity to our understanding of how roots sense and respond to changes in environmental conditions. It is important to unravel these adaptive mechanisms so that it is clear how the manipulation of one process will affect the function of the whole plant, and so that the effect on final yield and water use can be predicted. This complexity makes simple linear models inadequate as explanatory tools, and a systems approach is needed to incorporate the weave of interacting networks of signaling and response pathways. The real challenge is to discover how root growth can be improved, to supply breeders with the practical tools to identify or introduce superior alleles in crop species, and ultimately to ensure that discoveries lead to improvements in productivity in the field

Keywords: Root growth, water deficit, ABA, ethylene, ROS, DELLA proteins, cell wall

1. ROOT GROWTH AND DROUGHT

Roots are essential to plant survival and play a critical role in determining the yield of crops. However, they are hidden from view, often deep in the soil, and this makes them difficult to study and easy to ignore. Historically, plant breeders have

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made selections for crop improvement based on visible traits, or at least traits that were easy to identify because of the need to rapidly assess large numbers of plants. It is not surprising, therefore, that root characters rarely feature in lists of active breeding targets. This may change with new discoveries and the development of innovative screening techniques.

One of the most critical challenges land plants face is drought, and under such circumstances the role of roots in acquiring soil water and nutrients is absolutely essential. Drought is the largest single factor limiting crop productivity worldwide (Boyer, 1982), yet it is unfortunate that many aspects of how roots grow, develop and respond to changing soil conditions are poorly understood. It is the growth of roots that determines root system architecture and exploration of the soil profile. Nearly all of this growth occurs within several mm of root apices. What happens in this relatively small mass of cells can make a huge impact on yield under sub-optimum conditions. This chapter focuses on what we have learned about one aspect of plant adaptation to drought: how root growth is maintained in drying soils. Much of what we know about root behavior comes from studies done on simple systems in controlled environments; many of the examples we will use here are from the maize (*Zea mays* L.) primary root system. In addition, we consider examples of root growth of mature plants in field conditions.

The maize seedling root system consists of the primary root and additional sets of seminal roots emanating from the mesocotyl-radicle junction (Cahn et al., 1989). In addition, nodal roots successively form on the plant stem, some of which emanate above the soil surface. In the root system of the mature plant, these seedling and nodal roots can produce up to 70 axile roots, and first- and second-order lateral roots can emerge from these (Hoppe et al., 1986). With respect to the root system of mature plants, the primary root is a minor constituent; however, it is a convenient system to study, and although root types can respond differently (Volkmar, 1997), most physiological and molecular features of the primary root may be generally applicable.

In several crop species including maize, the growth of roots and shoots is inhibited during water deficit, but roots continue growing at low water potentials (ψ_w) that are completely inhibitory to shoot growth (Spollen et al., 1993). This differential growth sensitivity may confer an advantage to plants in water-limited conditions by favoring the allocation of carbon below ground to permit greater exploration of soil while limiting the loss of water from shoot tissues. Understanding how growth is regulated in response to water deficit is necessary in order to find ways to improve crop productivity.

1.1. Hormonal Regulation of Root Growth

Roots growing slowly at low ψ_w synthesize and accumulate the plant hormone abscisic acid (ABA). ABA applied to well watered roots inhibits root growth (Sharp, 2002; Sharp et al., 1994). Therefore, it might seem logical to conclude that the endogenous production of ABA at low ψ_w causes the root growth inhibition.

However, it has been shown that this is incorrect. In the maize primary root ABA accumulation is required for the maintenance of growth. This was discovered by conducting a series of classic hormone response experiments, following the rules set out years ago by Jacobs (Jacobs, 1959). Briefly, ABA accumulation was blocked either by adding an inhibitor of carotenoid and therefore ABA synthesis (fluridone), or by using maize mutants deficient in ABA, such as *vp14*, which contains a lesion in 9-cis epoxycarotenoid dioxygenase, the rate-limiting step in ABA synthesis (Tan et al., 1997). Roots deficient in ABA showed severe growth inhibition at low ψ_w, and when exogenous ABA was added back to the tissues to restore the normal endogenous concentration of ABA, growth rates were restored (Saab et al., 1990; Sharp, 2002; Sharp et al., 1994). When ABA was added to roots such that the endogenous concentration increased above the normal level, growth was inhibited. In fact, a tissue concentration of ABA that is required for root growth at low ψ_w causes growth inhibition under well watered conditions, showing that the sensitivity to ABA also changes with tissue water status (Sharp, 2002; Sharp et al., 1994). Thus, exogenous ABA application leading to supranormal tissue ABA concentrations can cause artefactual responses and incorrect conclusions about the role of endogenous ABA. These cautions most probably also apply to the assessment of other hormones that may be involved in root growth regulation under water deficit conditions.

Further experimentation with the maize primary root system revealed that an important function of endogenous ABA is to keep ethylene production under control (Spollen et al., 2000). Under water deficit conditions, elevated levels of ABA in roots are sufficient to suppress excess ethylene production and, hence, further growth inhibition is prevented. Interestingly, ABA accumulation in shoot tissues at low ψ_w can be insufficient to prevent ethylene-induced growth inhibition (Sharp, 2002). This may be part of the mechanism of greater sensitivity of shoot than root growth to low ψ_w, although additional factors clearly modulate maize leaf growth under water deficit conditions, and some of these may be independent of ABA/ethylene interactions (Voisin et al., 2006). In the absence of stress, ABA may also help promote shoot growth via ethylene suppression. This was shown in *Arabidopsis* (LeNoble et al., 2004) and tomato (Sharp et al., 2000) by decreased growth of ABA deficient mutants when shoot water status was maintained by high humidity (to overcome effects of impaired stomatal functioning).

The effects of ethylene on growth are complex and variable. In many species there is a biphasic response of growth to ethylene, whereby very low concentrations of ethylene can stimulate growth while greater concentrations inhibit growth (Pierik et al., 2006). In addition, developmental stage can affect the growth response to ethylene. For instance, during the post-germination development of maize seedlings at low ψ_w, ethylene shifts from growth promotion of the mesocotyl to growth inhibition (Sharp, 2002; Sharp and LeNoble, 2002). In contrast to maize, ethylene stimulates root growth in deep water rice (Steffens et al., 2006), and ABA inhibits root growth by competitive inhibition of gibberellin (GA) stimulation of ethylene synthesis. The rice and maize systems are similar in that ABA blocks ethylene, but ethylene can promote or inhibit growth in the different systems.

Recent evidence suggests that ethylene may inhibit growth by stabilizing the activity of DELLA proteins, which restrict root growth (Achard et al., 2006). In this study, *Arabidopsis* seedlings growing on saline substrate showed increased ethylene production and decreased growth, but not in mutants deficient in ABA signaling (*abi1-1*) or lacking four of the five DELLA proteins. Under severe stress conditions, the disadvantage of decreased growth was offset by increased plant survival in the mutants compared with wild type. One of the major DELLA proteins (GAI) regulates GA action: GA stimulates growth by stimulating the breakdown of DELLA proteins. A generic view may be that ABA restricts ethylene, which permits GA breakdown of DELLAs, releasing the 'brakes' on growth. Conversely, if low ψ_w-induced ABA accumulation is prevented, increased ethylene may inhibit GA, which then protects DELLAs and their inhibition of growth. An exception may be in submerged tissues of plants adapted to aquatic environments where ethylene-GA interactions have the reverse effect, allowing ethylene to stimulate GA levels and DELLA breakdown.

It is not yet clear how ABA and the ethylene synthesis pathway interact. Other hormones also affect root growth, including auxin (Fuand Harberd, 2003; Rahman et al., 2001), brassinosteroids (Müssig et al., 2003), and cytokinins (Riefler et al., 2006). Multiple hormone pathways interact to affect growth in a complex manner. Hormones also affect root initiation, which can be separated from expansive growth *per se*. For instance, work with the *lrd2* mutant in *Arabidopsis* showed that both auxin and ABA mediate lateral root initiation, and that the suppression of lateral root initiation by ABA at low ψ_w does not involve changes in primary root growth rate (Deak and Malamy, 2005). (In soils, however, it should be noted that low ψ_w can lead to increased lateral root development [Ito et al., 2006]). In tomato, overexpression of the tonoplast H^+-pyrophosphatase led to increased root growth and drought tolerance, which may have been caused by increased apoplast acidification and polar auxin transport (Park et al., 2005). Auxin and ABA pathways may converge at ABI3 (de Smet et al., 2006), and ABA and ethylene pathways can intersect at ERF4 (Yang et al., 2005).

Part of the complexity in interpreting hormone-growth relationships is the need to separate cause and effect, and to consider environmental conditions that can alter hormone levels. For instance, a set of near-isogenic maize hybrids were developed that contrast for leaf ABA concentration, and QTLs linked to this trait were identified (Giuliani et al., 2005). However, the interesting result is that the main QTL appears to control root system architecture, and the effects on leaf ABA are probably pleiotropic. The proposed model suggests that the lines that accumulate greater levels of leaf ABA do so because root density is greater in the more superficial soil layers, which tend to dry out even under irrigated conditions. These roots in drying soil may synthesize ABA and transport it to leaves in the transpiration stream. A beneficial result is that the 'high ABA' allele is associated with larger root systems that reduce lodging. Breeders need this kind of genotypic and phenotypic information in order to manipulate root architecture.

1.2. Root Growth Biophysics

Roots, as all plant tissues, grow by production of new cells by the meristem, followed by expansion of these cells until growth ceases. Although cell division is vital, it is mostly cell expansion that drives roots through the soil matrix. Biophysical models of tissue growth provide a clear framework for the interpretation of growth responses. The Lockhart (Lockhart, 1965) equation, originally developed to explain the linear expansion of single cells, also has been applied to multicellular organ growth, such as the primary root. In simplified form:

$$G = m(P - Y) \quad (1)$$

where G is expansive growth; m is cell wall extensibility; P is turgor; and Y is the minimum turgor threshold required to irreversibly extend the cell wall. This simplified form has been further elaborated, taking into account the driving forces and resistances to water flux through tissue (Passioura and Boyer, 2003), and soil penetration resistance (Bengough et al., 2006). It has been shown that m and Y are not merely physical constants describing the viscoelastic mechanical properties of the cell wall, but additionally comprise metabolic factors acting on wall polymer rheology (Passioura, 1994). This theoretical treatment of growth is important because it breaks down a complicated process into conceptually smaller components, each of which may be controlled by the expression of a gene or suite of genes that can be manipulated by plant breeding.

1.3. Root Growth Kinematics

In maize seedlings grown in darkness and near non-transpiring conditions at 29 °C, the primary root tip is advanced at about 3 mm h^{-1} in well watered vermiculite (an expanded phyllosilicate mineral that serves as a soil-like substrate) in which there is negligible penetration resistance (Sharp et al., 1988), or up to 4 mm h^{-1} in solution culture (Verslues et al., 1998). Roots are usually indeterminate structures: if optimum conditions could be maintained beyond the seedling stage, roots growing at this rate would reach a depth over 2 m in a month. It is not surprising, then, that the maize root systems excavated by Weaver (Weaver, 1926) in irrigated, deep prairie soils reached 2.5 m. In most cases, of course, environmental factors limit the full expression of the growth potential of the root system in the field.

In the seedling system, when the water content of the vermiculite decreased to a ψ_w of -1.6 MPa, root growth slowed to approximately 1 mm h^{-1} (Sharp et al., 1988). The rate of root growth is determined by the rate of cell production from the meristem, rates of cell expansion, and the final length of cells (or the time spent elongating). Following the first division in the meristem, cells at first expand anisotropically (Liang et al., 1997), then longitudinal expansion is favored over radial expansion. Each cell is displaced basipetally away from the root apex by further cell production and expansion. At a certain point cells cease

elongation. Mechanisms that control cell production and expansion within the root apex determine the growth rate and size of the entire root system. Other factors that are important, but not discussed here, are the production of new root apices (e.g. lateral root initiation), capacities for water uptake and transport, and the functional longevity of roots.

The process of root growth can be examined more closely, cell by cell if necessary, using the tools of growth kinematics (Silk, 1984; Figure 1). This is a powerful technique because it determines within the root apex the location of cells that are growing and those that have ceased growth. This is immensely important because the attributes–from gene expression patterns to wall mechanical properties–of growing cells are different from those that have ceased growth. This seems obvious, yet in most experiments, whole roots are ground up and analyzed as a single specimen. The fundamental bases of how root growth is regulated can be discovered best by first understanding the spatio-temporal organization of growth with fine resolution. Tools for determining the dimensions of the growth zone and rates of cell production now include, for instance, computational video image analysis (Peters, 2004; van der Weele et al., 2003; Walter et al., 2002).

Measurement of changes in local growth rates within the apical 10 mm of the root tip show that in the maize primary root, the length of the growth zone shortens under water deficit (Figure 1; Sharp et al., 1988). However, cells near the root apex maintain the same longitudinal expansion rate under well watered and water deficit conditions. Growth maintenance in this apical region depends on the accumulation of ABA (Figure 1; Ober and Sharp, 2003; Saab et al., 1992). The shape of the velocity curve may depend on species, growing conditions, and the resolution with which relative elongation rates are measured (Bengough et al., 2006).

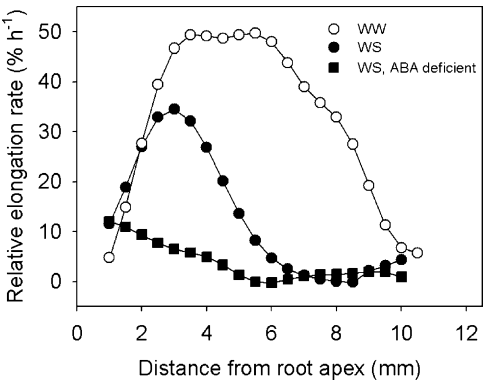

Figure 1. Spatial growth pattern of maize primary roots at high (WW) and low (WS) ψ_w. The root growth zone extends to 11 mm at high ψ_w, but is constricted to 7 mm at low ψ_w. Inhibition of ABA accumulation by treatment with fluridone, which inhibits ABA synthesis by a block in the carotenoid pathway, severely restricts root growth at low ψ_w. Data are taken from Ober and Sharp (Ober and Sharp, 2003)

1.4. Root Turgor Maintenance and Osmotic Adjustment

Equation 1 shows that the force of turgor is required for steady-state plastic deformation of cell walls. Turgor is maintained by accumulation of intracellular solutes, which decreases the ψ_w of the cell below that of the surrounding apoplast, driving water uptake. Cell expansion is a dynamic process, so that as water enters cells, solutes are diluted, and as walls relax, turgor would tend to diminish. Therefore, in actively growing cells, the rates of solute and water deposition in the cell are regulated to allow co-ordinated expansion of all the cells in the tissue (Bret-Harte and Silk, 1994). At low ψ_w, maize primary root growth is inhibited: both longitudinal and radial expansion rates decrease, causing shorter, thinner roots compared with roots grown at high ψ_w (Sharp et al., 1988). This reduction in volumetric expansion and dilution of solutes causes an increase in solute concentration. For example, the concentration of hexoses increases in the apical 6 mm of the root tip because net deposition rate of hexose is not affected by low ψ_w while water deposition decreases dramatically (Sharp et al., 1990). In addition, a surprising observation is that the net deposition rate of proline *increases* at low ψ_w in apical regions of the growing zone, resulting in even larger concentrations (Voetberg and Sharp, 1991). Thus, two mechanisms have developed to enable an increase in solute concentration at low ψ_w: changes in root morphology to allow less dilution, and increases in net solute deposition. This water deficit-induced shift in the ratio of solute to water deposition must be a regulated process, since under steady-state conditions the net deposition rate of potassium changes little with water deficit; i.e., the ratio of potassium to water deposition in the apical growth zone remains relatively constant at high and low ψ_w (Sharp et al., 1990).

The low ψ_w-induced accumulation of proline depends on the increased levels of ABA in the root tip (Ober and Sharp, 1994). Further studies showed that proline transport to the root tip was more important than de novo synthesis or protein catabolism in the root tip (Verslues and Sharp, 1999). Proline transporters in the plasmamembrane, regulated by ABA, may be important control points for root growth at low ψ_w.

In summary, the accumulation of inorganic and organic solutes is essential to build turgor that drives growth. Although growth rate cannot be predicted simply on the basis of the level of turgor (Frensch and Hsiao, 1995), it appears that maximum turgor is required to achieve maximum growth rates (Proseus et al., 2000). Pressure-driven symplastic transport capacity within the root tip limits growth (Bret-Harte and Silk, 1994; Gould et al., 2004). Evidence suggests that a turgor gradient favoring sucrose movement from phloem unloading sites into the elongation zone is crucial for root growth, and sucrose utilization and import must be linked (Farrar et al., 1995). Selection in breeding programs that increases solute transport to root tips and into growing cells could result in better root systems in water limited conditions.

1.5. Water Transport Within the Root

The transport of water into the growth zone is necessary to drive the expansive growth of cells, and water flux facilitated by aquaporins in the plasmamembrane

and vacuole play an important role (Luu and Maurel, 2005). Aquaporins are also important in regulating water flux between the soil and the root. However, the quantity of water necessary for growth is small compared to the volume that is removed from the soil to meet the transpirational demand of the shoot. Water uptake in maize occurs 20–30 mm from the root tip (Frensch et al., 1996), but the bulk of water transport occurs in mature roots at least 30 cm from the root tip (Varney and Canny, 1993). Significant water movement depends on open metaxylem elements, which are slow to mature in maize (Wenzel et al., 1989). Aquaporins are a large gene family showing differential tissue expression patterns (Hachez et al., 2006). Aquaporins only passively facilitate the movement of water across membranes and cannot alter the magnitude or direction of the driving force. Thus, temporal downregulation of aquaporins could restrict the loss of water from root tissues into dry soil, but it remains to be shown that increased aquaporin activity can aid the acquisition of water from the soil (Vandeleur et al., 2005).

There is a large body of research on hydraulic conductivity of roots and root systems, but we only make a few observations here. It has been long noted that drought (Brown et al., 1987; Sharp and Davies, 1985) and ABA (Hose et al., 2000) can increase root hydraulic conductivity, possibly via modulation of aquaporin activity (Hartung et al., 2005), which can also be affected by reactive oxygen species (Henzler and Steudle, 2004). It is also well known that different root types and root ages within a root system have different conductivities (Pierret et al., 2006). For these reasons there is not necessarily a good correlation between root length density and soil water uptake; the mere presence of roots does not mean they are fully functional.

1.6. Changes in the Cell Wall

Compared with well watered roots, in maize primary roots growing under severe water deficit (−1.6 MPa), the cumulative effect of increased solute concentrations is a two-fold decrease in the total osmotic potential. Nevertheless, despite the increased driving force for water uptake, turgor pressure throughout the apical 10 mm of the maize primary root is 60% smaller than at high ψ_w (Spollen and Sharp, 1991). How do cells near the root apex maintain linear rates of expansion despite lower turgor? Equation 1 shows that in order for G to remain unchanged as P decreases, m must increase and/or Y must decrease. This means that cell walls in the apical few mm of the maize root tip must be more extensible at low ψ_w than at high ψ_w. Measurements of growing root cells showed that wall properties can compensate quickly to changes in turgor (Frensch, 1997). Biochemical evidence for this was provided by Wu et al. (1996) who showed that in this region acid-induced extensibility increased at low ψ_w. This fits with evidence that cell wall pH is significantly lower 0–3 mm from the root apex than in more basal regions (Fan and Neumann, 2004).

Plastic deformation of the cell wall requires that bonds between load-bearing structural elements must be broken, allowing some slip so that previously slack

tethers take the strain (Passioura, 1994), fresh wall polymers are added (Proseus and Boyer, 2006), and then new bonds form to carry the tension in the wall produced by turgor pressure. Obviously this must happen in a controlled fashion, otherwise cells would explode with the internal force of hydrostatic turgor. The control occurs via activity of proteins acting on cellulosic, hemicellulosic and pectin components of the wall. Members of two protein families, xyloglucan endotransglycosylase (XET) and expansin, increase in activity specifically in the apical few mm of the elongation zone of maize primary roots at low compared to high ψ_w. XET activity at low ψ_w correlated with the spatial distribution of growth within the apical cm (Wu et al., 1994). The increase in XET activity at low ψ_w was dependent on ABA accumulation, and XET transcript levels also have been shown to be regulated by auxin in the *Arabidopsis* root tip (Osato et al., 2006). In contrast, effects of low ψ_w on expansin activity and transcript levels were not dependent on ABA in the maize primary root tip (Wu et al., 2001). Additional tests showed that tissues in the apical 5 mm of the maize primary root at low ψ_w exhibited increased susceptibility to expansin, whereas tissues in the zone of growth inhibition lose their sensitivity to expansin (Wu et al., 1996). The patterns of expansin activity, protein and transcript level are spatially and temporally complex (Wu et al., 2001). An important point to reinforce is that these discoveries would not have been made had whole roots been analyzed without regard to the spatial distribution of growth.

The synthesis of wall polymers and integration of new cell wall material is a complex process and is only partially understood. Construction of composite cell wall materials requires that deposition of cellulose microfibrils, which occurs outside the cell membrane, is coordinated with the synthesis of hemicellulose and pectin, which occurs in the Golgi apparatus. Furthermore, the rate of movement of these polymers from vesicles to the cell membrane depends on the supply of substrate for synthesis. A recently discovered family of wall-associated kinases, which are required for root growth, may play an important role in this regulation (Kohorn et al., 2006).

1.7. Functional Genomics and Proteomics

To discover further genes related to the pattern of growth within the root tip at high and low ψ_w, surveys of changes in the root transcriptome were undertaken (Figure 2; Poroyko et al., 2006). Results showed that EST profiles from distinct regions within the apical cm of the root tip, guided by the relative elongation rate distribution, showed populations of transcripts that were unique to accelerating, decelerating and non-growing cells. Other work also revealed a large number of specific genes up- or down-regulated by low ψ_w (Bassani et al., 2004). Cell wall proteomic studies of maize root tips (Zhu et al., 2006) are beginning to reveal information about the spatial and temporal regulation of proteins that may play a role in growth regulation. These approaches will supply a huge amount of new data, and an important challenge will be to narrow down large gene sets to a small number of key genes that can be studied in detail.

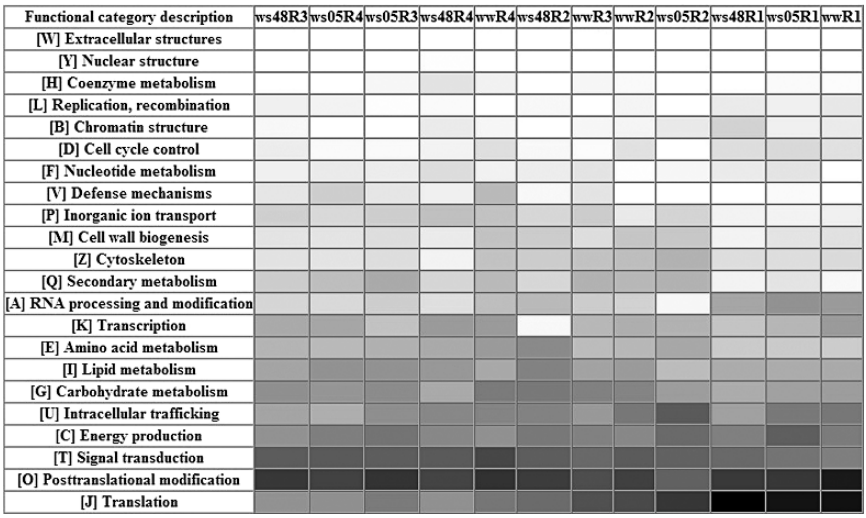

Figure 2. The relative abundance of transcripts, grouped according to functional category, expressed within the maize primary root tip at high (WW) and low (WS) ψ_w (Poroyko et al., 2006). The root tip was divided into four regions: 0–3 mm from the root apex-root cap junction (R1), 3–7 mm (R2), 7–12 mm (R3), 12–20 mm (R4). Refer to Figure 1 for the corresponding patterns in longitudinal growth rate. Roots were harvested at 5 and 48 h after transplanting seedlings to low ψ_w (−1.6 MPa). In the well watered treatment, roots were harvested at 5 and 48 h after transplanting and bulked together for analysis. Cells within the grid are shaded according to the proportional representation of each functional category within the unigene library for each experiment (columns). The scale ranges from 0 (white) to 12.6% (black)

1.8. The Role of Reactive Oxygen Species

In practically every study of stress-induced changes to the proteome there is a group of proteins classed under 'oxidative stress'. Reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot -}$) and hydroxyl radicals ($^{\cdot}OH$) accumulate under stress conditions and need to be kept under control to preserve the integrity of cellular macromolecules. The redox balance of cells is controlled by a series of enzymes and intermediate metabolites. Interestingly, ROS are not completely abolished, but also play important roles in signaling and growth regulation (Carol and Dolan, 2006). In growing cells, the controlled breakdown of cell wall polymers involves $^{\cdot}OH$ (Liszkay et al., 2004) and quenching ROS inhibits root growth (Demidchik et al., 2003). Overexpression in *Arabidopsis* of a peroxidase localized mainly in the root elongation zone stimulated root elongation (Passardi et al., 2006). Likewise, wall hardening via crosslinking polymers slows growth, and these reactions are controlled by ROS. For example, callose deposition and wall protein crosslinking via ROS production were induced by treating roots with ACC, the ethylene precursor, which reduced cell elongation (de Cnodder et al., 2005).

ABA also plays a role in regulating the balance between useful production and harmful over-production of apoplastic ROS. *Arabidopsis* mutants with defective NADPH oxidase could not generate the H_2O_2 required for ABA signaling during stomatal closure. However, root growth in the mutants was unaffected; only when exogenous ABA was added to well watered roots to cause root growth *inhibition* (see section 1.1) did it appear that ROS production mediated ABA (or induced ethylene?) effects on root growth (Kwak et al., 2003). In maize, ABA deficiency can cause uncontrolled ROS production and growth inhibition within the primary root apex (I-J Cho, M Sivaguru, RE Sharp, unpublished). Thus, too little or too much tissue ABA (in relation to normal physiological levels) can cause ROS-related growth inhibition. Another source of ROS is oxalate oxidase, a germin-type enzyme that releases H_2O_2 into the apoplast; other germin-type proteins show superoxide dismutase activity. Germins are a large and multi-functional family, and frequently appear in 'omic analyses of plants subjected to abiotic stresses (Bray, 2004). It is clear from current research that ROS play an important role in root growth regulation and the response to drought. However, finding a way to manipulate the control of cellular redox balance to favor root growth under dry conditions is a significant challenge.

1.9. Perception of Low ψ_w, Signal Transduction and Signal Maintenance

In the preceding sections we have described root growth responses to low ψ_w under steady-state conditions. An important question is how these responses are triggered as rhizosphere conditions begin to change. How do roots perceive a change in the surrounding ψ_w? One hypothesis is that the primary response occurs at the plasmamembrane-cell wall interface (Wojtaszek et al., 2005). Altered conformation of membrane-spanning proteins such as stretch-activated ion channels anchored to the cytoskeleton could affect ion fluxes and the electrochemical potential of the cell (Lew, 2004). This could then trigger a cascade of further events, perhaps including Ca^{2+} and pH, well-known intracellular signals (Gao et al., 2004).

Electrophysiological measurements showed that cortical cells within the elongation zone of maize primary roots undergo hyperpolarization (via activation of the plasmamembrane H^+-ATPase) in response to low ψ_w (Ober and Sharp, 2003). In another study, flux patterns of K^+, Cl^- and Na^+ at the root surface were transiently altered in response to low ψ_w (Shabala and Lew, 2002). A portion of the current in the elongation zone is also carried by Ca^{2+} (Kiegle et al., 2000), which is fundamental to growth in tip-growing cells such as pollen tubes and root hairs (Feijó et al., 2004). A hyperpolarized membrane potential would increase the driving force for increased K^+ uptake. This K^+ could contribute to short-term osmotic adjustment. However, under steady-state conditions during long-term exposure to low ψ_w, there is little change in tissue K^+ status (Sharp et al., 1990).

It is possible that short-term changes in cytoplasmic ion concentrations trigger the deposition of organic solutes, which accumulate during long-term exposure to low ψ_w, and as these compounds accumulate, ion concentrations return to normal

levels. Accumulation of organic "compatible solutes" prevents deleterious effects of high ionic strength in the cytoplasm. Such a sequence of events in osmotic adjustment occurs in other organisms (e.g. bacteria; Yim and Villarejo, 1994), and most likely in plant roots as well.

In maize root tips, root cells hyperpolarized during the initial exposure to low ψ_w, but eventually returned to resting potentials near to but significantly more negative than those at high ψ_w (Ober and Sharp, 2003). In ABA-deficient roots, however, membrane potentials remained hyperpolarized specifically in the region in which cell growth is responsive to ABA. This could be an indication that in roots, as in stomatal guard cells, setpoints for ion homeostasis shift in response to low ψ_w, and maintenance of these setpoints may depend on ABA (MacRobbie, 2006).

It is important to note that experimental procedures used to investigate stress perception and signal transduction have a large influence on the results. The mode in which ψ_w is altered and the rate at which it is applied can produce different effects (Kacperska, 2004). For instance, roots subjected to a rapid decrease in ψ_w showed a depolarization, while slow imposition of low ψ_w caused a hyperpolarization (Ober and Sharp, 2003). Mannitol is a common osmotic agent, but is toxic to maize roots (Hohl and Schopfer, 1991); polyethylene glycol solutions with inadequate aeration can induce symptoms of hypoxia (Verslues et al., 1998). The challenge for investigators is to understand which set of conditions lead to accurate conclusions that can be applied outside the laboratory.

1.10. Cell Production

The emphasis in this chapter has been on root cell expansion, but growth also depends on cell production rate, which is a function of the rate of cell division and meristem size (Beemster et al., 2005). Within the meristem, under a range of environmental situations affecting supply of water and nutrients to the root, the duration of the cell cycle is uniform, but the proportion of cells that are dividing can change (Baskin, 2000). Thus, under most conditions, meristems are well protected and meristematic activity is robust and not easily perturbed. However, under severe water deficit, cell division rate can be inhibited (Saab et al., 1992; Sacks et al., 1997). Merely increasing the rate of cell division would not necessarily increase root growth as it could result in a large number of very small cells. However, overexpression of cyclin B genes resulted in increased growth without any effect on final cell size (Doerner et al., 1996; Lee et al., 2003). Also, the *CRL2* mutant in rice shows increased meristem size, cell flux and cortical cell length compared with wild type (Inukai et al., 2001). These results suggest that cell production could be manipulated by breeding to benefit root growth, although pleiotropic effects on whole plant function would have to be examined carefully.

1.11. Root Growth in the Real World

Roots of crop plants growing in the field encounter a range of situations that are rarely matched in controlled experimental conditions. Crops often have to face

inhospitable subsoils: high penetration resistance, aluminum toxicity, pH extremes, and poor aeration are some of the problems roots face after they penetrate the surface layers (Passioura, 2006). In many cases, soils harden as they dry. In one study of wheat subjected to water deficit, soil penetration resistance inhibited yield more than soil water availability (Whalley et al., 2006).

The soil profile is often heterogeneous, with different soil textures and patches of water and nutrients (Hutchings and John, 2004). Roots do not grow in sterile media, but are surrounded by rhizosphere microflora (Watt et al., 2006) and many species are typically infected by mycorrhyzae. Neither is the rhizosphere a solitary root or neatly divided root system, but a complex weave of many roots, often clumped, and often of different species in weedy crops or intercropping systems. The highly variable and unpredictable nature of life in the field means that root systems must be equally flexible, as must be the models we employ to describe them.

Advantages to the plant provided by expression of 'phenotypic plasticity' in heterogeneous natural environments (Grime and Mackey, 2002) perhaps may be exploited for crop improvement. Examples of plasticity already mentioned are the ability of roots to grow thinner and longer, and changes in the number and length of lateral roots. Changes in root system architecture in response to P (Lynch et al., 2005) and N (Walch-Liu et al., 2006) status in local soil patches are well documented. The understanding of hydrotropism, the ability of roots to grow towards moist soil patches, is gaining ground with new studies utilizing mutants (Eapen et al., 2005; Tsuda et al., 2003), but the mechanisms remain unclear.

During water deficit as surface soil layers are depleted of moisture, root systems can proliferate deeper in the soil profile in permissive soils such that the density of roots is greater in stressed than non-stressed conditions (Klepper, 1990). Among numerous examples, this has been shown in maize roots growing in soil columns (Sharp and Davies, 1985), and in sugar beet (*Beta vulgaris* L.) roots in the field (Figure 3).

1.12. Selection Methods for Breeding for Improved Root Growth

One of the difficulties in breeding for complex quantitative traits such as root growth is the identification of a major character on which to base selections. One response is to make selections on phenotype without knowledge of what specific factors contribute to, say, greater root mass deep in the soil profile. With substantial effort and resources, this empirical approach can be used directly, or better, to identify QTLs conditioning these phenotypes. Markers linked to major QTLs can then be used for routine screening to select genotypes possessing superior alleles. This has been successful in rice (Steele et al., 2006). Another approach is to test the functional contribution of candidate genes, one by one, to determine which of the potential candidates has the greatest impact on the desired phenotypic trait. These efforts will be aided by innovations in laboratory and field techniques to observe and measure root systems. Rapid seedling screening techniques are essential when large numbers of genotypes need to be assessed (Bengough et al., 2004;

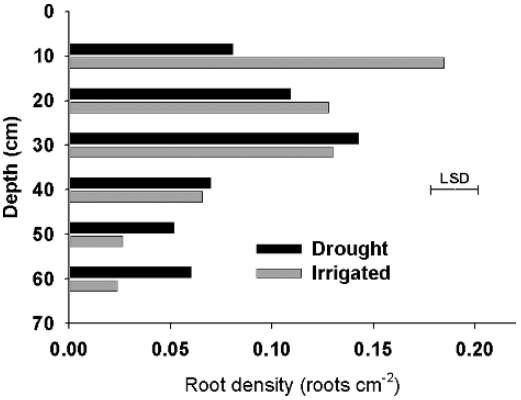

Figure 3. Plasticity in the proliferation of sugar beet roots within the soil profile under irrigated and droughted conditions in the field (CHG Smith, ES Ober, unpublished data). Note the significantly greater root density deep in the soil profile under droughted conditions. Root densities were determined using the trench profile face method, counting root contacts in 10 cm square grids. Bars indicate the mean of eight replicate plots. The LSD for the treatment x depth interaction term from ANOVA is shown

Kuchenbuch and Ingram, 2002). When the number of genotypes has been limited by this process, confirmation of genotypic rankings under field conditions can be considered. Most studies rely on the conventional techniques of quantifying root behavior using soil cores, but advances have been made in image analysis of samples (e.g. Vamerali et al., 2003). Another approach is to assess genotypic differences in rooting indirectly by measuring patterns of soil water depletion (Figure 4). Other useful field techniques that estimate different aspects of root systems are root pulling strength (Landi et al., 2002) and root electrical capacitance (van Beem et al., 1998).

1.13. Conclusions

Plants have evolved a highly complex regulation of root and shoot growth to achieve maximum fitness with the available resources. An important question to address is the extent to which plants have optimized this regulation, and what further advances are possible through genetic manipulation, whether via conventional plant breeding or transgenic technology. It is likely that benefits in one area may be balanced by increased costs in another. For instance, Passioura (Passioura, 1983) pointed out that “there is no point in a droughted crop investing a parcel of assimilate in its roots if the extra water thereby obtained does not allow the shoots to at least replenish the assimilate so spent”. Despite the inevitable stress-related trade-offs within the plant (Weih, 2003), plant breeders have managed to find improvements in yield for water-limited environments, although it is slow and difficult work overcoming the conservative nature of plants geared towards survival. (We have seen, for instance, how DELLA proteins inhibit growth, but increase survival under

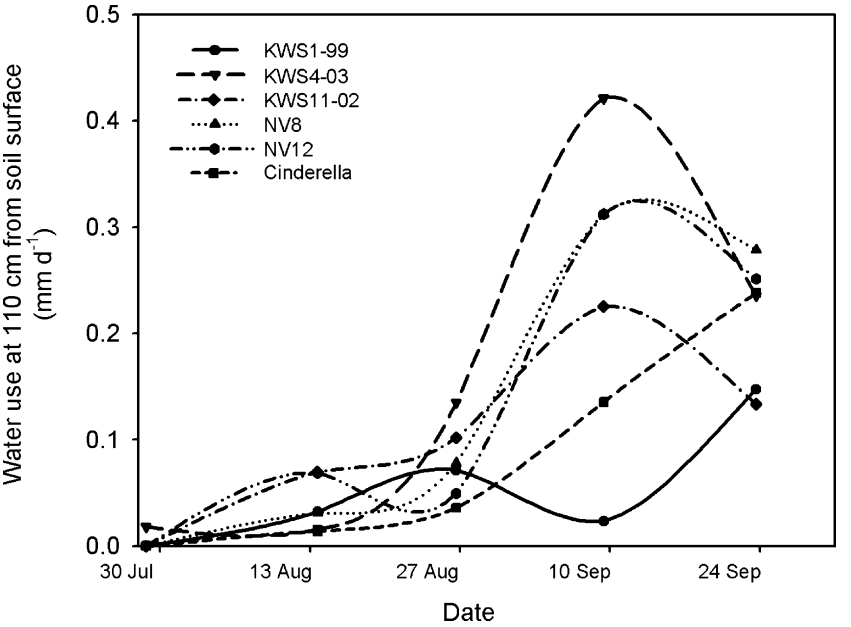

Figure 4. Patterns of soil water depletion during drought in five experimental sugar beet hybrids and one commercial cultivar (Cinderella). Differences between genotypes in summed water use from this layer were significant ($P<0.05$). Weekly changes in soil moisture content were made at 110 cm from the soil surface using a capacitance-type soil moisture probe (Ober et al., 2005). Smoothed lines connect mean values of four individual plots for each genotype

extreme conditions.) For a number of drought-related traits, there is substantial allelic variation among ecotypes within wild species (Mouchel et al., 2004), and within the pool of crop germplasm (Figure 4). These points provide encouragement for the process of crop improvement.

In this chapter we have reviewed a number of processes that are vital to root growth: cell wall modification, transport of organic solutes, ions and water, and control of oxidative stress. All of these were shown to be related to the spatial distribution of growth within the root tip, and most were regulated in some manner by ABA or ethylene. Factors that control hormonal synthesis or sensitivity, and the interactions between hormones are clearly important, but gross manipulation of hormone levels may produce confounding or undesirable side effects. Functional testing of candidate genes is necessary, but overexpression or silencing of genes must be done in a tissue-specific manner, using appropriate (e.g. stress-responsive) upstream elements. Growth kinematics is a useful tool to highlight which tissue regions should be targeted.

Functional genomic and proteomic studies are revealing numerous genes and proteins that are responsive to low ψ_w and spatially correlated with growth maintenance in the maize primary root. Taken together, so many interacting factors

must coincide at the right time and place that the complexity of growth becomes impossible to portray in a simple linear fashion. The methodology of systems biology (Aderem, 2005) is required to describe the process of growth and to identify limiting factors for given situations. Also, neural network models can be applied to describe the multiple parameters controlling growth (Ushada and Murase, 2006). The tools of genomics and computational biology are developing at a fast pace, and will continue to aid discovery and identification of potential breeding targets.

A potential limiting factor for these new technologies is the quality of the data that are fed into the system. For improvement of root growth and function, this means providing accurate phenotypic data under relevant conditions. An important caveat for all genetic improvement projects is that at some point plants must be grown in the field to measure yield. The differences between conditions in controlled laboratory or glasshouse conditions and the field are often huge. Phenotypic expression can be radically altered by growing conditions resulting in large genotype x environment interactions. What were permissive conditions for expression of a trait in potting compost, for instance, may not exist in the field, making the trait irrelevant to plant breeders. The need for a multi-disciplinary approach emphasizes the importance of co-operation between breeders, physiologists, molecular biologists, agronomists, statisticians and crop modelers. At all levels, key components in this research are patience and persistence. Weaver (Weaver, 1926), who excavated by hand entire root systems of several crop species, noted

"There is no easy method of uncovering the root system, and unless one is willing to spend considerable time and energy, and exercise a great deal of patience, it is better not to begin. But once started, the work, although difficult, is very interesting and in fact even fascinating."

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