Classification of Genes Differentially Expressed during Water-deficit Stress in *Arabidopsis thaliana*: an Analysis using Microarray and Differential Expression Data

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Many changes in gene expression occur in response to water-deficit stress. A challenge is to determine which changes support plant adaptation to conditions of reduced soil water content and which occur in response to lesions in metabolic and cellular functions. Microarray methods are being employed to catalogue all of the changes in gene expression that occur in response to specific water-deficit conditions. Although these methods do not measure the amount or activities of specific proteins that function in the water-deficit response, they do target specific biochemical and cellular events that should be detailed in further work. Potential functions of approx. 130 genes of *Arabidopsis thaliana* that have been shown to be up-regulated are tabulated here. These point to signal-ling events, detoxification and other functions involved in the cellular response to water-deficit stress. As micro-array techniques are refined, plant stress biologists will be able to characterize changes in gene expression within the whole genome in specific organs and tissues subjected to different levels of water-deficit stress.

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INTRODUCTION

Plant cellular water-deficit stress may occur under conditions of reduced soil water content. Under these conditions, changes in gene expression take place, with up- as well as down-regulation occurring. The changes in gene expression may be regulated directly by the stress conditions or may result from secondary stresses and/or injury responses (Hanson and Hitz, 1982). Changes in gene expression are induced by a complex series of signal transduction events that have not been clearly delineated. One of the signals involved is abscisic acid (ABA), although there are clearly other signal molecules that have not yet been identified. Signals that result in changes in gene expression may result from an injury response and/or may be responsible for inducing genes that may have an adaptive function.

The function of gene products that have altered abundance during water-deficit stress may be defined by the ability of the gene product to promote water-deficit stress survival. Some gene products may be involved in promoting stress tolerance and some may not. Those that are not may be expressed as a result of injury such as a block in metabolism. One of the important challenges is to understand which genes function to promote cellular and whole plant tolerance of water-deficit stress.

Gene expression patterns are influenced by the severity, extent and rate or application of the stress (Bray *et al.*, 2000). Gene expression patterns may be altered at the initial step—increasing the transcription rate of a specific gene—or at subsequent steps that control specific mRNA levels or

the translation of a specific mRNA. Together a complex pattern of gene expression is established that is a result of the specific stress conditions. Hanson and Hitz (1982) argued that when stress is imposed rapidly a greater number of responses will be injury-induced than under a slower long-term application of water-deficit stress.

GENES REGULATED BY WATER-DEFICIT STRESS

Microarray analyses provide a powerful method to study the many changes in gene expression that occur in an organism in response to developmental and environmental cues. For water-deficit stress, microarray analyses using full-length cDNAs obtained from *Arabidopsis thaliana* plants subjected to different stresses have been published (Seki *et al.*, 2001). To identify changes in expression patterns in response to water-deficit stress, Seki *et al.* (2001) dehydrated 3-week-old *Arabidopsis thaliana* plants on filter paper (60 % RH, 22 °C) for 2 h (Yamaguchi-Shinozaki and Shinozaki, 1994). From an array containing 1300 full-length cDNAs isolated from libraries prepared from stressed plants, 44 genes were confirmed to be induced by the water-deficit treatment after RNA blot analyses (Table 1; Seki *et al.*, 2001). Sixty-eight per cent had not been previously identified as water-deficit-induced genes. A microarray study, using arrays originally designed for studies in wound induction of gene expression, on water-deficit gene induction using a similar method of stress imposition was also published (Reymond *et al.*, 2000; supplemental information at http://www.unil.ch/ibpv/docs/

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TABLE 1. *Genes identified as being induced under conditions of water-deficit stress*

Functional process	Genbank ID	Protein entry code*	Predicted targeting[†]	Reference
Metabolism				
Cytochrome P450	D78598	At4g31500	1 TM	Reymond *et al.* (2000)
Hydroxynitrile lyase	Z25670	At4g16690	0 TM	Reymond *et al.* (2000)
Enolase (2-phospho-D-glycerate dehydratase)	B050561	At2g36530	1 TM	Seki *et al.* (2001)
Amino acid biosynthesis and degradation				
Anthranilate synthase (α-subunit)	M92353	At5g05730	0 TM	Reymond *et al.* (2000)
Anthranilate synthase (β-subunit)	L22585	At5g57890	1 TM	Reymond *et al.* (2000)
Tryptophan synthase (α-subunit)	U18993	At3g54640	cpt?, 0 TM	Reymond *et al.* (2000)
Tryptophan synthase (β-subunit)	M23872	At5g54810	cpt, 0 TM	Reymond *et al.* (2000)
Δ¹-Pyrroline-5-carboxylate synthetase	AB050546	At2g39800	1 TM	Seki *et al.* (2001)
S-Adenosylmethionine synthetase	M33217	At4g01850	0 TM	Reymond *et al.* (2000)
Lactoylglutathione lyase-like	AB050576	At1g11840	0 TM	Seki *et al.* (2001)
Chorismate mutase	Z26519	At3g29200	cpt, 0 TM	Reymond *et al.* (2000)
Aromatic metabolism				
4-Coumarate : CoA ligase	U18675	At1g51680	1 TM	Reymond *et al.* (2000)
Cinnamyl alcohol dehydrogenase	L37883	At4g39330	0 TM	Reymond *et al.* (2000)
Cinnamyl alcohol dehydrogenase	X67816	At4g37980	0 TM	Reymond *et al.* (2000)
Chalcone synthase	M20308	At5g13930	0 TM	Reymond *et al.* (2000)
Phenylalanine ammonia-lyase	L33677	At2g37040	1 TM	Reymond *et al.* (2000)
Cinnamoyl–CoA reductase	T41765	At1g15950	0 TM	Reymond *et al.* (2000)
Dihydroflavonol-4-reductase	T20927	At2g33590	0 TM	Ostergaard *et al.* (2001)
O-Methyltransferase	U70424	At5g54160	0 TM	Reymond *et al.* (2000)
Fatty acid multifunctional protein				
Hydroperoxide lyase	AF087932	At4g15440	0 TM	Reymond *et al.* (2000)
Acyl-CoA oxidase	AF057043	At5g65110	0 TM	Reymond *et al.* (2000)
Acyl-CoA oxidase	AF057044	At4g16760	Perox, 1 TM	Reymond *et al.* (2000)
Epoxide hydrolase	D16628	At2g26740	1 TM	Reymond *et al.* (2000) Kiyosue *et al.* (1994*c*)
Fatty acid multifunctional protein	AF123254	At3g06860	1 TM	Reymond *et al.* (2000)
Omega-3 fatty acid desaturase	D14007	At3g11170	cpt, 1 TM	Reymond *et al.* (2000)
Lipoxygenase	L23968	At3g45140	cpt, 0 TM	Reymond *et al.* (2000)
Allene oxide synthase	X92510	At5g42650	cpt, 0 TM	Reymond *et al.* (2000)
Energy				
Oxygen-evolving complex	X52428	At5g66570	cpt, 0 TM	Reymond *et al.* (2000)
PSI, reaction centre sub II	AB050572	At4g02770	cpt, 0 TM	Seki *et al.* (2001)
Transcription				
14–3–3 like protein, GF14	U60445	At3g02520	0 TM	Reymond *et al.* (2000)
Ethylene response element binding protein 4	AB008106	At1g53170	0 TM	Reymond *et al.* (2000)
AREB1	AB017160	Not annotated		Uno *et al.* (2000)
DREB2A	AB007790	At5g05410	0 TM	Liu *et al.* (1998)
ATMYB2	D14712	At2g47190	0 TM	Urao *et al.* (1996)
ATHB-6	AF104900	At2g22430	0 TM	Söderman *et al.* (1999)
ATHB-7	X67032	At2g46680	0 TM	Söderman *et al.* (1996)
ATHB-12	AF001949	At3g61890	0 TM	Lee *et al.* (2001)
His1-3	U72241	At2g18050	0 TM	Ascenzi and Gantt (1999)
RNase RNS1	U05206	At2g02990	Sig seq, 1 TM	Reymond *et al.* (2000)
Cell growth, cell division and DNA synthesis				
Nitrilase (indole-3-aceto-nitrile hydrolysis)	U09958	At3g44300	0 TM	Reymond *et al.* (2000)
Protein synthesis				
None identified				
Protein destination				
Ubiquitin (UBQ4)	X12853	At5g20620	0 TM	Reymond *et al.* (2000)
Ubiquitin (UBQ1)	J05507	At3g52590	0 TM	Kiyosue *et al.* (1994*a*)
AtHsp81–2	AB011476	At5g56030	1 TM	Kiyosue *et al.* (1994*a*)
AtHSP70–1	M23105	At5g02500	0 TM	Kiyosue *et al.* (1994*a*)
rReg ATP subunit of CLP protease	AB000615	At5g51070	cpt	Kiyosue *et al.* (1993*a*)
DNAJ homologue	AB050562	At3g62600	Sig seq, 1 TM	Seki *et al.* (2001)
Cysteine protease	D13043	At3g19390	Sig seq, 1 TM	Koizume *et al.* (1993)
Cysteine protease	D13042	At4g39090	Sig seq, 1 TM	Seki *et al.* (2001)
Cysteine protease	AB050573	At4g16190	Sig seq, 1 TM	Seki *et al.* (2001)
Cysteine protease	X74359	At2g21430	Sig seq, 1 TM	Williams *et al.* (1994)
Cysteine protease inhibitor	AB044405	At2g40880	Sig seq, 1 TM	Seki *et al.* (2001)
Metallopeptidase	Y13577	At1g51760	ER, 1 TM	Reymond *et al.* (2000)
Transport				
Aquaporin 2C	D13254	At2g37180	5 TM	Yamaguchi-Shinozaki *et al.* (1992)
Aquaporin	AB050549	At2g39010	4 TM	Seki *et al.* (2001)

T A B L E 1. *Continued*

Functional process	Genbank ID	Protein entry code*	Predicted targeting†	Reference
γ-TIP2	AB050557	At3g26520	4 TM	Seki *et al*. (2001)
Sugar transporter (ERD6)	D89051	At1g08930	7 TM	Kiyosue *et al*. (1998)
Cellular transport				
Intracellular transport protein	AB050567	At2g24420	Sig seq, 2 TM	Seki *et al*. (2001)
HVA22-like (YIP2-like)	AB015098	At4g24960	1 TM	Seki *et al*. (2001)
Cell communication/signal transduction				
Ca-binding EF-hand protein	AB039924	At2g33380	0 TM	Seki *et al*. (2001); Takahashi *et al*. (2000)
CDPK1	D21805	At1g18890	1 TM	Urao *et al*. (1994)
CDPK2	D21806	At1g35670	1 TM	Urao *et al*. (1994)
CDPK	D28582	At2g17290	0 TM	Urao *et al*. (1994)
AtPIP5K1 (phosphatidylinositol-4-phosphate-5-kinase)	AB050902	At1g77740	0 TM	Mikami *et al*. (1998)
AtPLC1	D38544	At5g58670	0 TM	Hirayama *et al*. (1995)
ATMEKK1 (MAPKKK)	D50468	At4g08500	0 TM	Mizoguchi *et al*. (1996)
ATMPK3	D21839	At3g45640	0 TM	Mizoguchi *et al*. (1993)
ATPK19	D42061	At3g08720	0 TM	Mizoguchi *et al*. (1995)
Cell rescue, defence, cell death and ageing				
Oxidative stress				
Glutathione-*S*-transferase	D17672	At1g02930	0 TM	Kiyosue *et al*. (1993); Reymond *et al*. (2000)
Glutathione-*S*-transferases	D17673	At2g30870	1 TM, sig seq?	Kiyosue *et al*. (1993)
Glutathione-*S*-transferase	D44465	At2g29450	0 TM	Reymond *et al*. (2000)
Glutathione-*S*-transferase	AJ012571	At1g78380	0 TM	Reymond *et al*. (2000)
L-Ascorbate peroxidase	AB050564	At1g07890	0 TM	Reymond *et al*. (2000); Seki *et al*. (2001)
GSH-dep dehydroascorbate reductase	AB050550	At1g19570	0 TM	Seki *et al*. (2001)
Catalase 3	AB050551	At1g20620	0 TM	Seki *et al*. (2001)
Glutathione peroxidase GPX2	AJ000470	At2g31570	0 TM	Reymond *et al*. (2000)
Cu, Zn Superoxide dismutase	X60935	At1g08830	0 TM	Reymond *et al*. (2000)
Thioredoxin, FL3-2C6	AB050571	At5g42980	0 TM	Seki *et al*. (2001)
Peroxiredoxin TPX1	AB050556	At1g65980	0 TM	Seki *et al*. (2001)
Pathogenesis-related				
Basic chitinase, PR3B1	M38240	At3g12500	Sig seq, 1 TM	Reymond *et al*. (2000)
β-1–3-Glucanase, PR2	M90509	At3g57260	Sig seq, 1 TM	Reymond *et al*. (2000)
Extensin-like	T41880	At2g43150	Sig seq, 1 TM	Reymond *et al*. (2000)
Proline-rich	R64825	At2g14890	2 TM	Reymond *et al*. (2000)
PR1 (antifungal protein)	M90508	At2g14610	Sig seq, 1 TM	Reymond *et al*. (2000)
PR5 (thaumatin-like, acidic)	M90510	At1g75040	Sig seq, 1 TM	Reymond *et al*. (2000)
Thionin	L41244	At1g72260	cpt target	Reymond *et al*. (2000)
Putative lectin	N38164	At3g16460	0 TM	Reymond *et al*. (2000); Seki et al., 2001
AIG2-like (phosphate acetyltransferase)	U40857	At3g28930	0 TM	Reymond *et al*. (2000)
Metallothionein-like	L15389	At1g07600	0 TM	Reymond *et al*. (2000)
Antifungal protein-like	T04323	At5g44420	Sig seq, 1 TM	Reymond *et al*. (2000)
Ionic homeostasis				
None identified				
Cellular organization				
Ferritin	AB050569	At5g01600	cpt target	Seki *et al*. (2001)
β-glucosidase-like	AB050566	At1g52400	Sig seq, 1 TM	Seki *et al*. (2001)
Polygalacturonase-like (*rd22*)	D10703	At5g25610	Sig seq, 1TM	Seki *et al*. (2001)
Xyloglucan endo-transglycosylase	AB050552	At1g14720	Sig seq, 1 TM	Seki *et al*. (2001)
Ripening-related protein	AB046991	At5g62350	Sig seq, 1 TM	Seki *et al*. (2001)
Polygalacturonase-inhibiting	AB010697	At5g06870	Sig seq, 1 TM	Reymond *et al*. (2000)
Reversibly glycosylated polypeptide-2	AB050560	At5g15650	0 TM	Seki *et al*. (2001)
Unclassified				
Unclassified hydrophilic proteins				
rd29B (Lti65)	D13044	At5g52300	0 TM	Yamaguchi-Shinozaki and Shinozaki (1994)
rd29A (Lti78)	D13044	At5g52310	0 TM	Seki *et al*. (2001)
Cor15a	U01377	At2g42540	cpt, 0 TM	Seki *et al*. (2001)
Kin1	X51474	At5g15960	0 TM	Seki *et al*. (2001)
Kin2 COR6·6	X55053	At5g15970	0 TM	Seki *et al*. (2001)
LEA14	Y10085	At1g01470	0 TM	Reymond *et al*. (2000)
LEA 76 type 1	AB050548	At1g52690	0 TM	Seki *et al*. (2001)
Group II LEA (Erd10)	D17714	At1g20450	0 TM	Seki *et al*. (2001)
Group II LEA (rd17, cor47)	AB004872	At1g20440	0 TM	Seki *et al*. (2001)
Group II LEA (Erd14)	D17715	At1g76180	0 TM	Seki *et al*. (2001)

T A B L E 1. *Continued*

Functional process	Genbank ID	Protein entry code*	Predicted targeting†	Reference
Group II LEA (XERO2)	U19536	At3g50970	0 TM	Reymond *et al.* (2000)
Classification not clear cut/unclassified				
Drought-induced protein like	AB050563	At4g02380	0 TM	Seki *et al.* (2001)
Erd7	AB039929			Seki *et al.* (2001)
Erd3	AB039927			Seki *et al.* (2001)
Erd4	AB039928			Seki *et al.* (2001)
Steroid dehydrogenase-like	X99793	At4g24220	0 TM	Reymond *et al.* (2000)
Lectin-like (JIP)	N37581	At3g16420	?	Reymond *et al.* (2000)
ERD15	D30719	At2g41430	0 TM	Kiyosue *et al.* (1994)
REM3	R90622	At2g41870	0 TM	Reymond *et al.* (2000)
Remorin-like	M25268	At2g45820	0 TM	Reymond *et al.* (2000)
Glutamate-rich protein	AB050570	At2g05380	0 TM	Seki *et al.* (2001)
Major latex protein-like	AB050543	At4g23670	0 TM	Seki *et al.* (2001)
Ozone and pathogen induced	U20347	At4g00860	Sig seq, 0 TM	Reymond *et al.* (2000)
Non-specific lipid transfer protein	AB050558	At2g38540	Sig seq, 1 TM	Seki *et al.* (2001)
FL5-2H15	AB050559	At5g61820	Sig seq, 1 TM	Seki *et al.* (2001)
Non-specific LTP	AB050544	At5g59320	Sig seq, 1 TM	Seki *et al.* (2001)
ENOD20-like	AB050542	At4g27520	Sig seq, 2 TM	Seki *et al.* (2001)
Cold acclimation protein	AB044404	At2g15970	5 TM	Seki *et al.* (2001)

Sig seq, predicted signal sequence; cpt target, predicted chloroplast transit peptide.

* Protein entry code refers to the chromosome and the gene placement on the chromosome (http://mips.gsf.de/proj/thal/db/index.html).

† TM, number of predicted transmembrane domains.

WWWPR/Docs/listegenes.html). This resulted in the identification of further genes that are up-regulated at the mRNA level (Table 1). The experiments completed thus far have involved a severe, rapid stress and were not completed with microarrays that contain the whole genome. Even though the stress imposed in these two studies was rapid and severe, it is still worthwhile evaluating the patterns of gene expression that are altered, and comparing these changes with what is know about alterations in metabolism. In addition to genes identified by these arrays, genes identified using standard differential expression methods have been compiled for this analysis of water-deficit alterations in gene expression.

PREDICTED FUNCTIONS OF ARABIDOPSIS GENE PRODUCTS

A system to categorize gene function has been applied to known plant and animal genomes. Genes are broken down into at least 13 general categories (http://mips.gsf.de/proj/thal/db/tables/tables_func_frame.html). In the *Arabidopsis thaliana* genome, the largest category is that of unclassified, or genes whose function has not been determined (Arabidopsis Genome Initiative, 2000). Many genes involved in water-deficit stress also fall into this category. Some, such as the LEA (late embryogenesis abundant) proteins, have been much studied, yet their function remains unknown (Garay-Arroyo *et al.*, 2000). Others are completely unknown in that an mRNA (or EST) has not been previously identified and there is no amino acid sequence homology with other proteins of known function. The categories include one called 'cell rescue, defence, cell death and ageing'. Many of the genes known to be induced by water deficit are placed in this category even though an

exact function is not understood. Instead of using this category, the present analysis places genes into a potential functional category or into the unclassified category, if the function is not known. In Table 1, it is noted if these genes are targeted to a specific cellular compartment or if they contain predicted transmembrane domains. Genes induced by water-deficit stress have been allocated to 11 different functional categories (Table 1; Fig. 1). Since a whole genome analysis of the genes expressed in response to water-deficit stress has not yet been published, the categories and conclusions drawn are only preliminary. More genes will almost certainly be identified as further microarray analyses are completed, and more information will be gained about gene expression and function as different techniques for stress imposition are used.

In addition to the functional categories of proteins established at MIPS (Münich Information Centre for Protein Sequences), it is also appropriate to consider whether the potential function of a gene product has an adaptive role in water-deficit stress. Induction of gene expression does not necessarily imply that a gene will play an adaptive role. Depending upon the conditions to which the plant was subjected, some of genes that are expressed may indicate that the plant has been subjected to an injury and/or a secondary stress. For example, 11·4 % of the genes in Table 1 are likely to be involved in detoxification of oxidative stress. It is uncertain whether these genes are induced directly by cellular water deficit or by the resulting oxidative stress. The increased production of ROS (reactive oxygen species) may arise from the reduced CO_2 available for photosynthesis causing a decrease in re-oxidation relative to reduction (Smirnoff, 1993; Asada, 1999). This may lead to the generation of O_2^{*-} and H_2O_2. Therefore, the
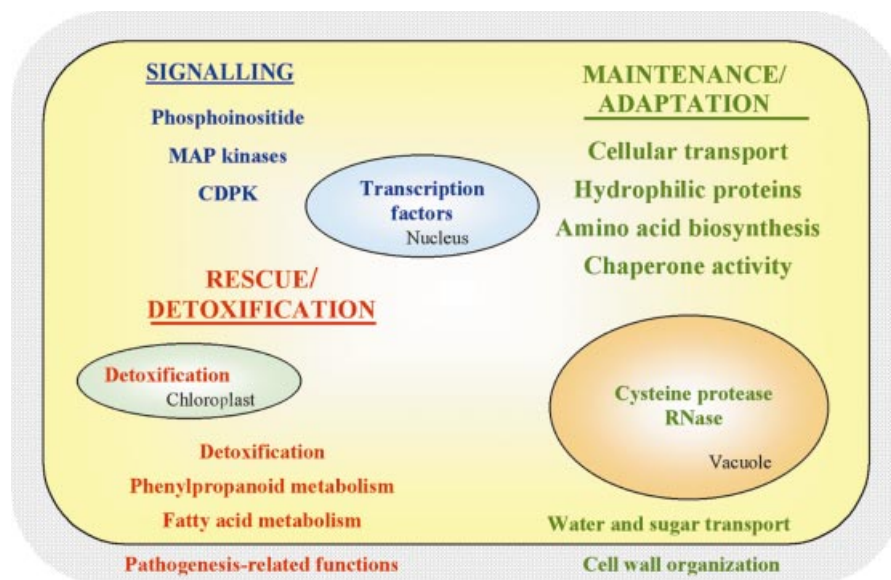
FIG. 1. Cellular model identifying the functional categories of genes that are up-regulated by water deficit stress.

signals that induce the genes may also provide valuable insight into gene function.

It must also be considered whether cellular functions can be inferred from RNA expression levels. Of course, mRNA accumulation is not the only level of gene expression that is regulated. It can be argued that both protein accumulation and activation should be the targets of gene expression studies. Therefore, studies at the mRNA level should be used to target particular processes for further study. Water-deficit studies have already documented an increase in mRNA content when there is no increase in protein content. For example, an aquaporin *RD28* is induced at the mRNA level (Yamaguchi-Shinozaki *et al*., 1992) but when the protein levels were studied no change in protein content was observed in response to different levels of water deficit (Daniels *et al*., 1994). Also, the gene *ERD1*, which encodes a ClpC-like ATPase that may interact with chloroplast-localized ClpP protease, is induced by stress and senescence at the mRNA level, but the protein levels declined with senescence (Weaver *et al*., 1999).

Metabolism

It has been known for some time that cellular metabolism is altered by water-deficit stress (Hsiao, 1973); however, detailed analyses on the effect of stress on the majority of the enzymes in individual metabolic pathways is lacking. Of changes that are known, it remains unclear which are brought about as an adaptive response and which represent lesions in metabolic pathways. Most work has concentrated in the areas of photosynthesis (carbon and energy metabolism; reviewed by Chaves, 1991), carbon and nitrogen utilization (Foyer *et al*., 1998), and the synthesis of small molecules that potentially play a role in osmotic adjustment (reviewed by Bray *et al*., 2000). The activities of a number of enzymes have been demonstrated to increase or decrease in response to water deficit (reviewed by Todd, 1972). It is

generally unknown if these are regulated at the level of enzyme activity or through alterations in the transcription and translation of specific genes. A portion of these changes can be substantiated by known changes in gene expression at the mRNA level. The genes identified in the two microarray studies that fall into the metabolism category are largely involved in three main processes: amino acid, phenylpropanoid and fatty acid metabolism (Table 1). The majority of these were identified on the wound microarray, which is heavily biased towards genes known to be regulated in a defence response. Nitrate reductase activity has been shown to be decreased by water-deficit stress in a number of species (e.g. Foyer *et al*., 1998). This gene was also strongly down-regulated at the mRNA level in arabidopsis (http://www.unil.ch/ibpv/docs/WWWPR/Docs/listegenes.html).

Energy

It is well documented that photosynthetic carbon and energy metabolism is decreased by water-deficit stress (Chaves, 1991). Considerable discussion continues over the mechanism for the decrease. It has been demonstrated that there is a decrease in the mRNA that encodes the small subunit of Rubisco (Bartholomew *et al*., 1991). In tomato plants subjected to water-deficit stress the mRNA is decreased through inhibition of transcription (Cohen *et al*., 1999). In addition, dark respiration is decreased (Hsiao, 1973). The microarrays only note two genes of central energy metabolism that are up-regulated in response to water-deficit stress.

Transcription

Transcription factors are required to regulate changes in gene expression in response to water-deficit stress. The *Arabidopsis thaliana* genome contains more than 1500

transcription factors, many of which are specific to plants (Riechmann *et al*., 2000). Several different classes of transcription factors are induced by water-deficit stress (Table 1), including bZIP (AREB1), homeodomain (ATHB-6, –7 and –12), AP2 domain (DREB2A), MYB (ATMYB2) and MYC-related factors. These are all likely to be involved in the up-regulation of genes, many of which are signalled through ABA. A gene encoding a transcription factor involved in the ethylene response is induced as well as a 14-3-3 like protein (Reymond *et al*., 2000). Many of these transcription factors are members of large multigene families. It will be important to determine if some members have a role as repressors prior to water-deficit stress. Mutant studies will allow questions to be asked about other responses that are controlled by these transcription factors.

A stress-induced histone H1 was also identified (Ascenzi and Gantt, 1999). This is similar to a stress-induced histone H1, H1-S, identified in tomato and its relatives (Scippa *et al*., 2000). This histone may play a role in maintaining chromatin structure during water deficit.

Protein destination

Many genes that are induced are involved in the fate of proteins that have been synthesized. Water deficit may cause proteins to become aggregated or malformed which may either require protein degradation or chaperone activity. Accordingly, some mRNAs of genes that encode proteases and chaperones are induced. However, the proteases induced are cysteine proteases and metallopeptidases that are predicted to reside in the vacuole (or the cell wall) rather than in the cytoplasm. Protease inhibitors are also induced which may indicate that a balancing mechanism is also required. There are a number of likely roles for the cysteine proteases and metallopeptidases that have increased mRNA levels in tissues responding to water-deficit stress. First, cysteine proteases have been shown to be induced by oxidative stress in soybean cells, and to play a role in programmed cell death (Solomon *et al*., 1999). Since other genes involved in oxidative stress are induced in arabidopsis, it is possible that these cysteine protease genes are induced by the oxidative stress that accompanies water deficit and may be a harbinger of cell death. Secondly, they may be important for breakdown of stored proteins to be used when the stress is relieved as a source of free amino acids for increased protein synthesis (Vierstra, 1996). It is also possible that the degraded proteins are transported into the vacuole during stress, although a mechanism for this has not been established (Vierstra, 1996). Thirdly, once a cell has been lysed, these types of proteases might also be involved in defence of cells that have not been damaged.

Transport

The only genes induced in the transport category in response to water-deficit stress are involved in water transport and sugar transport. Aquaporin mRNAs are increased in response to water deficit, yet, as mentioned above, interpretation of these results should be undertaken with caution as protein levels were not found to increase in response to water deficit in one study (Daniels *et al*., 1994). Three of the 23-plus aquaporins (Weig *et al*., 1997) that have been identified in the *Arabidopsis thaliana* genome are known to be induced by water-deficit stress at the mRNA level. Two are predicted to function in the plasma membrane and one in the tonoplast. For these proteins, the programme used at MIPS to predict transmembrane domains may underestimate the number of domains. Only four or five transmembrane domains (Table 1) are predicted when all models of aquaporins indicate six transmembrane domains. The tissues and organs in which the genes are expressed will play an important role in the function of these genes (Hsiao and Xu, 2000). Although protein levels do not increase in whole organs, when specific cell types are evaluated there may be a change in protein content and activity. It is important to identify the specific cells of expression since increased expression in all cell types could increase water loss because water movement through aquaporins is dependent upon the water potential gradient. A potential sugar transport gene has also been shown to be up-regulated by water-deficit stress, yet genes involved in ion transport have not been identified in microarrays or in other gene expression studies.

Cell communication/signal transduction

In the microarrays, few potential signalling molecules were identified. Is this because the mRNA levels were not altered, were not very abundant or cDNAs were not included in the array? However, many genes have been identified that play a potential role in the regulation of cellular processes in response to water-deficit stress (Table 1). The Shinozaki/Yamaguchi-Shinozaki groups in Japan identified many of these genes in stress libraries. These implicate MAP kinase cascades as well as phosphatidylinositide signalling pathways. In addition, these genes provide impetus to further study the role of calcium in the regulation of the water-deficit response.

Cell rescue, defence, cell death and ageing

A number of genes that may be classified in the cell rescue or defence category are up-regulated in response to water-deficit stress. The two largest groups identified thus far are in the amelioration of oxidative stress and/or in defence against pathogens. Increased expression of antioxidant enzymes, including ascorbate peroxidase, glutathione reductase, catalase and superoxide dismutase (Jagtap and Bhargava, 1995; Sairam and Saxena, 2000; Borsani *et al*., 2001) correlate with increased stress resistance. The mRNAs corresponding to these genes are induced by water deficit. The genes involved in phenylpropanoid metabolism categorized under metabolism also have a potential role as antioxidants. Phenolics and phenylpropanoids have free radical trapping properties.

Cellular organization

Cell wall function and characteristics may also be changed in response to cellular water-deficit stress (Wu and Cosgrove, 2000). Growth as described by the Lockhart equation (Lockhart, 1965) measures a relative rate of irreversible volume increase as the product of volumetric extensibility (m) and the difference between turgor pressure (Ψ_p) and the yield threshold turgor pressure (Y).

$$\mathrm{d}V/V \cdot \mathrm{d}t = m\,(\Psi_p - Y)$$

Therefore, growth of a cell will only occur when turgor pressure is greater than a minimum threshold. Water stress may decrease growth through a decrease in turgor. However, this may be compensated by changes in both m and Y, which are measures of the plasticity of the cell wall (Hsiao and Xu, 2000). For growth to continue under reduced turgor, cell wall extensibility may be increased or the yield threshold decreased, making cell expansion possible under conditions of reduced turgor. In addition, cell wall shrinkage with water loss will reduce the loss of cell turgor (Tyree and Jarvis, 1982). In a study on jack pine roots subjected to PEG treatment, cell walls were shown to constrict around the shrinking protoplast which served to maintain turgor pressure (Marshall and Dumbroff, 1999). It is argued that new proteins accumulating in the cell wall played a role in the alterations in cell wall elasticity that occurred throughout the stress period. A number of genes induced by water deficit may be predicted to play a role in altering the characteristics of the cell wall. These include a polygalacturonase-like gene product and a polygalacturonase inhibitor. A β-glucosidase and a gene product with pectinesterase activity may also be involved in altering the extracellular matrix. Other gene products that may be secreted into the cell wall to alter cell wall properties have not been identified in *Arabidopsis thaliana*. Although studies have shown that proteins accumulate in cell walls in response to water-deficit stress (Bozarth *et al*., 1987; García-Gómez *et al*., 2000), the function of these proteins has not been identified. The proline-rich proteins identified in bean cell walls subjected to water-deficit stress were shown to interact with a membrane protein.

Uncharacterized/classification not yet clear cut

Notably, the hydrophilic proteins that have been called LEA proteins remain a very interesting group. These proteins have been classified into several different groups based on amino acid sequence (Bray *et al*., 2000). New work continues to uncover the important role of these proteins in the dehydrating cytoplasm (Wolkers *et al*., 2001). The different characteristics predicted from the protein structure indicate that the proteins may play distinct roles in cells subjected to water-deficit stress, although the molecular function remains unclear.

FUNCTION OF DOWN-REGULATED GENES

Much less effort has been devoted to the expression of genes that are down-regulated by water-deficit stress; however, it is known that a few genes have reduced mRNA levels. In *Arabidopsis thaliana*, proline accumulation occurs in response to water deficit. Genes involved in proline accumulation are induced by water-deficit stress and genes involved in proline breakdown are repressed (Kiyosue *et al*., 1996). This, and other possible controls, results in an accumulation of proline in *Arabidopsis thaliana*. In addition, two other genes, hydroxynitrile lyase and a remorin-like gene, were down-regulated and genes of similar predicted function were simultaneously up-regulated (see Reymond *et al*., 2000).

CONCLUSIONS

Future experiments using microarray and gene discovery techniques will continue to add to the broad picture of the cellular plant stress response. The use of full genome arrays will permit the characterization of the vast majority of the genes that are altered by water-deficit stress. Since the experiments that have been published thus far are based on a rapid, severe water-deficit treatment, it is important that microarray experiments be completed in experimental conditions that more closely approximate stress development in the field. The widespread use of these techniques will permit many different experimental designs to be used which will permit a better understanding of the complexities of water-deficit stress and the potential functions of the induced genes. It is important that multidisciplinary teams of scientists with broad expertise in plant stress biology join forces to analyse and interpret the microarray data. In this way, we will be able to distinguish genes that are induced by secondary stresses and genes that are induced by lesions in metabolic pathways in order to identify the genes involved in adaptation. The gene patterns will point to biochemical and cell biology experiments that should be undertaken to understand fully the cellular response. Further investigations using whole plant studies will finally be needed before this information can used to assess the impact of gene expression on the whole plant and to apply the findings to practical agriculture.

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