Root Growth Maintenance during Water Deficits: Physiology to Functional Genomics

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Abstract
Progress in understanding the network of mechanisms involved in maize primary root growth maintenance under water deficits will be reviewed. These include adjustment of growth zone dimensions, turgor maintenance by osmotic adjustment, and enhanced cell wall loosening. The role of the hormone abscisic acid (ABA) in maintaining root growth under water deficits will also be addressed. The research has taken advantage of kinematic analysis, i.e. characterization of spatial and temporal patterns of cell expansion within the root growth zone. This approach revealed different growth responses to water deficits and ABA deficiency in distinct regions of the root tip. In the apical 3 mm region, elongation is maintained at well-watered rates under severe water deficit, although only in ABA-sufficient roots, whereas the region from 3-7 mm from the apex exhibits maximum elongation in well-watered roots but progressive inhibition of elongation in roots under water deficit. This knowledge has greatly facilitated discovery of the mechanisms involved in regulating the responses. The spatial resolution with which this system has been characterized and the physiological knowledge gained to date provide a unique and powerful underpinning for functional genomics studies. Characterization of water deficit-induced changes in transcript populations and cell wall protein profiles within the growth zone of the maize primary root is in progress. Initial results from EST and unigene analyses in the tips of well-watered and water-stressed roots highlight the strength of the kinematic approach to transcript profiling.

Media summary
Interdisciplinary research approaches encompassing physiology to functional genomics to identify mechanisms of root growth maintenance during water deficits are reviewed.

Keywords
Kinematics, osmotic adjustment, cell wall extensibility, expansins, abscisic acid, ESTs

Introduction

Drought is the major abiotic stress factor limiting crop productivity worldwide, and understanding the genetic and biochemical mechanisms which control drought tolerance is a central question in plant biology. As water resources for agricultural uses become more limiting, the development of drought-tolerant lines will become increasingly important. One aspect of principal importance in this arena is the response of root growth and development to water deficit conditions.

The physiology of maize primary root elongation at low water potentials has been studied extensively by Sharp and co-workers, and the findings are summarized in this report. This work has provided the foundation for an understanding of the complex network of responses involved. The research has taken advantage of a kinematic approach, i.e. the study of spatial and temporal patterns of cell expansion within the growth zone (Erickson and Silk 1980; Silk 1984), which has greatly facilitated discovery of the mechanisms involved in the growth responses. The spatial resolution with which this system has been characterized and the physiological knowledge gained to date provide a unique and powerful underpinning for functional genomics studies which are underway and will be introduced here. This combined approach can be expected to yield much novel and valuable information towards the goal of a comprehensive understanding of the regulation of root growth during water deficits.

Root growth maintenance during water deficits

In plants growing in drying soil, the development of the root system is usually less inhibited than shoot growth, and may even be promoted (Sharp and Davies 1989). Maintenance of root growth during water

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deficits is an obvious benefit to maintain an adequate plant water supply, and is under genetic control (O'Toole and Bland 1987; Sponchiado et al. 1989).

An important feature of the root system response to soil drying is the ability of some roots to continue elongation at water potentials that are low enough to completely inhibit shoot growth. For example, this occurs in nodal (adventitious) roots of maize, which must penetrate through the often dry surface soil (Sharp and Davies 1979; Westgate and Boyer 1985), and in primary roots of a range of species, which helps seedling establishment under dry conditions by ensuring a supply of water before shoot emergence (Sharp et al. 1988; Spollen et al. 1993; van der Weele et al. 2000). Figure 1 shows for several agronomic species that the primary root maintains substantial elongation rates at water potentials lower than –1.5 MPa, whereas shoot growth is completely inhibited at much higher water potentials.

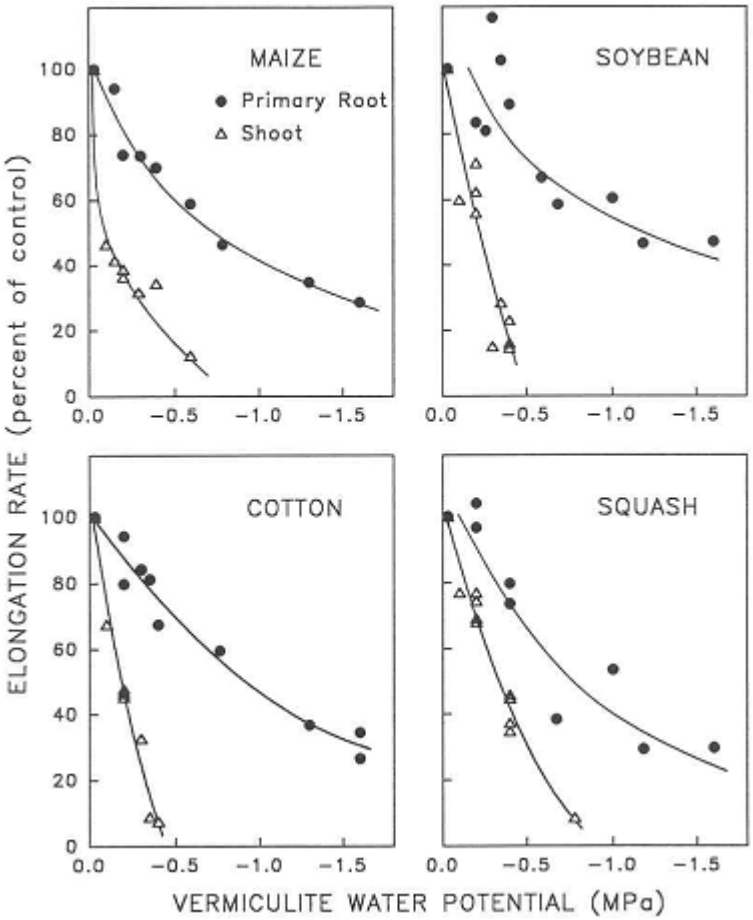

Figure 1. Elongation rates of the primary root (●) and shoot (Δ) of seedlings of four species at various water potentials. After germination, seedlings were transplanted to vermiculite at different water potentials (obtained by thorough mixing with different amounts of water) and grown at 29°C in the dark and near saturation humidity to minimize transpiration. For roots, data were evaluated when elongation rates were steady. For shoots, data represent maximum elongation rates obtained after transplanting. Data are plotted as a percentage of the rate at high water potential. (From Spollen et al. 1993.)

Taking advantage of a kinematic approach

As emphasized by Erickson and Silk (1980), knowledge of the spatial and temporal variation in growth rates within tissues can be a powerful tool in physiological studies. Under well-watered conditions, the elongation zone of the maize primary root encompasses the apical 12 mm (Figure 2). Cells are produced in the meristem near the apex, and during expansion are displaced basally by the production and expansion of new cells. The relative elongation rate peaks at about 4.5 mm from the apex, and then gradually declines to zero. In water-stressed roots, it was discovered that elongation is maintained preferentially towards the apex (Sharp et al. 1988). Remarkably, even at a water potential of –1.6 MPa, the relative elongation rate is unaffected in the apical 3 mm, but is progressively inhibited at more basal locations, reaching zero at 7 mm from the apex. Clearly, different mechanisms can be expected to underlie the responses to water stress in the apical and basal regions, and investigation of mechanisms maintaining cell elongation must focus on the apical region. However, the inhibition in the basal region is

also important to understand, because this is probably part of a coordinated response to allow preferential use of limited resources (water and growth substrates) in the apical region.

In addition, roots growing at low water potential become thinner (Sharp et al. 1988), and this change in morphology is also believed to be adaptive such that roots can further concentrate their use of resources (Sharp et al. 1990). The thinning is caused by restriction of the lateral expansion rate of cells in both the stele and cortex, although only in the apical 5 mm. Basal to this, lateral expansion rates are nearly identical to those in well-watered roots (Liang et al. 1997). This pattern is opposite to the response of longitudinal expansion, showing that the response of cell expansion to water stress is regulated independently in longitudinal and radial directions. The regulatory mechanisms that determine these responses are unknown.

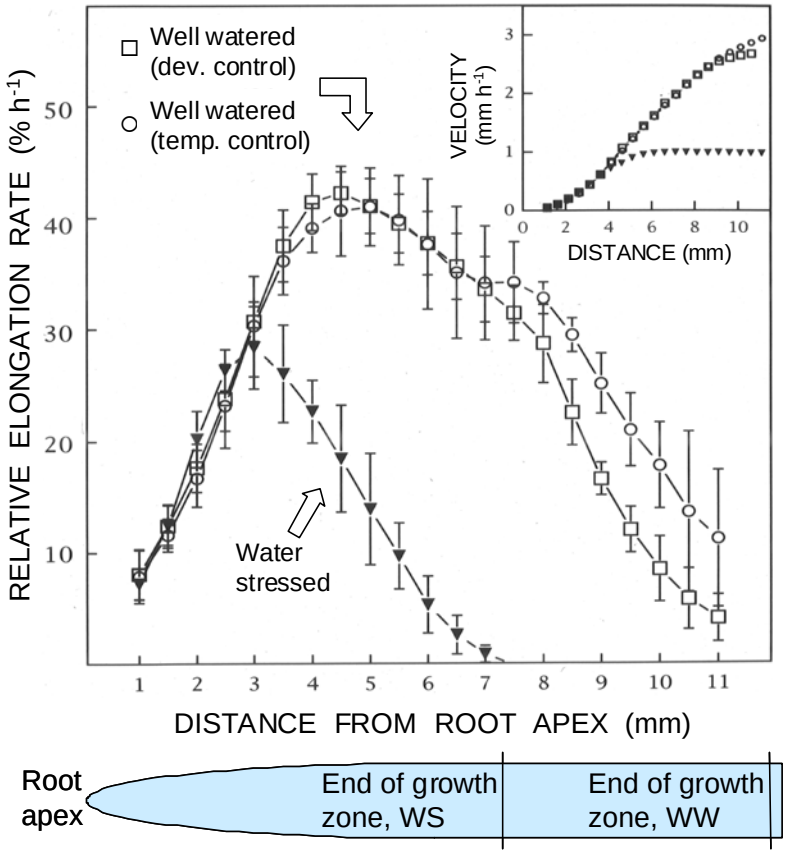

Figure 2. Relative elongation rate as a function of distance from the apex of the primary root of maize (cv FR27 x FRMo17) seedlings growing under well-watered (WW, water potential of -0.03 MPa) or water-stressed (WS, water potential of -1.6 MPa) conditions (grown as described in Figure 1). Two well-watered controls are shown, a developmental control (roots of the same length as at low water potential, 5 cm) and a temporal control (roots of the same age as at low water potential, 48 h after transplanting). The inset shows longitudinal displacement velocity profiles for the same roots. (Modified from Liang et al. 1997.)

Osmotic adjustment

Early work showed that there is substantial osmotic adjustment in the tips of both maize nodal roots (Sharp and Davies 1979; Westgate and Boyer 1985) and primary roots (Sharp et al. 1990). Osmotic adjustment in growing regions can result from two overall mechanisms, either an increase in the net rate of osmoticum deposition (including effects on solute synthesis, uptake, catabolism, import and utilization), or a decrease in the rate of tissue expansion and, therefore, in the rate of osmoticum dilution. Clearly, the former is more likely to represent an adaptive response that could contribute to growth maintenance. In the maize primary root tip, hexoses are the primary contributor to osmotic adjustment in the basal region of the growth zone, but the accumulation of hexose can be accounted for by the reduced rates of volume expansion in this region; in fact, hexose deposition rates beyond 3 mm from the apex are decreased in water-stressed compared to well-watered roots (Sharp et al. 1990). In contrast, in the apical few mm, proline concentration increases dramatically in water-stressed roots, and contributes up to 50% of the osmotic adjustment. This response involves a several-fold increase in the net rate of proline

deposition (Voetberg and Sharp 1991), which in turn involves an increase in the rate of proline transport to the root tip (Verslues and Sharp 1999). Additional work showed that accumulation of the hormone abscisic acid (ABA) is required for the increase in proline deposition at low water potential (Ober and Sharp 1994), suggesting that ABA may play a role in regulating proline transport to the root tip. In this regard, it is noteworthy that there are reports that water stress can increase expression of proline transporters (Rentsch et al. 1996) as well as cause large increases in proline concentration in the phloem sap (Girousse et al. 1996). The solutes that account for most of the other 50% of the osmotic adjustment in the apical few mm of the water-stressed roots remain to be identified. The other measured solutes (hexose, sucrose, other amino acids, potassium) made only a small contribution to osmotic adjustment in this region (Voetberg and Sharp 1991).

Enhanced cell wall loosening

The extent of osmotic adjustment in the maize primary root tip, although substantial, is insufficient to maintain turgor at well-watered levels in roots growing under severe water deficits. At a water potential of –1.6 MPa, turgor is reduced by over 50% throughout the growth zone (Spollen and Sharp 1991). In the apical few mm, the complete maintenance of elongation rate (Figure 2) despite the decrease in turgor suggested that longitudinal cell wall extensibility is enhanced in this region under water stress. This was confirmed by direct assessment of cell wall extension properties, which showed a large increase in acid-induced extensibility in the apical 5 mm (and a decrease in the 5-10 mm region) of water-stressed compared to well-watered roots (Wu et al. 1996).

Cell wall-loosening proteins are believed to play key roles in controlling cell wall extension. Therefore, activities of expansins and xyloglucan endotransglycosylase (XET) were examined to see if they correlated with the increase in wall extensibility in the apical region of the water-stressed roots. Expansin activity and extractable expansin protein increased substantially in the apical 5 mm of water-stressed compared to well-watered roots (Wu et al. 1996). The susceptibility to expansin action also increased, indicating changes in wall structure or chemistry that facilitated expansin accessibility or action. A subsequent study (Wu et al. 2001) showed that four expansin genes are expressed specifically in the growth zone in well-watered roots, and that three of these are rapidly up-regulated in the apical 5 mm and down-regulated in the 5-10 mm region after transplanting to low water potential (Figure 3), correlating with the maintenance of elongation and the increase in cell wall extensibility in the apical region. These results illustrate the advantage of using a kinematic approach, because the up- and down-regulation of expression of the same genes in adjacent regions would have precluded observation of these changes if the whole tip had been studied. XET activity was also enhanced specifically in the apical 5 mm of the water-stressed roots, and this response was shown to be dependent on ABA accumulation (Wu et al. 1994). In contrast, the evidence suggested that the changes in expansin gene expression are not mediated by ABA. Proteomic analyses are in progress to gain a comprehensive understanding of how cell wall protein composition changes in association with the differential growth responses to water deficits in the different regions of the root tip.

Growth-maintaining role of ABA accumulation in water-stressed roots

Hormones are likely to play important regulatory roles in the adaptation of root growth to water deficits, but the involvement of most of these compounds has not been elucidated. The exception is the accumulation of ABA, which has been shown to be required for maintenance of maize primary root elongation at low water potentials (reviewed in Sharp 2002). When maize seedlings are grown at a water potential of -1.6 MPa, the ABA content of the root growth zone increases about five-fold. Three approaches have been used to study the effect on root growth of reducing the accumulation of ABA: (i) the inhibitor fluridone, which blocks carotenoid synthesis and, thereby, inhibits ABA synthesis although at an early step of the pathway; (ii) the *vp5* mutant, which has a defect at the same step as that blocked by fluridone; (iii) the *vp14* mutant, which has a defect in the synthesis of xanthoxin (Tan et al.

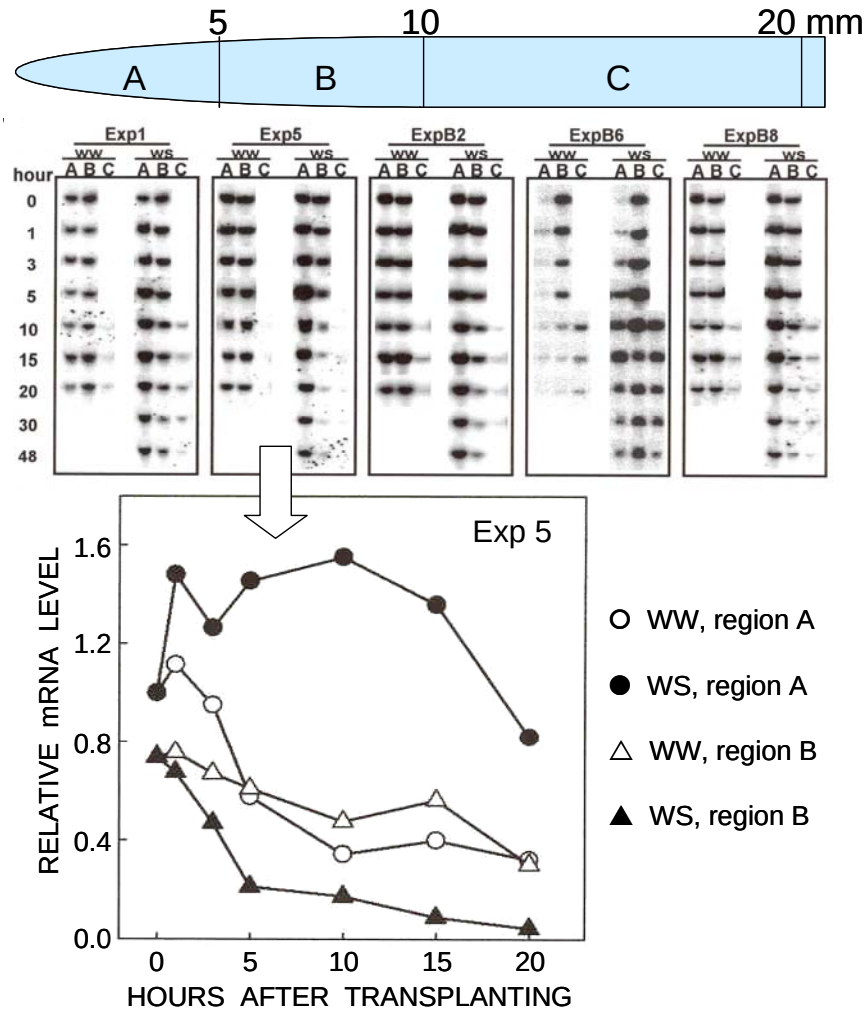

Figure 3. Time course for expression of five expansin genes (northern-blot analysis) in the primary root tip of maize (cv FR27 x FRMo17) seedlings growing under well-watered (WW, water potential of -0.03 MPa) or water-stressed (WS, water potential of -1.6 MPa) conditions (grown as described in Figure 1). Quantification of the signals for *Exp 5* is shown. The first 20 mm of the root was cut into three regions from the apex: A, 0-5 mm; B, 5-10 mm; C, 10-20 mm. Roots were shorter than 20 mm during the first 5 h after transplanting, so samples could not be collected for region C during this period. (Modified from Wu et al. 2001.)

1997). Xanthoxin synthesis is considered a key regulatory step in water stress-induced ABA production (Qin and Zeevaart 1999). Initial studies used fluridone and *vp5* (Saab et al. 1990; Saab et al. 1992; Sharp et al. 1994), and studies of *vp14* were recently undertaken to strengthen the conclusion that the results were due to ABA deficiency and not to other effects. The results obtained with the three approaches were very similar (Figure 4). At high water potential, root elongation rates (and ABA contents) were minimally affected. At low water potential, in contrast, reduced ABA accumulation was associated with more severe inhibition of root elongation than in wild type or untreated seedlings. In all cases, root elongation rate fully recovered when the ABA content of the growth zone was restored to normal levels with exogenous ABA, confirming that the normal accumulation of ABA is necessary for root growth maintenance during water stress.

It is important to note that the conclusion that the accumulation of ABA in water-stressed roots helps to maintain elongation cannot be inferred by applying ABA to well-watered seedlings to simulate the increase in content under water stress (Sharp et al. 1994). Figure 4 shows that in well-watered seedlings treated with ABA, root growth was substantially inhibited at the root tip ABA content that occurs in water-stressed roots. These results illustrate that the maintenance of root elongation at low water potential by ABA is not solely a function of the increase in ABA content, but also requires the change in environmental conditions that modifies the growth response to ABA.

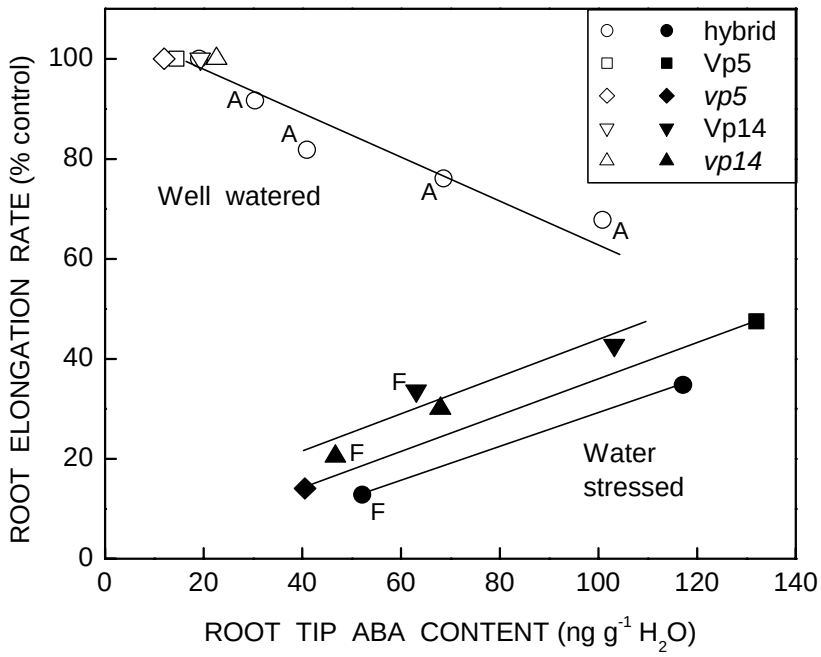

Figure 4. Primary root elongation rate as a function of root tip (apical 10 mm) ABA content for various maize genotypes growing under well-watered (water potential of -0.03 MPa, open symbols) or water-stressed (water potential of -1.6 MPa, closed symbols) conditions (grown as described in Figure 1). At high water potential, the root ABA content of hybrid (cv FR27 x FRMo17) seedlings was raised above the normal level by adding various concentrations of ABA (A) to the vermiculite. At low water potential, the root ABA content was decreased below the normal level by treatment with fluridone (F) or by using the *vp5* or *vp14* mutants. Data are plotted as a percentage of the rate for the same genotype at high water potential. Elongation rates of the mutants under well-watered conditions were similar to their respective wild types. (Modified from Sharp 2002; original data from Saab et al. 1990, Sharp et al. 1994 and unpublished data of I-J Cho, B-C Tan, DR McCarty and RE Sharp.)

The role of ABA in determining plant growth responses to water deficits is a long-standing question, and the finding that the accumulation of ABA is necessary for root growth maintenance at low water potentials contrasts with the commonly proposed growth-inhibitory function of increased ABA concentrations in water-stressed plants (Trewavas and Jones 1991). Recent studies have also shown that the normal ABA levels in well-watered plants are required to maintain shoot growth in tomato (Sharp et al. 2000) and *Arabidopsis* (LeNoble et al. 2004). In all cases, the action of ABA has been shown to involve suppression of ethylene production (Sharp et al. 2000; Spollen et al. 2000, LeNoble et al. 2004), but taken together, the studies indicate that water-deficient compared to well-watered plants require increased levels of ABA to prevent excess ethylene production (Sharp 2002). This difference may be related to a role of ABA accumulation in promoting the antioxidant system to maintain reactive oxygen species (ROS) at non-damaging levels during water deficits (Figure 5). This hypothesis is based on evidence that ROS are produced in greater amounts in stressed tissues, that ABA treatments have been shown to increase expression of genes for antioxidant enzymes, e.g. catalase in maize leaves (Guan et al. 2000), and that excess ROS levels can cause increased ethylene synthesis (Overmyer et al. 2000).

In recent studies, the effect of ABA deficiency on levels of ROS in the maize primary root growth zone was studied using the *vp14* mutant, in which ABA levels are deficient in water-stressed but not well-watered roots (unpublished data of I-J Cho, M Sivaguru and RE Sharp). Under well-watered conditions, ROS levels were low in roots of both wild-type and *vp14* seedlings. Under water deficits, ROS levels were slightly greater in the growth zone of wild-type roots and increased dramatically in *vp14*. The increased ROS levels in *vp14* were prevented when ABA was restored to the wild-type level by exogenous application. The effect of ABA deficiency on ROS levels occurred specifically in the region 1-3 mm from the root apex where cell elongation is normally maintained under water deficits but is inhibited by ABA deficiency (Saab et al. 1992; Sharp et al. 1994; Ober and Sharp 2003). Loss of plasma membrane integrity occurred in the same region of the ABA-deficient roots, and results indicate that the increase in ROS levels preceded and caused the membrane impairment. The relation of these events to the increases in ethylene production and inhibition of root elongation caused by ABA-deficiency under water deficits is under investigation.

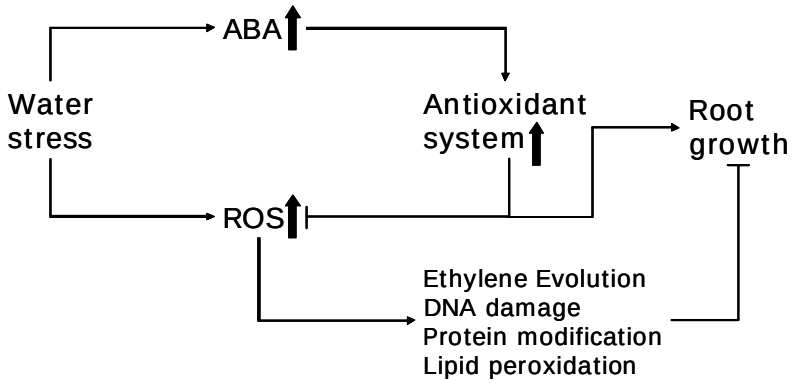

Figure 5. Hypothesized role of ABA accumulation in promoting the antioxidant system in the growth zone of water-stressed roots, and thereby preventing high levels of reactive oxygen species (ROS), excess ethylene production and growth inhibition.

From physiology to functional genomics

As detailed above, the maize primary root system is ideally suited for the application of genomic analyses to gain a comprehensive understanding of the mechanisms that control the responses of root growth to water stress. Characterization of water deficit-induced changes in gene transcript populations within the growth zone of the maize primary root is in progress. Initial results from expressed sequence tag (EST) and unigene analyses in the tips of well-watered and water-stressed roots highlight the strength of the kinematic approach to transcript profiling.

At 5 h and 48 h after transplanting to well-watered or water deficit (water potential of -1.6 MPa) conditions, primary roots were harvested and cut into four regions from the apex (Figure 6):

- Region 1 (0-3 mm), in which elongation rates are completely maintained under water deficit.
- Region 2 (3-7 mm), in which elongation rates are maximal in well-watered roots but progressively inhibited under water deficit.
- Region 3 (7-12 mm), in which elongation decelerates in well-watered roots and is completely inhibited under water deficit.
- Region 4 (12-20 mm), which is non-elongating in well-watered and water-stressed roots.

By comparison with regions 1-3, region 4 helps to identify stress-induced changes that are specifically associated with growth, and its maintenance or inhibition (as in the similar studies of expansin expression shown in Figure 3). The 48 h time point was chosen to characterize changes that are associated with the steady state patterns of relative elongation rate in acclimated seedlings (Figures 2 and 6). The 5 h time point was chosen to facilitate identification of regulatory/primary changes in gene expression (as opposed to secondary effects) after the initiation of the water stress treatment. The well-watered samples from 5 h and 48 h were combined, and 12 primary cDNA libraries (well-watered; water-stressed 5 h; water-stressed 48 h; four regions each) were generated such that each library carried a region-identifying sequence tag. After combining the libraries for each condition, three normalized libraries were generated and ~6,000 ESTs from each were sequenced. The whole 3'-EST collection was then grouped into clusters of sequences with high similarity. The relative size of each cluster in a library-region combination was calculated and the distribution of cluster sizes for each of the 12 combinations then compared to produce similarity scores. These scores are represented in the hierarchical tree shown in Figure 6.

The results illustrate that EST populations were distinctly different both in adjacent regions within a treatment and in particular regions between treatments. The most distinct profile was found in region 2 of the well-watered roots, corresponding to the uniqueness of this region in exhibiting maximal elongation rates. Under water-deficit conditions, region 2 acquired a transcript profile which was more similar to those of the decelerating region (region 3) of well-watered roots. Functional annotations of the EST populations indicate that processes which reflect the active root apical meristem, including translation, posttranslational modification, RNA processing and modification,

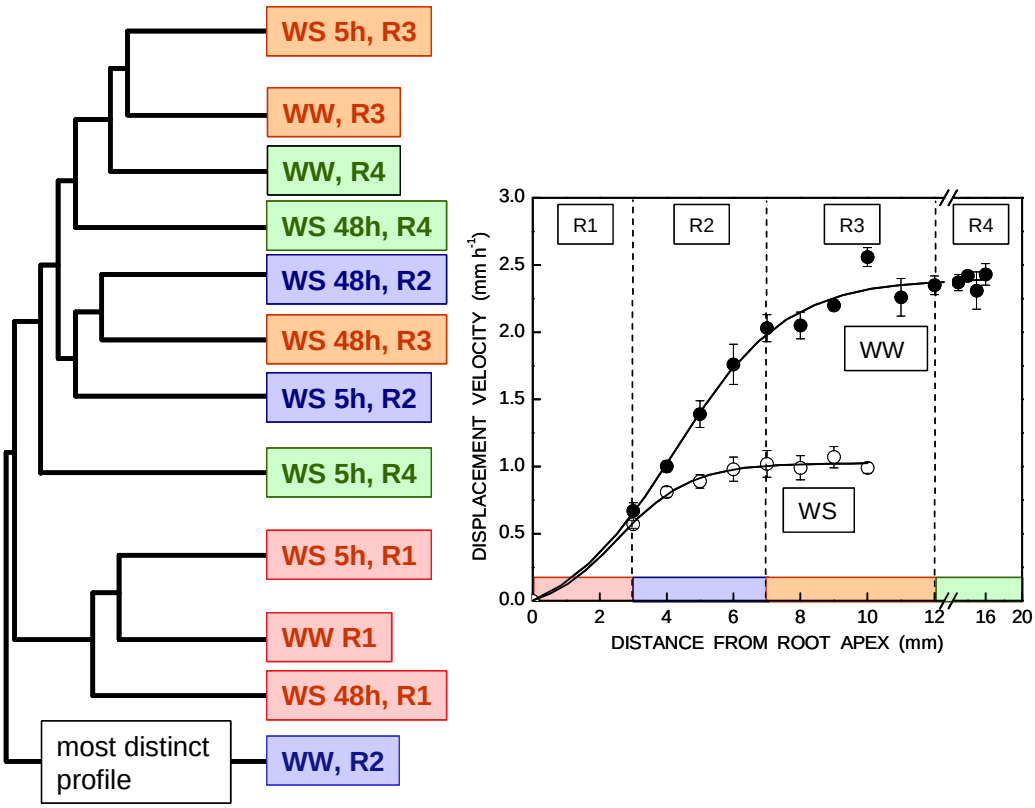

Figure 6. Similarities in EST profiles among different regions of the primary root of maize (cv FR697, water stress-tolerant) under well-watered (WW, water potential of -0.03 MPa) or water-stressed (WS, water potential of -1.6 MPa) conditions (grown as described in Figure 1). Roots were harvested at 5 h and 48 h after transplanting and cut into four regions (R) from the apex: R1, 0-3 mm; R2, 3-7 mm; R3, 7-12 mm; R4, 12-20 mm. The well-watered samples from 5 h and 48 h were combined. Further details are provided in the text. The graph shows displacement velocity as a function of distance from the root apex (calculated from root elongation rates and cortical cell length profiles at 48 h after transplanting; Silk et al. 1989), showing that water stress caused a similar change in the pattern of relative elongation rate to that shown in Figure 2. Regions 1-4 are color-coded in correspondence to the EST clusters. (Unpublished data of V Poroyko and HJ Bohnert [ESTs], and LG Hejlek and RE Sharp [displacement velocities].)

chromatin structure and dynamics, and cell cycle control were not greatly affected by the water deficit treatment. However, water deprivation for 48 h led to the repression of transcripts in categories of intracellular trafficking and carbohydrate metabolism and to significant up-regulation of transcripts related to lipid metabolism. Transcripts associated with the cytoskeleton and cell wall formation declined during water stress in regions 2-4, although region 2 exhibited recovery in the expression of these transcripts at 48 h, indicating acclimation.

To date, a total of 7,688 unigenes have been identified in the root tips from the well-watered and water-stressed roots, including 992 from a subtracted cDNA library in which transcripts present under well-watered conditions were removed. Of the total of 6,696 unigenes identified in the three tagged libraries, a surprisingly high number, 4,517, were specific to individual libraries, and moreover, the majority of those were also specific to individual regions within the individual libraries (Table 1). These results again illustrate the advantage of the kinematic approach to this study. While experiments in the 1980s estimated the number of genes expressed in roots to be less than 10,000 (Kamalay and Goldberg 1980; 1984), recent analyses have altered these estimates. The analysis of SAGE tags and an analysis of microarray data in *Arabidopsis* indicated a much higher number of genes expressed in (total) root tissues; at least 15,000 expressed sequence tags or transcripts have been identified (Birnbaum et al. 2003; Ekman et al. 2003). Accordingly, our results suggest that from the sampling of ~21,000 sequenced ESTs from which the root tip unigene set was derived, approximately half of the maize root transcriptome has been obtained.

Table 1. Distribution of library-specific unigenes among regions 1-4 of the maize primary root under well-watered or water-stressed conditions (see Figure 6 for details of root regions, experimental treatments and cDNA library generation). From a total of 6,696 unigenes identified in the three libraries, 4,517 were specific to individual libraries. "No region identity" indicates that the region-identifying tags were not found. (V Poroyko and HJ Bohnert, unpublished data.)

Library-specific unigenes	Well watered	Water stressed, 5 h	Water stressed, 48 h
R1 specific	353	355	149
R2 specific	292	69	91
R3 specific	145	362	879
R4 specific	173	358	361
In multiple regions	108	108	135
No region identity	226	189	164
Total	1297	1441	1779

Probing the maize root transcriptome by SAGE

An additional dimension has been introduced by the generation of a SAGE library from well-watered roots (regions 1-4). SAGE (Serial Analysis of Gene Expression) technology results in concatenated clones that may contain 3'-end located portions of up to 70 genes per clone (Velculescu et al. 1995). From the number of repeat detections of particular SAGE-tags, a true abundance profile of a tissue is an additional outcome with this technique. In the well-watered maize root, more than 14,000 individual SAGE tags were found (V Poroyko and HJ Bohnert, unpublished data). This number of unique tags represents a minimum number of expressed genes, but most likely does not represent the entire maize root transcriptome.

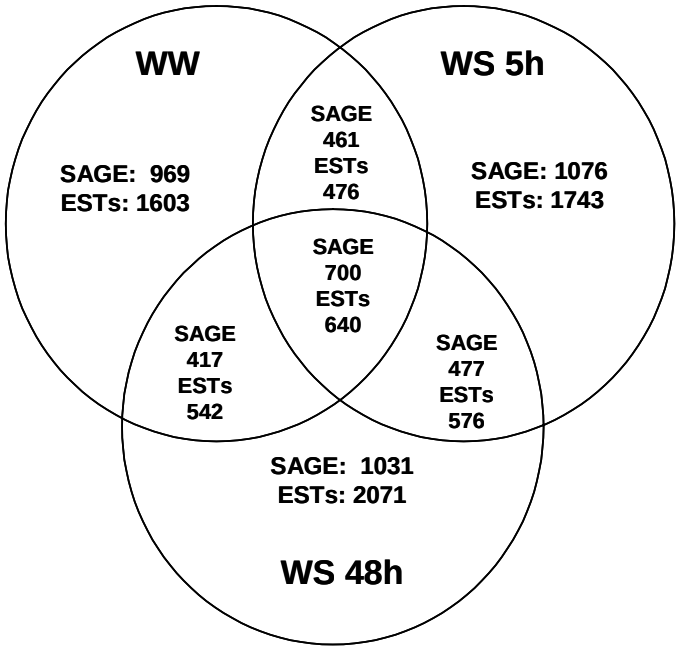

Figure 7. Venn diagram outlining the distribution of ESTs and corresponding SAGE tags in three maize root tip cDNA libraries (WW, well-watered; WS water-stressed; see Figure 6 for details of experimental treatments). Indicated are numbers of unigenes, identified by ESTs and SAGE tags, that are specific for each library. The uniform distribution of the SAGE tags indicates that during water stress, transcriptome complexity remained largely constant, especially during short-term stress. After 48 h, differences in complexity became apparent. (V Poroyko and HJ Bohnert, unpublished data.)

To identify genes corresponding to the different SAGE tags our maize root EST database was queried to obtain a virtual maize root transcript population. In total, >18,000 maize cDNA sequences that had been determined in our EST sequencing project were analyzed, resulting in the identification of 5,630 cDNAs that could be assigned to SAGE tags. Correlating SAGE tag and EST number in each library provided an interesting aspect (Figure 7). Although the SAGE tags were derived from well-watered root RNA, they were almost evenly distributed between the libraries, indicating that transcript complexity is largely unchanged by water stress and that most genes are expressed irrespective of the physiological state of the root. What is different, however, is transcript abundance. This observation suggests that gene expression, affected by water stress, primarily alters abundance levels (reported as the number of a SAGE

tag) and does not completely alter the structure of the expressed part of the genome. Closer inspection (Figure 7) indicates that complexity changes after longer-term water stress (48 h), with extrapolation indicating that approximately 20% of the transcriptome could be specific for the water-stressed state.

Conclusion

The studies reviewed here illustrate the complexity of mechanisms involved in root growth maintenance during water deficits. Understanding is being enhanced by an integrated approach, working across disciplines from physiology to functional genomics to gain a comprehensive knowledge of the gene networks, proteins and metabolites involved. This knowledge will lead to novel approaches for improving drought tolerance through genetic and metabolic engineering of root function.

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