Genes commonly regulated by water-deficit stress in *Arabidopsis thaliana*

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Abstract

Cellular water-deficit stress triggers many changes in gene expression which can be used to define the response of a plant to an environmental condition. Microarray technology permits the study of expression patterns of thousands of genes simultaneously, permitting a comprehensive understanding of the types and quantities of RNAs that are present in a cell in response to water-deficit stress. The expression of specific genes was compared in three different experiments designed to understand changes in gene expression in response to water-deficit stress. Surprisingly, there was a relatively small set of genes that were commonly induced or repressed. There were 27 genes commonly induced and three commonly repressed; 1.4% and 0.2% of the genes analysed in common to all three experiments. The induced genes fell into six different functional categories: metabolism, transport, signalling, transcription, hydrophilic proteins, and unknown. The three commonly repressed genes indicated that repression of gene expression supported a frequently observed response to water-deficit stress, decreased growth. A more detailed analysis of genes involved in cell wall metabolism, indicated that there was a global decrease in expression of genes that promote cell expansion.

Key words: *Arabidopsis*, gene expression, microarray, water-deficit stress.

Introduction

Drought has a major impact on plant growth and development, limiting crop production throughout the world. Soils too dry for crop production cover 28% of the earth’s land. Other abiotic stresses that include a component of cellular water deficit—most usually noted are salinity and low-temperature stresses—can also severely limit crop production. Major research efforts are currently directed at understanding the mechanism of plant response to conditions in which water limits plant growth and development in order to identify gene products that confer adaptation to water-deficit stress.

Mechanisms of response to water-deficit stress can be measured at many different levels from the whole plant to the molecular level. Since responses are controlled by the plant genome, recent efforts have focused on the molecular response of the plant to water-deficit stress. A better understanding of the genes that are expressed in response to water deficit are needed to characterize fully the mechanisms that permit adaptation to limiting water conditions. Many research programmes have focused on the cellular signalling mechanisms that are activated by water-deficit stress (reviewed by Shinozaki et al., 2003; Xiong et al., 2002). Several model plants, with the greatest emphasis being placed on the model plant *Arabidopsis*, are being exploited to further understanding of the molecular mechanisms that underlie the plant response to abiotic stress. One hope in the extensive studies involving this model plant is that a thorough understanding of the model plant will translate to applications in the improvement of crop growth and production (Zhang et al., 2004).

Several major classes of genes have been noted that are altered in response to water-deficit stress; genes involved in signalling and gene regulation and gene products that are proposed to support cellular adaptation to water-deficit stress are among the most frequently altered in gene
expression. Yet, the functions of the majority of the genes with altered expression remain unknown and there are probably more genes yet to be discovered. Microarray analyses, especially for the model plant Arabidopsis thaliana, have reached an advanced stage and are contributing to an understanding of the types and quantities of genes that are regulated by water-deficit stress (Seki et al., 2003, 2004). The significance of these many changes in gene expression is difficult to evaluate without a means of comparison. The objective of this study was to identify genes that were altered in expression pattern in response to different methods of water-deficit stress imposition. It is proposed that genes commonly induced or repressed under different laboratory conditions designed to simulate conditions of water limitation are more likely to play a general role in adaptation to water-deficit stresses.

Methods of analysis

The only microarray studies currently published in Arabidopsis to understand water-deficit stress used three different means of imposing water-deficit stress. Seki et al. (2002b) analysed the expression of approximately 7000 Arabidopsis genes in response to water deficit, salinity, and low-temperature stress. A microarray of full-length cDNAs was used in this analysis. Plants (A. thaliana Columbia) grown in agar for 3 weeks were transferred to dry filter paper and were desiccated for 1, 2, 5, 10, or 24 h under dim light. Sodium chloride (250 mM) and low temperature (4 °C) treatments were also applied to the 3-week-old plants. A companion study analysed the genes altered by ABA treatment (Seki et al., 2002a). Genes with an increased or decreased expression of more than 5-fold compared with the control in at least one of the treatments at one or more of the time points were reported in a supplemental table; 299 genes were induced by water-deficit conditions, 54 by low temperature, and 213 by salinity. Genes from this list whose expression was induced by ≥3.0-fold or repressed by ≤0.5-fold in one of the desiccation time points were used for the analyses reported here. The genes were identified by the Arabidopsis Genomics Initiative (AGI) locus name. In the few cases in which an AGI name was not provided in the supplemental data table, an AGI name was identified, if possible, by using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) to match the sequence of the full-length cDNA to an annotated gene in the Arabidopsis genome.

Kreps et al. (2002) analysed the expression of approximately 8100 Arabidopsis genes using an Affymetrix GeneChip®. Plants (A. thaliana Columbia) were grown in liquid culture for 4 weeks and were subjected to three different stress treatments in liquid culture. Cellular water-stress treatments were administered by transferring plants to media containing 200 mM mannitol for 3 h or 27 h. Plants were also treated with NaCl (100 mM) or low temperature (4 °C) for 3 h or 27 h. Samples were divided into shoots and roots before RNA isolation. Genes that were increased or decreased more than 2-fold at one of the time points were reported.

In the third set of data, Arabidopsis plants (Columbia) grown in soil were subjected to progressive soil-water deficit (Kawaguchi et al., 2004). Relative water content (RWC) was measured at intervals and leaves were collected after 8 d when the RWC reached 65%. The leaf samples were separated into polyosomal and non-polyosomal fractions and RNA was isolated for hybridization to the 8.2k Affymetrix GeneChip®. The change in abundance of individual mRNAs was estimated from the polyosomal and non-polyosomal RNA hybridizations (Kawaguchi et al., 2004). Probe pair sets from the GeneChip® with ≥2.0-fold or ≤0.5-fold change in response to soil-water deficit were identified and matched to an AGI name.

The data sets of AGI names representing genes up- or down-regulated by different methods to decrease cellular water potential were compared based on the AGI number using a Perl routine. The microarrays used by Kreps et al. (2002) and Kawaguchi et al. (2004) contained a similar set of genes. There are 1919 genes (Shinozaki et al., 2003) represented in common on the full-length cDNA microarrays used by Seki et al. (2002b) and the Affymetrix 8.2k GeneChip® used by Kreps et al. (2002) and Kawaguchi et al. (2004).

Common up-regulation of gene expression

Three sets of microarray data in which the experiments were designed to identify global changes in gene expression in response to water-deficit stress were compared to determine if a common set of genes was induced. In total there were 806 unique Arabidopsis genes induced in the microarray experiments of Seki et al. (2002b), Kreps et al. (2002), and Kawaguchi et al. (2004) (Fig. 1). 1.4–5.7% of the genes analysed were induced when separate experimental conditions were considered (Table 1). The comparison revealed 27 genes that were induced under all three conditions (Fig. 1; Table 2). All of the genes, except one (At2g43570) were also induced by ABA (Seki et al., 2002a) and the majority was induced in at least one of the cold experiments and one of the salt-stress experiments. Four genes were not induced by low-temperature stress and one was not induced by salt treatment (Table 2). Using the 8.2k Affymetrix GeneChip®, Kreps et al. (2002) and Kawaguchi et al. (2004) identified 535 unique genes induced by the experimental water-deficit conditions and 50 of these genes were induced under both conditions. Of these 50, 27 of them were also identified using the full-length cDNA microarray as employed by Seki et al. (2002b; Fig. 1). Although all of the experimental treatments completed on the same genotype of Arabidopsis (Columbia) were designed to identify genes involved in the plant
response to cellular water deficit, the observed increases in gene expression were finely tuned to the specific experimental conditions resulting in a set of genes induced by the specific experimental conditions rather than a set of genes generally induced by cellular water-deficit stress.

The Kreps et al. (2002) and Seki et al. (2002b) studies also analysed the expression of genes in response to other experimental conditions that produce cellular conditions of water-deficit stress. 33 genes were commonly up-regulated in roots and 39 in leaves after 3 h of low temperature, salt, or mannitol treatment, although there were fewer similarities after the 27 h stress treatment (Kreps et al., 2002).

Interestingly, there were more similarities between the low temperature, salt, and mannitol treatments completed by Kreps et al. (2002) than in the experiments done in the three different laboratories to identify water-stress-responsive genes. Seki et al. (2002a, b) also analysed changes in gene expression in response to salt and low-temperature stress. In total, 351 unique genes were induced more than 5-fold by at least one of the abiotic stress conditions. 6.3% of the stress-induced genes (22 genes) were induced commonly by all three of the stress conditions. One of these 22, ERD7, was also found to be commonly induced by water-deficit conditions in this study.

To determine if the lack of similarity between the different means of imposing water-deficit stress treatments was unique to the water-deficit experiments, published microarray experiments in which Arabidopsis plants were subjected to cold-stress treatments were also compared (Fowler and Thomashow, 2002; Kreps et al., 2002; Seki et al., 2002b). Like the water-deficit result, only 25 out of a total of 910 unique induced genes were induced in common. Thus, the lack of similarity between experiments completed in different laboratories was not a special characteristic of the water-deficit stress experiments.

There are many possible differences between these experiments that may lead to the lack of similarity in the expression of genes in these ‘drought’ stress experiments. First, there were 1919 genes represented in common between the filter paper dehydration experiment (Seki et al., 2002b) and the experiments using the Affymetrix GeneChip® (Kreps et al., 2002; Kawaguchi et al., 2004). However, the number of genes in common between the mannitol and soil-water-deficit experiment and between the filter paper and soil-water-deficit experiments was similar (50 and 44, respectively). Even in the experiments that were common in both sets.

Table 1. Number of genes identified by microarray analyses with altered regulation in response to three different water-deficit-stress experimental systems

<table>
<thead>
<tr>
<th>Published paper</th>
<th>Experimental conditions</th>
<th>No. of genes with increased/decreased mRNA</th>
<th>Total genes in microarray analysis</th>
<th>% Genes increased/decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seki et al., 2002b</td>
<td>3-week-old plants were removed from agar and desiccated on filter paper for 1–24 h</td>
<td>359/161</td>
<td>~7000 (full-length cDNA array)</td>
<td>5.1/2.3</td>
</tr>
<tr>
<td>Kreps et al., 2002</td>
<td>4-week-old plants subjected to 200 mM mannitol in liquid culture for 3 h or 27 h. Leaf and root samples were analysed separately</td>
<td>466/573</td>
<td>~8100 (Affymetrix)</td>
<td>5.8/7.1</td>
</tr>
<tr>
<td>Kawaguchi et al., 2004</td>
<td>7-week-old plants subjected to progressive soil-water deficit until leaves reached 65% RWC after 8 d</td>
<td>119/654</td>
<td>~8200 (Affymetrix)</td>
<td>1.4/4.0</td>
</tr>
</tbody>
</table>

* In the supplementary material genes that were induced by 5-fold in one of the three treatments, cold, salt, and water deficit, were listed. Genes were chosen for this analysis that were increased by at least 3.0-fold in response to water-deficit stress.
* 2.0-fold increase or more in one of the treatments.
* Genes with a $\geq 2.0$-fold or $\leq 0.5$-fold change in response to soil-water deficit were identified. The fold increase was estimated as described in Kawaguchi et al. (2004).
The culturing of plants in mannitol solution is likely initiated to conditions of cellular water deficit. Third, the majority of the common changes occurred in the leaves, although two of the changes were root specific (Table 2). Thus the sampling procedure used by Seki et al. (2002b) and Kawaguchi et al. (2004) preferentially characterized genes expressed in the shoot.

Table 2. Genes induced in common when expression patterns are compared between three different water-deficit stress conditions done in three different laboratories (Kreps et al., 2002; Seki et al., 2002b; Kawaguchi et al., 2004)

The genes are grouped by potential functional categories. The induction of the genes in response to ABA application (ABA), low-temperature stress (cold), and salt stress (salt) is noted. The organ in which the genes were expressed is listed in the last column.

<table>
<thead>
<tr>
<th>AGI locus</th>
<th>Gene name and description</th>
<th>Cold&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cold&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Salt&lt;sup&gt;c&lt;/sup&gt;</th>
<th>ABA&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Organ&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Leaf</td>
<td></td>
</tr>
<tr>
<td>At1g09500</td>
<td>Cinnamyl alcohol dehydrogenase, putative</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>leaf</td>
<td></td>
</tr>
<tr>
<td>At1g54100</td>
<td>Aldehyde dehydrogenase, putative; similar to 26S rDNA turgor protein from pea</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>leaf</td>
<td></td>
</tr>
<tr>
<td>At1g62570</td>
<td>Flavin-binding monoxygenase-like domain</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>leaf and root</td>
<td></td>
</tr>
<tr>
<td>At2g38400</td>
<td>β-alanine-pyruvate aminotransferase, putative</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>leaf</td>
<td></td>
</tr>
<tr>
<td>At2g45370</td>
<td>Endochitinase isolog</td>
<td>X</td>
<td></td>
<td></td>
<td>leaf and root</td>
<td></td>
</tr>
<tr>
<td>Transporters</td>
<td>Mitochondrial dicarboxylate carrier protein, putative</td>
<td>X</td>
<td></td>
<td></td>
<td>leaf and root</td>
<td></td>
</tr>
<tr>
<td>At2g22500</td>
<td>Amino acid/polyamine transporter, family II</td>
<td>X</td>
<td></td>
<td></td>
<td>leaf and root</td>
<td></td>
</tr>
<tr>
<td>Signal transduction</td>
<td>Similar to β-transducin, one of three subunits of G proteins</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Root</td>
<td></td>
</tr>
<tr>
<td>At1g49450</td>
<td>PP2CA, protein phosphatase 2C</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>leaf and root</td>
<td></td>
</tr>
<tr>
<td>At4g26080</td>
<td>ABI1, protein phosphatase 2C</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Leaf</td>
<td></td>
</tr>
<tr>
<td>Transcription</td>
<td>ATHB-7, homeobox-leucine zipper protein</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
</tr>
<tr>
<td>At2g46680</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At3g11410</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At4g27410</td>
<td>RD26, transcription factor, no apical meristem family member</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
</tr>
<tr>
<td>At2g41190</td>
<td>Amino acid/polyamine transporter, family II</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
</tr>
<tr>
<td>Heterogeneous, heat-soluble</td>
<td>Lea14-A-like cotton (Class VI)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
</tr>
<tr>
<td>At1g01470</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At1g20440</td>
<td>Dehydrin, COR47/d17 (Class II)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
</tr>
<tr>
<td>At1g52690</td>
<td>LEA76-like Brassica napus (Class III)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
</tr>
<tr>
<td>At5g60760</td>
<td>LEA-like (Class I)</td>
<td>X</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At5g66400</td>
<td>Dehydrin, RAB18 (Class II)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
</tr>
<tr>
<td>At5g73200</td>
<td>LT158/RD29B; low-temperature-induced 65 kDa protein</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Leaf</td>
<td></td>
</tr>
<tr>
<td>At5g25310</td>
<td>LT78/COR78/RD29a; low-temperature-induced 78 kDa protein</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>SAG29, senescence-associated protein; similar to MtN3/saliva protein</td>
<td>X</td>
<td></td>
<td>Leaf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At5g13170</td>
<td>X</td>
<td>Leaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At2g47770</td>
<td>TspO- and benzodiazepine receptor-like</td>
<td>X</td>
<td></td>
<td>Leaf and root</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At2g40000</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At2g17840</td>
<td>ERD7, senescence-related</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
</tr>
<tr>
<td>At1g05340</td>
<td>Unknown</td>
<td>X</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At1g27200</td>
<td>Unknown</td>
<td>X</td>
<td>X</td>
<td>Leaf and root</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Fowler and Thomashow (2002).
<sup>b</sup> Kreps et al. (2002).
<sup>c</sup> Seki et al. (2002b).
<sup>d</sup> Seki et al. (2002c).
Functional classification

The genes in the group overlapping between Kreps et al. (2002) and Seki et al. (2002b) (Fig. 2A) and between all three experimental conditions (Fig. 2B) were classified into functional categories. When comparing between the mannitol and filter paper dehydration conditions, the greatest proportion of genes were tentatively characterized in metabolic pathways, although they did not group in a particular metabolic category. Genes of unknown function make up the second greatest category.

The set of 27 common genes were analysed in more detail. There were genes found in six different categories (metabolism, transport, signalling, transcription, hydrophilic, and unknown) with the greatest number of genes categorized as hydrophilic proteins.

Cellular metabolism

There were five genes in the common set in which the predicted function is in cellular metabolism (Table 2). Two of the genes may be involved in cellular detoxification and one in defence against pathogens. The aldehyde dehydrogenase family (www.ebi.ac.uk/interpro; IPR002086) is a large family of enzymes that oxidize aliphatic and aromatic aldehydes using NADP as a cofactor. Thus the substrate of this enzyme cannot be identified from the amino acid sequence alone. At1g54100 is a member of the aldehyde dehydrogenase family and is highly similar to a water-deficit-induced gene first identified in pea (Guerreiro et al., 1990). It also has strong similarity to a human protein named antiquitin 1, a member of the subfamily 7 of the aldehyde dehydrogenases that is thought to play a role in the detoxification of aldehydes generated by alcohol metabolism and lipid peroxidation (Lee et al., 1994). At1g62570 is a flavin-containing monoxygenase family member (IPR000960). These proteins are xenobiotic metabolizing enzymes, requiring NADPH and FAD for activity.

At2g43570 contains two domains found in chitinases: a chitin binding domain and a domain found in glycoside hydrolase family 19 (IPR000726). This family contains only enzymes with chitinase activity. O-Glycosyl hydrolases hydrolyse the glycosidic bond between two or more carbohydrates, and chitinases specifically hydrolyse the β-1,4-N-acetylglucosamine linkages in chitin polymers. Chitinases function in plants in the defence against fungal and insect pathogens through the destruction of their chitin-containing cell walls. Thus, this gene is probably induced under water-deficit stress as a defence mechanism.

At2g38400 is classified as an alanine-glyoxylate amino-transferase, a class III aminotransferase (IPR005814). These enzymes are pyridoxal-phosphate-dependent enzymes that transfer amino groups. There are many different substrates and potential functions for these types of enzymes. The cinnamyl-alcohol dehydrogenase family, a large family in the Arabidopsis genome, may be involved in lignin biosynthesis. The common induction of a cinnamyl-alcohol dehydrogenase, At1g09500, may indicate that cell wall modifications are induced by water-deficit stress.

Cellular transport

Two genes potentially functioning in cellular transport processes were found to be commonly up-regulated in response to cellular water deficit. Based on its DNA sequence, At2g22500 is likely to be a mitochondrial substrate carrier protein (IPR001993). Since a variety of substrate carriers contain this domain, the exact function cannot be determined from the amino acid sequence. At2g41190 belongs to family II of the amino acid/polyamine transporters and has low similarity to a vesicular GABA transporter from Rattus norvegicus. The predicted protein has 11 transmembrane domains. The function of these common changes in gene expression cannot be clearly identified without further studies.

Signal transduction

The ability of the cell to sense water-deficit stress and transduce that signal to downstream events is a poorly understood process. Within the common set of genes, there are three genes that shed some light on the signalling mechanism. The Arabidopsis genome contains at least 69 genes encoding protein phosphatases belonging to the class 2C (PP2C). Two genes in the common set (ABI1 and PP2CA) encode PP2C-related gene products that group in the same clade (Saéz et al., 2004). These genes are induced by water deficit, low temperature, salinity, and ABA (Táhtiharju and Palva, 2001). Both ABI1 and PP2CA are proposed to be negative regulators of ABA responses (Táhtiharju and Palva, 2001).

The common set of genes also contains a gene A1tg49450 with WD-40 domains that are found in β transducin, the β-subunit of G-protein (guanine nucleotide binding protein). It is not known if this gene functions in Arabidopsis as a G-protein subunit.
Transcriptional regulation

In this category there are three transcription factors and one H1 histone. Two of the transcription factors contain a homeodomain (HD) linked to a leucine zipper motif (zip). These HDzip genes, ATHB-7 and ATHB-12, are induced by ABA and water-deficit stress (Lee et al., 2003; Söderman et al., 1996). ATHB-7 strictly requires an increase in ABA concentration to be induced and its induction is also impaired in the ABA-insensitive mutant ab1 (Söderman et al., 1996). ATHB-7 was not induced by low-temperature stress in any of the microarray experiments (Table 2). Overexpression of ATHB-7 causes reduced stem elongation (Hjellström et al., 2003) indicating that this gene may be involved in the growth inhibition response to water-deficit stress. The final transcription factor is a member of the no apical meristem (NAM) gene family, and was identified as a water-deficit-induced gene (Yamaguchi-Shinozaki et al., 1992). Other members of this gene family have a role in the development of the shoot apical meristem (Sablowsky and Meyerowitz, 1998). The role in water deficit has not been defined.

The last gene in this category, his1-3, is a member of the H1 histone gene family in Arabidopsis. This Arabidopsis gene is induced in the meristem of the root by water deficit and ABA (Ascenzi and Gantt, 1999). Under or over-expression of this gene in Arabidopsis did not cause any notable changes in the response to drought (Ascenzi and Gantt, 1999). However, when long-term experiments were completed in tomato in which the tomato water-deficit stressed histone H1, H1-S, was reduced by antisense expression, the transgenic plants had greater stomatal conductance and photosynthetic rate during the first two weeks of a period of soil-water deficit (Scipps et al., 2004).

Hydrophilic, heat-soluble proteins

Genes induced in seeds during the late stages of seed development when seed desiccation tolerance is developing, known as late embryogenesis abundant (lea) genes, are also induced in vegetative plants in response to cellular water deficit. These genes tend to be hydrophilic and remain soluble when subjected to heat treatment in vitro. Several classes of these gene products have been noted and a new classification scheme has recently been proposed (Wise, 2003). Five genes that are classified as LEA proteins are present in the common set. The gene At5g06760 is annotated as LEA-like and contains a domain (IPR005513), which is attributed to LEA Class I proteins. The common set contains two members of Class II, which are generally referred to as dehydrins, COR47/RD17 and RAB18. One gene product is similar to LEA76 from Brassica napus and is a member of Class III. At1g01470 is similar to LEA14 of cotton, a member of Class VI in the new classification (Wise, 2003). The function of these different types of LEA genes remains uncertain. Recent reports implicate dehydrins in the inhibition of lipid peroxidation (Hara et al., 2003) and evidence has been presented that a dehydrin binds lipid vesicles (Koag et al., 2003).

Two other hydrophilic proteins are also in the common set of genes, although these genes have not been identified as late embryogenesis abundant. These two genes are related, COR78/LTI78/RD29A and LTI65/RD2Bb, and were discovered by three different groups (Horvath et al., 1993; Nordin et al., 1993; Yamaguchi-Shinozaki and Shinozaki, 1993). It is suggested that these genes play a protective role in cells subjected to cellular water deficit, but a molecular function has not been elucidated.

Gene products of unknown function

There are seven genes commonly induced that are classified as genes with unknown function. Two of these genes were previously shown to be induced by stress conditions. The remainder of these genes has not been previously studied. SAG29 (senescence-associated gene 29), At5g13170, is predicted to be a membrane protein containing seven transmembrane domains and is similar to a membrane protein isolated from Medicago trunculata nodules, MtN3. SAG29 has domains that provide some clues to its function. It contains domains found in the MtN3/saliva family domain (IPR004316), a family of transmembrane proteins with undefined function, a signature domain for proteins that are components of phosphoenolpyruvate-dependent sugar phosphotransferase systems involving serine phosphorylation (IPR002114), and a domain found in N-methyl-D-aspartate receptors which are members of the glutamate receptor channel superfamily (IPR001508).

At2g47770 contains weak similarity to a peripheral-type benzodiazepine receptor studied in humans and to the tryptophan-rich sensory protein (TspO) from the bacteria Sinorhizobium meliloti. It is likely to be a membrane protein but its function in plants is uncertain.

At2g40000 is annotated as a putative nematode resistance protein because of its similarity to a nematode resistance gene, HsIpro-1, positionally cloned from sugar beet (Cai et al., 1997). The gene product from beet contains domains found in disease resistance proteins including leucine-rich repeats and a membrane spanning domain.

At2g17840 is similar to the partial cDNA isolated as an early response to dehydration ERD7. The function of this gene has not been determined. At1g05340 is a gene of unknown function. It is predicted to be targeted to the chloroplast. At1g27200 is also an unknown protein. It is predicted to have a signal peptide and contains a domain of unknown function found in several C. elegans proteins.

Common down-regulation of gene expression

Out of a total of 1369 genes that were down-regulated, there were only three genes repressed in all three experiments (Fig. 3; Table 3). The specific set of genes down-regulated
in response to each of the different methods of delivering water-deficit stress was tuned closely to the particular experimental conditions and did not yield a large common set of repressed genes. This was very similar to the situation observed for gene induction, but even more dramatic. In two microarray experiments (Kreps et al., 2002; Seki et al., 2002b), down-regulation of gene expression was compared in response to three different stress treatments having a water-deficit component. Seventeen genes were commonly repressed out of 162 unique genes (Seki et al., 2002). Thus a greater proportion of genes involved in cell wall biology were categorized for log2-fold change in expression under soil-water-deficit compared with well-watered conditions. Frequency plots were made for seven categories of genes (Fig. 4A, cell wall degradation: pectate lyases, polygalacturonases, and cellulases; Fig. 4B, cell wall modification: expansins, XTHs, and glucanases; Fig. 4C, cell wall synthesis: galactosyl transferases, and cellulose synthases). In all cases the average log2-fold regulation was less than zero, indicating that, in general, the expression of genes involved in cell wall metabolism was repressed by soil-water deficit.

### Cell wall biology

Leaf growth inhibition by water stress can result from the reduced production of cells and the reduced expansion of existing cells. Inhibition of cell expansion can result from reduced cellular turgor as well as biochemical changes that affect cell expansion through processes that control wall extensibility (Lu and Neumann, 1998). Cell wall extensibility is controlled by the structure of the cell wall as well as the activity of cell-wall-modifying proteins such as expansins (McQueen-Mason et al., 1992), XTHs, and glucanases (Li et al., 2003). Three categories of genes involved in cell wall biology were categorized for log2-fold change in expression under soil-water-deficit compared with well-watered conditions. Frequency plots were made for seven categories of genes (Fig. 4A, cell wall degradation: pectate lyases, polygalacturonases, and cellulases; Fig. 4B, cell wall modification: expansins, XTHs, and pectin esterases; Fig. 4C, cell wall synthesis: galactosyl transferases and cellulose synthases). In all cases the average log2-fold regulation was less than zero, indicating that, in general, the expression of genes involved in cell wall metabolism was repressed by soil-water deficit.

### Cell wall modifications

Three classes of genes involved in cell wall modification were analysed. Decreased expression of these types of genes is likely to signify decreased cell wall extensibility resulting in a decreased ability of cells to expand. The Arabidopsis genome has three subfamilies of expansin proteins, totaling 38 open reading frames (Li et al., 2003). In the soil-water-deficit experiment conducted by Kawaguchi et al. (2004), 16 expansins were analysed with an average log2-fold change of −0.69 (Fig. 4A).

Xyloglucan endotransglucosylase/hydrolases (XTHs) are also involved in controlling cell wall extensibility through the cleavage and reformation of bonds between xyloglucan chains (Hyodo et al., 2003). The XTHs comprise a large gene family with at least 42 genes annotated in the genome of Arabidopsis thaliana. The average log2-fold change in expression in response to water deficit was −1.25, when 23 genes annotated in this class were analysed (Fig. 4A).
Pectin esterases, such as pectinmethylesterase, catalyse the demethylesterification of homogalacturonans releasing acidic pectins and methanol (Micheli, 2001). Depending upon the pH and the availability of divalent cations in the cell, pectinmethylesterases may act linearly, promoting cell wall stiffening or randomly resulting in cell wall loosening (Micheli, 2001). Pectinmethylesterase activity is also required before pectin-degrading enzymes such as polygalacturonase, pectate lyase, and pectin esterase can degrade the pectin of the cell wall. The expression of 53 genes annotated as members of the pectin esterase family was analysed (Fig. 4A). The log₂-fold change was an average of −0.63, indicating that this class of genes as a whole was also down-regulated by these water-deficit stress conditions.

**Cell wall degradation**

The pectin portion of the extracellular matrix can be degraded by enzymes such as pectate lyase, exo- and endo-polygalacturonases, and β-galactosidase. Pectin degradation has been implicated in the process of cell expansion through cell wall loosening. Pectate lyases cleave...
pectin to give oligosaccharides with 4-deoxy-\(\alpha\)-D-gluc-4-enuronosyl groups at their non-reducing ends (http://www.godatabase.org/cgi-bin/go.cgi?action=replace_tree&query=GO:0030570&search_constraint=terms). Sixteen Arabidopsis genes annotated as pectate lyases were analysed with an average log₂-fold change in response to soil-water deficit of \(-1.00\) (Fig. 4B).

Endo-polygalacturonases cleave homogalacturonan regions, made up of unesterified galacturonic acid, along the backbone of pectin in the middle lamella of the cell wall. The Arabidopsis genome contains a large family of polygalacturonases—as many as 57 genes. Twenty-four polygalacturonases were analysed with an average log₂-fold change in gene expression in response to soil-water deficit of \(-0.78\) (Fig. 4B). Polygalacturonases have been connected with processes of cell expansion as well as fruit ripening, abscission, and pathogen defence (Hadfield and Bennett, 1998). Thus, down-regulation of genes encoding polygalacturonases may also inhibit cell expansion. Alternatively, the polygalacturonases may be involved in controlling cellular water relations. Overexpression of a fruit-specific polygalacturonase on a constitutive promoter in apple trees resulted in plants with the inability to close their stomata (Atkinson et al., 2002).

**Cellulas**

The glycoside hydrolases include a range of enzymes that hydrolyse the glycosidic bond. There are 94 different families of these enzymes (http://afmb.cnrs-mrs.fr/CAZY/). The cellulase endo-1,4-\(\beta\)-glucanase (cellulase) catalyses the endohydrolysis of 1,4-\(\beta\)-D-glucosidic linkages of cellulose. Nineteen genes from Arabidopsis annotated as glycosyl hydrolases were used to calculate log₂-fold change in expression in response to water-deficit stress. There was a \(-0.27\) decrease in expression for this group of cellulase genes (Fig. 4B).

**Cell wall synthesis**

Several gene families have been characterized that are involved in the synthesis of cell walls, including cellulose synthase and galactosyl/xylosyl transferases involved in hemicellulose synthesis. A classification of the gene families can be found at the following location: http://afmb.cnrs-mrs.fr/CAZY/GT_intro.html. The processes of cellulose biosynthesis and the genes involved are just beginning to be unravelled (Saxena and Brown Jr, 2000). AtCslB4, a member of the glycosyltransferase family II, was the most strongly down-regulated. The average of the log₂-fold change in expression of genes involved in cell wall synthesis in response to water-deficit stress was less than zero \((-0.43\) for cellulose synthases and \(-0.30\) for galactosyltransferases; Fig. 4C).

In concert, the down-regulated expression of genes involved in cell wall metabolism is likely to decrease the ability of the cell to expand during periods of water-deficit stress. In agreement with the long-noted growth inhibition response to water deficit (Hsiao, 1973), these findings underscore the specific genes that should be studied further. An important question that can be posed to understand these responses better is, ‘Are these changes in growth an aspect of cellular adaptation required for crop survival in a single growing season, or does this change support plant survivability on an evolutionary scale?’

**Germin-like proteins**

It was also observed that two germin-like genes (GLP) were down-regulated under all three experimental conditions (Table 3). Although the function of GLPs is not clearly understood, one hypothesis is that these genes are involved in the regulation of cell wall extensibility. Germin proteins were first identified in wheat as genes that were expressed during germination (Lane et al., 1991; IPR001929). The germin-like protein family is a large family consisting of at least three different phylogenetic groups (Khuri et al., 2001). The true germins have oxalate oxidase and SOD activity and may be involved in the alleviation of oxidative stress. Although the GLPs have similar structure and are members of the cupin superfamily, indicating that they have two conserved histidine-containing domains (Khuri et al., 2001), those that have been tested do not have oxalate oxidase activity (Membré et al., 2000). One possible role for GLP is in alteration of cell wall properties that control growth. In cotton fibres, it was shown that a GLP is expressed highly during the period of cotton fibre elongation and is repressed once the rate of growth has slowed (Kim and Triplett, 2004). The expression of GLPs in fibres subjected to abiotic and biotic stresses is also repressed. The genes annotated as GLPs on the 8.2k Affymetrix GeneChip® were collected and the log₂-fold change in gene expression of 10 germin-like genes in response to soil-water deficit was evaluated. The average log₂-fold change in gene expression in response to soil-water deficit was \(-0.75\) (data not shown).

**Summary**

In three different microarray experiments, using three different experimental treatments designed to identify changes in gene expression patterns in response to cellular water deficit, there was a small number of common changes in gene expression. In general, the common genes were also induced by ABA and two other stresses that have a water-deficit component, salt stress and low-temperature stress. Thus the 27 common genes are likely to play a general role in the response to cellular water deficit. A few of the gene products, notably ABI1 and PP2CA, can be predicted to play an important role in the signalling pathways that are required for the stress response, yet the signalling pathways have not yet been completely defined. Several of the genes are likely
to be involved in metabolic processes, but it is difficult to assign these genes to a particular function without further detailed functional studies. Seven of the genes are categorized as hydrophilic, heat-soluble proteins, of which five are noted to fall into the late embryogenesis abundant class of genes. Yet, the exact role of these genes in the response to cellular water deficit has thus far defied definition. The function of the remaining genes cannot be predicted. Interestingly, there were even fewer genes commonly down-regulated. These genes are likely to play a role in the inhibition of growth that is a central part of the response to reduced cellular water content. To determine the role of the individual commonly regulated genes in the water-deficit response, mutant *Arabidopsis* plants must be obtained in which the individual genes are disrupted and physiological and molecular techniques should be used to evaluate the importance of the each gene in the water-deficit response.

An important question that must now be addressed is, ‘Can gene expression studies completed on *Arabidopsis* in the laboratory be used to understand the response of crop plants to drought stress in the field?’ The conclusions drawn in this report indicate that a single study on gene expression completed in the laboratory is not likely to pinpoint a set of individual genes that will be important in a crop response to soil-water deficit in the field. Experimental approaches in the laboratory designed to identify short-term responses to water-deficit stress cannot provide a full picture of the water-deficit stress transcriptome. Changes in gene expression in the field under water stress conditions will mirror the multiple environmental stimuli resulting in a unique pattern of gene expression. Comparisons of gene expression using multiple highly defined field conditions combined with physiological studies on the impact of altering individual genes is needed to develop a clear picture of the molecular mechanisms that control adaptation to soil-water-deficit stress. The 30 genes identified in this study are a good starting place.

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