Available online at www.sciencedirect.com
ScienceDirect
Plant Science 171 (2006) 745–758
PLANT SCIENCE
www.elsevier.com/locate/plantsci

Accessions of *Solanum tuberosum* ssp. *andigena* show differences in photosynthetic recovery after drought stress as reflected in gene expression profiles

Jonathan I. Watkinson [a], Lori Hendricks [a], Allan A. Sioson [b], Cecilia Vasquez-Robinet [a], Verlyn Stromberg [a], Lenwood S. Heath [b], Mary Schuler [c], Hans J. Bohnert [c], Merideth Bonierbale [d], Ruth Grene [a,*]

[a] *Department of Plant Pathology, Physiology and Weed Science, 103 PMB, 435 Old Glade Road, Virginia Tech, Blacksburg, VA 24061, United States*
[b] *Department of Computer Science, Virginia Tech, Blacksburg, VA 24061, United States*
[c] *University of Illinois at Champaign-Urbana, United States*
[d] *Centro Internacional de la Papa, Lima, Peru*

Received 16 May 2006; received in revised form 10 July 2006; accepted 12 July 2006
Available online 8 August 2006

Abstract

A drought screen is described that identifies accessions of *Solanum tuberosum* ssp. *andigena* that show varying degrees of physiological acclimation or adaptation to repeated drought stress. VTSA01 shows adaptation, recovering photosynthesis after a first cycle and maintaining this ability upon subsequent stress exposure. VTSA03 shows acclimation, recovering photosynthesis only after a second cycle of stress. VTSA02 shows an intermediate phenotype with partial recovery after one cycle and, later, improved recovery. Using statistical methods, we correlated changes in expression, of specific pathways and genes with the adaptive and acclimation-related phenotypes exhibited by the three accessions. These pathways and genes include transcripts in anti-oxidant defense, metallothioneins, flavonoid biosynthesis, and cytochrome P450. Genes involved in chromatin remodeling and transcriptional control of gene expression were also up-regulated. In the latter group, two types of zinc finger transcription factors, and several Myb and bZIP factors stand out, not previously associated with stress responses. Distinct and diverse responses to drought exist and attest to germplasm complexity within these potato land races.

Keywords: Anthocyanin; Basic leucine zipper (bZIP); Cytochrome P450 (CYP); Histone deacetylase; Metallothionein; Small heat shock protein

1. Introduction

The necessity to broaden the range of food production brings crops into environments that tend to reduce yield potential. The main contributing factor to yield reduction in these extended ranges is variation in climatic conditions such as high light, shortage or excess of water and high or low temperature. These factors impact processes such as photosynthesis and the subsequent metabolism and partitioning of carbohydrates, that are crucial for the realization of yield potential as well as consistent yield quality. Drought is a primary abiotic stress that not only reduces yield, but also negatively affects product quality and leads to inconsistency in harvestable biomass from year to year.

Drought stress has been well studied physiologically and by molecular genetic approaches. The effects of drought stress have been correlated with the expression of certain genes through the use of single gene studies and microarray data [1–6]. ABA has been implicated in drought stress responses where its activity is modulated by transcription factors such as bZIPs (At4g34000) [7,8] and a MYB (AtMyb2, At2g47190) [9]. Repression of transcription is also important to stress tolerance as shown for the action of Cys2/His-type zinc-finger transcription factors under drought, cold and salt stress in Arabidopsis [10]. Analysis of transcript profiles using clustering algorithms has revealed suites of genes or regulons

* Corresponding author. Tel.: +1 540 231 6761; fax: +1 540 231 5755.
E-mail address: grene@vt.edu (R. Grene).

0168-9452/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.plantsci.2006.07.010

that are stress responsive [3]. One such regulon is the Arabidopsis CBF cold-response pathway, which includes three transcriptional activators (CBF1, CBF2, CBF3) that activate downstream cold-response genes [11] and shares commonalities with drought stress [12]. Zhang et al. demonstrated that, while these genes are present in tomato, the tomato homologs are regulated differently by cold [13], suggesting that selective pressure on the upstream elements controlling expression of these genes has allowed plants to adapt to different environments. Inan et al. and Taji et al. demonstrate that *Thellungiella halophila*, a salt-tolerant relative of Arabidopsis, has higher constitutive levels of expression for genes that are drought and salt responsive in Arabidopsis and, also, contains paralogous members of Arabidopsis stress-responsive gene families [14,15]. Differences in stress resistance, therefore, may also be associated with the presence of paralogs and/or a greater degree of readiness as evidenced by constitutively expressed defense genes.

In clonal cuttings of loblolly pine (*Pinus taeda*), we compared the effects of two levels of drought stress (mild and severe) on physiological responses and compared that with changes in gene expression on cDNA microarrays [6]. Physiologically, the trees were able to recover photosynthetic rate to near control levels in cycles of mild drought stress subsequent to an initial cycle of drought stress. Trees exposed to severe drought stress did not show this capability. We defined the responses to the mild stress as acclimation. Specific gene expression profiles were associated with acclimation as evidenced by our finding that 80% of the genes showing changes in expression were unique to trees grown under mild stress. Genes of the flavonoid pathway, and specific classes of LEAs and HSPs were up regulated during acclimation.

Potato is highly susceptible to drought stress and extracts less of the available water from the soil in comparison with other crops [16]. Withholding water reduces leaf number, leaf size, shoot height [17], and the rate of photosynthesis [18]. Dalla Costa et al. showed that limiting water supply to plants in lysimiters significantly reduced the photosynthetic rate of potato plants, which was reflected in lower tuber yields and biomass [19]. The relative inability of potato to withstand drought limits its productive range to areas with adequate rainfall or suitable irrigation and means that it has a high and costly water requirement for maximal yield.

Responses to drought stress are cultivar-dependent in *S. tuberosum*, indicating genetic variability and suggesting the potential for discovery of more robust resistance mechanisms. Levy identified different osmotic responses to drought stress in *S. tuberosum* cultivars [20]: (1) a short duration osmotic adjustment (high solute concentration coupled with lower osmotic potential) 24–48 h after stress reflecting short term stress acclimation and (2) constitutively/continuously high solute concentration and low osmotic potential reflective of an adapted genotype that is prepared to cope with transient water stress. This demonstrates that mechanisms for both adaptation and acclimation are present in Solanum germplasms.

We are interested in exploring genetic diversity in potato, making use of materials from the Andes, the center of origin and diversity of the potato, where farmers have long selected numerous cultivars with superior stress tolerance. These selections represent a vast genetic resource for traits that could contribute to the development of more robust potato varieties for other regions. The harsh climate of the Andes, home to the *S. tuberosum* ssp. *andigena* (hereafter referred to as Andigena) complex generates great variability in the ability to adapt or acclimate to drought stress. We propose that adaptation and acclimation to drought stress involves the coordinated engagement of regulatory networks and, specifically, of genes associated with signaling, gene transcription, and metabolic pathways involved in resource partitioning and stress defense and protection. We developed a drought stress evaluation protocol that allowed for the measurement of responses in a number of Andigena accessions. The primary goal was to search for lines that, based on their capacity to recover photosynthesis after drought stress, could be placed into one of three categories: stress-adaptation capacity, acclimation potential, or sensitivity to drought stress. Within these categories, patterns of gene expression have been recorded that can be used to distinguish the distinct molecular genetic response circuits of adaptation and acclimation.

2. Materials and methods

2.1. Plant material and drought stress

Open-pollinated accessions (6) of Andigena chosen to represent the broad geographical range of Andigena were acquired as true seed from the US potato germplasm resource (USDA, Sturgeon Bay, WI). Seeds from open-pollinated sources are maintained by the germplasm repository with the understanding that genetic heterogeneity exists within each accession. The seeds were soaked in 1000 ppm GA for 24 h prior to sowing into 601 seed-flat inserts (Wetsel, Harrisonburg, VA) and allowed to germinate under natural light (July, Blacksburg, VA) in a greenhouse (15–25 °C). The media used for all phases of plant growth was Pro-Mix BX (Wetsel). Seedlings were grown in 9 cm square pots in the greenhouse. At 8 weeks, plants were transferred to 28 cm × 19 cm containers, allowed to condition for 2 weeks, and at 10 weeks water was withheld. The progression of the stress was monitored by photosynthetic measurements (LiCor 6400, Lincoln, NE). The stress condition was defined as the physiological state of the plants when photosynthetic rates were 1 ± 1 μmol $CO_2/m^2/s$. Control plants were watered when photosynthetic rate approached 15 μmol $CO_2/m^2/s$ and averaged between 15 and 20 μmol $CO_2/m^2/s$ throughout the experiment. Photosynthesis was monitored every 3–4 days on the second (from the top) most fully expanded leaf until stressed plants neared complete inhibition of photosynthesis after which rates were monitored daily. When the maximum stress level had been reached, plants were rewatered and photosynthesis measurements were taken 24 h later. The relative degree of photosynthetic recovery was determined in each case. Leaves (third to sixth fully expanded

from growing shoots) and tubers (all) were harvested 24 h after recovery, weighed, flash frozen in liquid nitrogen and stored at −80 °C for RNA extraction. Two cycles of stress were imposed. One group of plants was harvested at the first recovery (cycle 1), whereas the second group was allowed to dry down again and harvested after the second re-watering (cycle 2). There were two plants of control and treated per accession for each cycle of stress giving a total of eight plants analyzed per accession.

2.2. RNA isolation

RNA was isolated from 5 to 10 g of tuber tissue. The frozen tuber sample was crushed with a hammer and 5–10 g of the resultant sample was used for RNA extraction. RNA was isolated using a phenol-based method as instructed by TIGR http://www.tigr.org/tdb/potato/microarray_SOPs.shtml.

2.3. Microarrays

RNA from tubers of the selected Andigena accessions were processed at TIGR for microarray analysis according to established protocols http://www.tigr.org/tdb/potato/microarray_SOPs.shtml.

2.4. Statistical analysis

Microarray slides were scanned at TIGR using GenePix Array Scanner (Axon Instruments, Union City, CA), and the slide images were analyzed using GenePix Pro 3.1 (Axon Instruments). For each spot, the Lowess normalized intensity value was collected, except for spots that GenePix Pro had flagged as absent.

The sensitivity of individual genes to the experimental treatments was estimated using the two-stage analysis of Wolfinger et al. [21]. The first stage addresses global effects, whereas the second stage estimates the interaction between individual genes and experimental treatments. The technique is highly sensitive to small differential changes in relative expression values [22] and is increasingly used for data analysis [6,22–24].

In stage one, the values of the major global factors are estimated by fitting the intensity data to this ANOVA normalization model:

$$y_{gijkn} = \mu + T_i + A_j + D_k + (T \times A)_{ij} + \varepsilon_{gijkn}$$

The y_{gijkn} values are $\log_2$-transformed intensity signals with indices for gene (g), treatment (i), array (j), dye (k), and spot (n). The value μ is the mean of all the y_{gijkn} values. The remaining variables are: treatment (T_i, two levels, treated and control), array (A_j, the microarray slides per experimental treatment comparisons), dye (D_k, two dyes, Cy3 and Cy5), the interaction $[(T \times A)_{ij}]$ of treatment (i) and microarray slide (j), and the model residual (ε_{gijkn}).

Stage 2 of the analysis uses the residual values (ε_{gijkn}) computed in the first stage to estimate the interaction between individual genes and treatments (stress level and control) at $\alpha = 0.05$. The ANOVA gene model for this stage is

$$e_{gijkn} = G_g + (G \times T)_{gi} + (G \times A)g_j + (G \times S)_{gn} + \lambda_{gijkn}$$

In this equation, the variables are: the mean (G_g) of the residual values for gene (g), the interaction $[(G \times T)_{gi}]$ of gene (g) and treatment (i), the interaction $[(G \times A)_{gj}]$ of gene (g) and microarray slide (j), the interaction $[(G \times S)_{gn}]$ of gene (g) and spot (n). The λ_{gijkn} are the residual stochastic errors.

Both models are implemented using SAS (SAS/STAT Software version 8.2, SAS Institute Inc., Cary, NC). The least square means of $(G \times T)_{gi}$ and the differences in least square means between the stress level and control were estimated for each gene. Each difference constitutes the estimated $\log_2$-fold change for that gene.

A total of nine experimental treatment comparisons were analyzed. Nominally, each microarray contained 11,243 potato cDNA targets, each replicated four times. A gene with positive (respectively, negative) estimated fold change is marked as positively (respectively, negatively) expressed. The remaining genes are marked unchanged. Genes marked N/A could not be statistically analyzed due to the absence of data as determined by the GenePix Pro analysis.

2.5. Data mining by ILP

The result of statistical analysis is mined using inductive logic programming (ILP), a data mining technique for finding relationships among data [25] used in our earlier work [6]. Signature patterns of gene expression reflecting changes across experimental treatments presented as rules are derived from the ILP component of the Expresso project [26], which is implemented as an extension of the Postgres database management system [27]. The main input to ILP is a table consisting of cDNA accession number, functional categories, experimental conditions, and expression levels. The ILP data mining technique [28] searches through the space of possible relationships among the fields of the said input table to automatically identify the most promising rules [5]. We require that each implication has strength of at least 80% and a support of at least 2. (The rule A :- B, is read ‘‘If B then A,’’ its strength is the fraction of genes satisfying B that also satisfy A, while its support is the number of genes that satisfy both A and B.) ILP rules provide in descriptions that summarize the data and suggest new and testable biological hypotheses.

2.6. Real-time PCR

We reverse transcribed RNA (5 μg) using an oligo $(dT)_{18}V$ and superscript reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturers protocol. The reaction was performed at 42 °C for 1 h. Appropriately diluted samples were stored at −20 °C.

Real-time PCR primers were designed from cloned sequence of candidate genes to generate an amplicon of 80–120 bp (see Table 1 for a list of primers). An 183 bp amplicon of

Table 1
Primer sequences of gene used in real-time PCR analysis of gene expression

Gene name	Forward primer	Reverse primer
Adenosine kinase	5′-AAGGCCTCTACAAGCTAGCTCG-3′	5′-CGGTAATTCCCTCTGCTGAGTC-3′
Chalcone isomerase	5′-GGTGGCAGAAAATTGTGTTG-3′	5′-GTGACCATGCTGTCGAGCC-3′
Isoflavonoid reductase	5′-CCTTCAGCCTCAACAATGCG-3′	5′-GTGACCATGCTGTCGAGCC-3′
Leucocyanidin reductase	5′-TTCTGGTTGGGGACATTTGG-3′	5′-GGGTTGGGATTGGAAGAAGG-3′
Anthocyanidin 3-*O*-glucosyltranferase	5′-TGTATGTGGACTGGCTCC-3′	5′-AAAGGAACGGGACTTCGC-3′

adenosine kinase served as an internal control. Plasmid DNA of each candidate gene was diluted in a 1:10 dilution series and used to generate a standard curve.

Real-time PCR reactions were performed using SYBR green master mix (Applied Biosystems, Foster City, CA), 5 μl of template and 40 nM of each primer in a 25 μl reaction. Negative controls were performed with 5 μl of nuclease free water instead of template cDNA. All reactions were run in triplicate. The ABI PRISM® 7700 Sequence Detection System (Applied Biosystems) was used to perform the reactions. The PCR mixtures were pre-heated at 95 °C for 10 min followed by multiple cycles of amplification (95 °C for 15 s; 56 °C for 30 s; 72 °C for 30 s). The threshold value was selected based on standard curve for each gene.

3. Results

3.1. Physiology and growth

Plants from three accessions are listed in Table 2, while seeds of two of the six accessions failed to germinate and a third succumbed to the drought stress. Plants grew uniformly and, despite not being clonal, the eight plants of each accession had similar characteristics. Plants tuberized rapidly under short days (∼12 h day) and were tuberizing at the start of the stress experiment (October, Blacksburg, VA). Tuber formation was readily apparent because green tubers formed at the surface of the medium. Plants reached maximum stress level ∼4 weeks after watering was terminated, when photosynthesis was inhibited. All plants behaved similarly in their phenotypic response (Table 3). At recovery, both plants of accession VTSA01 had photosynthesis rates that were near (80% of) maximal levels accession VTSA02 showed partial recovery with rates about 30% of the maximum. The other accession showed no recovery of photosynthesis at the end of cycle one, with rates about 10% of the maximum.

After the second cycle of stress (8 weeks after commencement of the experiment), all plants in groups VTSA01, VTSA02, and VTSA03 showed photosynthetic recovery to 50% or greater of the maximum 24 h after re-watering (Table 3).

Table 2
Accessions of *Andigena* used for the drought screen

USDA accession #	VT ID	Provenance	Altitude
PI214431	VTSA01	Junin, Peru	3249 m
PI232841	VTSA02	Pasco, Peru	4338 m
PI160215	VTSA03	Mexico	3930 m
PI280896	VTSA04	Argentina	Unknown

Table 3
Photosynthesis rates of stressed plants at time of maximum stress (stress) and 24 h after rewatering (recovery)

Accession	Cycle 1		Cycle 2	
	Stress	Recovery	Stress	Recovery
VTSA01	2.74	16.37	−0.94	12.94
VTSA02	1.52	6.7	0.97	9.32
VTSA03	0.64	1.91	1.07	9.43

Results are expressed as μmol $CO_2/m^2/s$ and are an average of 10 separate measurements, 5 each per plant of each accession. Control plants were watered when photosynthetic rate approached 15 μmol $CO_2/m^2/s$ and averaged between 15 and 20 μmol $CO_2/m^2/s$.

3.2. Microarrays

Microarray hybridizations were performed on tuber tissue from VTSA01, VTSA02 and from tuber tissue from VTSA03. The Wolfinger, two-stage ANOVA analysis was applied to the array data as described [5,6,24] (see supplemental data), because the model effectively removes various sources of error. All reported expression changes are thus statistically significant at alpha = 0.05. TIGR arrays consist of ∼10,000 elements, but, due to problems with PCR amplification for spotting, many genes annotated as null are not considered in the analysis. For some other genes, the statistical significance could not be assessed because some datapoints were flagged. These genes are marked as not analyzed (N/A). In general, within a comparison 20% of the 10,000 genes spotted on the array showed a response and across all comparisons greater than 50% of genes giving signal showed differential regulation (Table 4).

Table 4
The number of *Andigena* genes that showed significant positive (Pos) or negative (Neg) changes in gene expression as identified by Wolfinger analysis

	VTSA01 (adapted)			VTSA02 (intermediate)				VTSA03 (acclimated)	
	Lvs1	Tub1	Tub2	Lvs1	Lvs2	Tub1	Tub2	Tub1	Tub2
Pos	2560	2741	1113	2246	1499	2470	2381	986	1468
Neg	1937	2123	921	1540	1198	3005	1667	711	1227

Accessions are indicated. L1: leaves cycle 1, L2: leaves cycle 2, T1: tubers cycle 1, T2: tubers cycle 2.

Clone ID	Contig	Annotation	VTSA01 L1	VTSA01 T1	VTSA01 T2	VTSA02 L1	VTSA02 L2	VTSA02 T1	VTSA02 T2	VTSA03 T1	VTSA03 T2
STMEW16	59325	Metallothionein-like protein type 2 A	0	+	+	0	0	0	0	0	+
STMIF26	68245	Metallothionein-like protein type 2 A	0	+	+	0	0	0	+	0	+
STMGS26	57824	Metallothionein-like protein type 2 B.	0	+	+	-	0	-	+	+	+
STMIO10	57824	Metallothionein-like protein type 2 B.	0	+	+	-	0	-	+	+	+
STMCA73	66263	Metallothionein-like protein type 2.	0	0	+	0	0	0	0	0	+
STMHV42	66263	Metallothionein-like protein type 2.	0	NA	NA	NA	0	0	0	NA	NA
STMHX32	66263	Metallothionein-like protein type 2.	0	0	NA	NA	NA	0	0	NA	0
STMIU81	66263	Metallothionein-like protein type 2.	-	0	+	0	0	0	0	0	+
STMFB27	49541	Metallothionein-like protein type 3.	0	+	+	-	0	-	+	0	0
STMFB42	49541	Metallothionein-like protein type 3.	0	0	+	-	0	-	0	0	0
STMCO76	65685	putative metallothionein-like protein	0	0	+	-	0	-	0	0	+

Fig. 1. Expression profile of genes associated with the putative class of antioxidants metallothioneins in plants of Andigena recovering from drought stress. Genes encoding metallothioneins were identified by ILP as being associated with adaptation and acclimation in plants of Andigena plants recovering from drought stress. The genes identified by ILP are identified here. Accessions are indicated. Organ and cycle are indicated as L1 (leaves first cycle), L2 (leaves second cycle), T1 (tubers first cycle) and T2 (tubers second cycle). Significant (as identified by Wolfinger analysis) changes in transcript profiles are shown as positive, negative, (0) unchanged or (NA) no data.

Inductive logic programming (ILP), which has been tested before [5,6], was used here to identify genes within functional categories that have the same expression profile. To aid in ILP analysis, we categorized the potato genes deposited on the array into a modification of the functional category scheme we had previously established for loblolly pine [6]. ILP seeks to find a relationship between genes in a particular expression cluster and genes in a specific functional category and these relationships are expressed as rules. We present below genes in several categories that were identified by ILP as having unique expression profiles. It is important to note that while ILP finds groups of genes with similar expression patterns, other genes not falling into a recognizable pattern did show statistically significant changes in gene expression.

3.3. Metallothionein

Redox, a sub-category within the major category ''Environment'', includes genes associated with detoxification of reactive oxygen species and other anti-oxidant processes, including the metallothioneins. Several of these genes show induced expression in tubers of VTSA01 after both cycles of stress (Fig. 1). Likewise, the metallothioneins are induced in tubers of VTSA03 after the second cycle of stress (Fig. 1), when plants of this accession show recovery from drought stress. An EST of metallothionein 2B and an EST of metallothionein 2A show significant induction, as identified through analysis by the Wolfinger model, in tubers of VTSA02 (cycle 2). Other, better studied antioxidant genes encoding glutathione-*S*-transferase, superoxide dismutase and

Clone ID	Contig	Annotation	VTSA01 L1	VTSA01 T1	VTSA01 T2	VTSA02 L1	VTSA02 L2	VTSA02 T1	VTSA02 T2	VTSA03 T1	VTSA03 T2
STMJP47	41240	bZIP DNA-binding protein	-	+	0	0	0	0	0	+	+
STMIZ83	41240	bZIP DNA-binding protein	-	0	+	0	0	+	0	0	0
STMCD81	48761	bZIP protein	+	+	NA	+	0	-	+	0	0
STMEO01	49403	bZIP transcription factor	0	0	0	+	0	-	0	+	+
STMIM52	49402	bZIP transcription factor	0	0	+	+	0	-	0	+	0
STMIK68	47080	bZIP transcription factor-like	+	0	NA	+	0	0	0	NA	+
STMDO44	45361	GATA transcription factor 1	+	+	0	+	+	-	+	+	+
STMJH83	45361	GATA transcription factor 1	+	+	+	0	+	-	+	0	+
STMCJ23	45361	GATA transcription factor 1	0	+	0	+	+	-	0	0	+
STMGA19	45361	GATA transcription factor 1	+	+	+	0	+	-	0	0	+
STMIR09	41358	zinc finger transcription factor-like	0	0	0	+	+	-	-	0	-
STMCR23	44837	histone deacetylase-like protein	+	+	-	+	-	0	0	0	-
STMDE18	44837	histone deacetylase-like protein	+	+	-	+	0	0	0	+	0
STMGJ95	44837	histone deacetylase-like protein	+	+	-	0	0	0	0	+	0
STMEI54	45028	putative histone deacetylase HD2	0	+	-	+	0	0	+	+	0
STMGC44		putative histone deacetylase HD3	0	+	-	+	0	+	+	+	-
STMDV49	45028	putative histone deacetylase HD2	0	+	-	+	0	+	+	+	0

Fig. 2. Expression profile of genes encoding transcription factors in plants of Andigena recovering from drought stress. Genes within the category of transcription factors were identified by ILP as having altered transcript profiles in Andigena plants recovering from drought stress. The genes identified by ILP are labeled here. Figure is labeled as in Fig. 1.

thioredoxin did not respond to drought stress, except in the tubers of VTSA01 after the first cycle where they were repressed (supplemental data).

3.4. Chromatin remodeling and transcription factors

ILP identified two subsets of transcriptional regulators with unique patterns of expression (Fig. 2). One of these includes histone deacetylases (At3g44750), which control chromatin structure (and accessibility of the transcriptional machinery to the DNA) by modulating histone modifications that affect the packing of nucleosomes [29]. These genes show induction in tubers of VTSA01 (adapted accession) after the first cycle of stress but repression after the second cycle (Fig. 2). Furthermore, these same genes were induced in tubers of VTSA03 (acclimated accession) after the first cycle of stress and some were induced in VTSA02 after both cycles of stress. Histone acetyltransferases, which have the opposite effect on chromatin structure, were unchanged in VTSA01 and VTSA03 (supplemental data). The results suggest regulation of chromosome structure or accessibility as an important requisite for resistance to drought stress.

Among transcription factors with significant regulation were zinc-finger transcription factors that ILP identified as differentially regulated by drought in the various accessions of Andigena. The zinc-finger genes (GATA transcription factor 1, At3g24050; Zn finger transcription factor, At2g40140) were induced in leaves of VTSA02 after the second cycle of stress

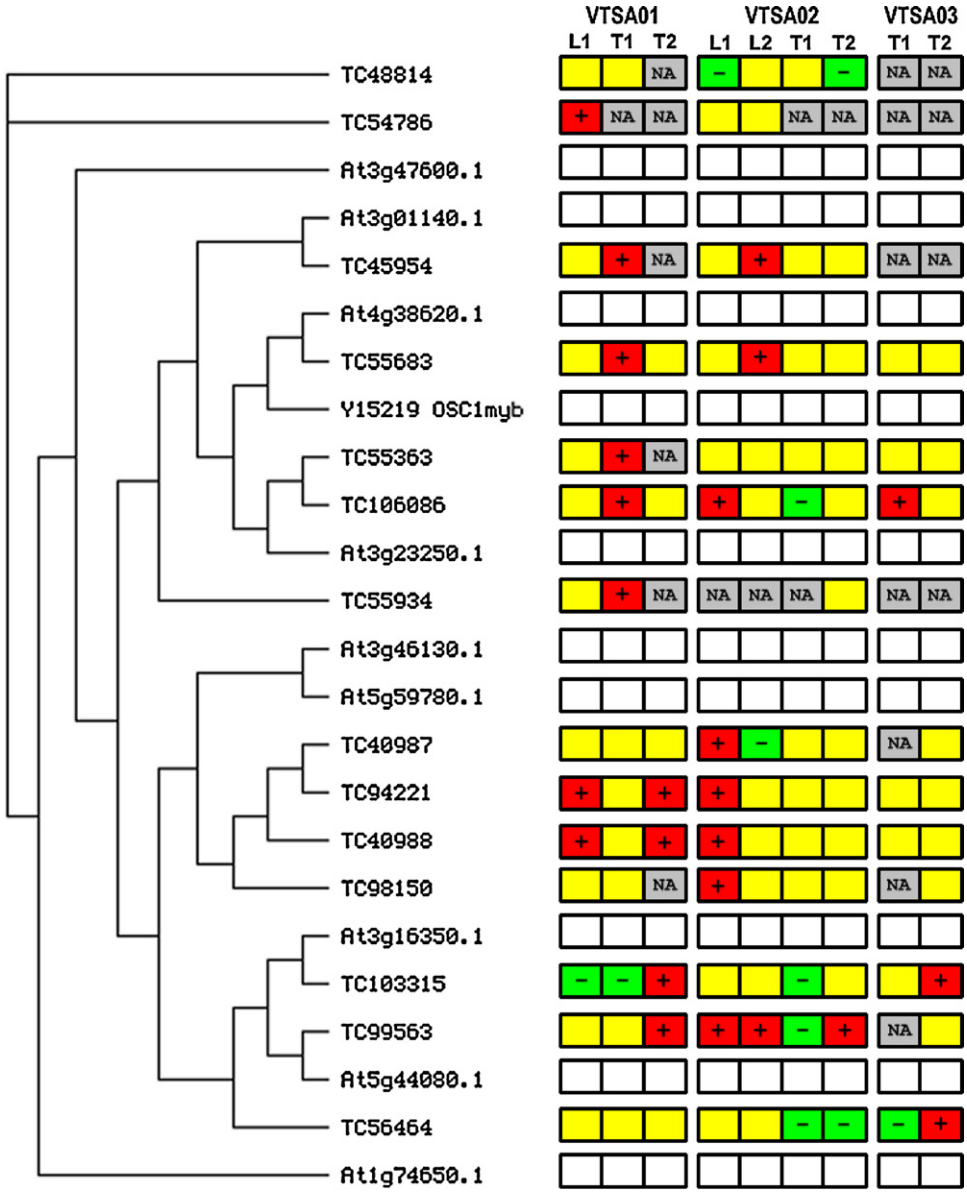

Fig. 3. Phylogenetic tree of *S. tuberosum* and Arabidopsis Myb transcription factor genes and the associated expression pattern of the potato clones in Andigena. Contig sequences of potato Myb transcription factors were downloaded from TIGR and the Arabidopsis homolog was downloaded from NCBI. The sequences were aligned using ClustalX and phylogenetic analysis performed using the heuristic search parameter of PAUP. The microarray expression data is represented along side the phylogenetic tree. Labeling for the expression table is as in Fig. 1.

Clone ID	Contig	Annotation	VTSA01 L1	VTSA01 T1	VTSA01 T2	VTSA02 L1	VTSA02 L2	VTSA02 T1	VTSA02 T2	VTSA03 T1	VTSA03 T2
STMGX79	51257	putative flavonol synthase-like protein	0	0	NA	0	0	0	0	0	+
STMJJ75	45254	Flavonone- 3-hydroxylase	0	-	NA	0	-	+	-	NA	-
STMEM28	120836	Chalcone isomerase	0	0	0	-	0	0	-	0	0
STMIU56	129877	Chalcone isomerase	0	-	NA	NA	+	+	+	NA	NA
STMGV21	121842	Isoflavone reductase	0	0	+	0	0	0	-	0	+
STMJM54	52615	putative flavanone 3-hydroxylase	0	+	NA	NA	NA	+	0	NA	NA
STMDB35	49846	flavanone 3 beta-hydroxylase	0	-	NA	0	+	+	+	NA	0
STMHI60	57312	flavonoid 3'-hydroxylase	0	0	0	0	0	0	0	0	-
STMHO88	50435	flavonoid 3'-hydroxylase (TT7)	0	0	0	-	+	-	0	0	0
STMCA17	49231	flavonoid 3'-hydroxylase (TT7)	NA	0	NA	NA	+	0	0	NA	NA
STMHR18	49232	flavonoid 3'-hydroxylase (TT7)	0	0	NA	0	+	-	0	0	0
STMHT73	49232	flavonoid 3'-hydroxylase (TT7)	0	+	NA	0	+	-	0	NA	0
STMCR55	52458	putative flavonoid 3'-hydroxylase (TT7)	+	NA	NA	-	0	0	NA	NA	NA
STMDZ37	49808	dihydroflavonol 4-reductase-like (CAD-like)	+	+	-	0	0	+	-	0	0
STMCF20	49666	flavonoid 3-O-glucosyltransferase	0	-	NA	0	+	+	+	NA	-
STMGM14	41629	putative anthocyanidine rhamnosyl-transferase	0	-	0	+	-	+	0	-	0
STMCL27	49387	anthocyanidin-3-glucoside rhamnosyltransferase	0	-	NA	0	+	+	+	NA	0
STMCX70	49388	anthocyanidin-3-glucoside rhamnosyltransferase	0	0	0	+	+	+	+	-	0

Fig. 4. Expression profile of genes associated with the production of flavonoids and anthocyanins in Andigena. Genes encoding enzymes of anthocyanin and flavonoid biosynthesis were grouped and their expression profiles are represented here. Figure is labeled as in Fig. 1.

when plants were acclimated. These same genes were repressed in tubers of VTSA03 after the first cycle of stress (Fig. 2), when acclimation had not yet taken place. Likewise, these genes were induced in tubers of VTSA01 after the first cycle of stress, and in tubers of VTSA02 after the second cycle. Results following the Wolfinger statistical analysis revealed that individual clones of other transcription factors showed similar patterns of expression. For example, the bZIP transcription factors showed induction in leaves of VTSA02 as well as other accessions (supplemental data). Another example is myb-related transcription factors that show induction in tubers of VTSA01 (cycle 1; Fig. 3).

3.5. Resistance genes

3.5.1. Comparisons with Arabidopsis genes of known function

Transcripts of genes in this group cover a number of different, biochemical or molecular pathways that have been implicated in stress responses. Genes for enzymes of flavonoid biosynthesis, which we had identified as responsive during drought acclimation in pine [6], were induced in tubers and leaves of VTSA02 (intermediate accession, Fig. 4). Other transcripts that responded were associated with anthocyanin production, including flavonol synthase, flavonoid 3′-hydroxylase, dihydroflavonol reductase, and anthocyanodin 3-*O*-glycosyltransferase. These genes showed no trends in expression in other tissues or accessions, though individual isoforms of flavonoid 3′-hydroxylase showed both repression and induction in leaves and tubers of the other accessions. Finally, a gene encoding a dehydration-induced rd22 (ABA responsive transcription factor) was repressed in most accessions and tissues, except tubers of VTSA02 where it was induced in the first cycle (supplemental data).

Cytochrome P450s (CYP) represent a multi-faceted, sequence-divergent, family of genes with functions in diverse biosynthetic pathways. CYPs, which form a large gene family in Arabidopsis, catalyze oxidation reactions, many of which have been associated with stress. Many of the CYPs showed differential regulation in response to stress in potato (Fig. 5). Alignments of the potato CYPs with those from Arabidopsis showed that several CYPs associated with brassinolide metabolism were induced in leaves of VTSA02. CYPs involved in indole glucosinolate synthesis (aligning with At4g31500) were induced in leaves and tubers of all accessions, while CYPs associated with the production of terpenoids (At3g52970) were induced in leaves and tubers of VTSA01 and VTSA02. The expression of terpenoid genes could be related to the production of defense compounds including carotenoids which have been shown to interact with CDSP34, a drought responsive, nuclear encoded, chloroplastic protein from potato [30].

Finally, ESTs encoding small heat shock proteins (sHSP) had significant expression changes in leaves and tubers of VTSA01 during the first cycle of stress and tubers of VTSA03 (Fig. 6) during the second cycle, but were unchanged in VTSA02. ESTs encoding the peptidyl–prolyl *cis–trans* isomerases (PPiases) show differential expression within and between accessions (Fig. 7). In VTSA01, ESTs encoding ROF1 (Rotomase-FKBP) type PPiases are induced in leaves and tubers after both cycles (Fig. 7). The same ESTs are repressed in tubers of VTSA02 after the first cycle but induced after the second cycle. A subset of these ESTs show induction in tubers of VTSA03 after the second cycle of stress. ESTs of a ROC7 (Rotomase-cyclophilin) type PPiase are induced in tubers of VTSA02 after the first cycle of stress, but unchanged after the second cycle (Fig. 7). These same ESTs are repressed in VTSA01.

3.6. Real-time PCR

Real-time PCR was used to confirm the expression data generated by microarrays and to further explore expression

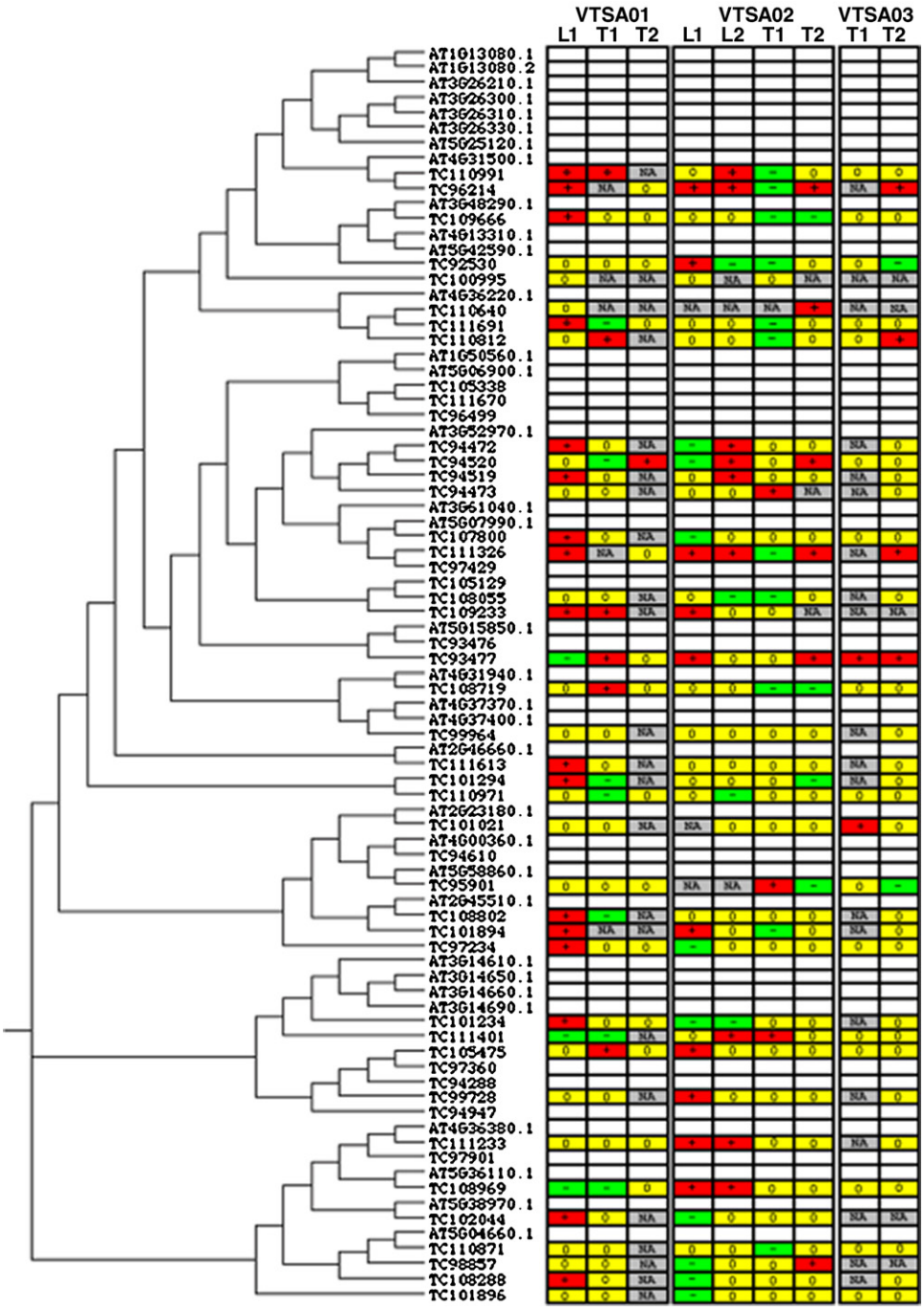

Fig. 5. Phylogenetic tree of *S. tuberosum* and Arabidopsis cytochrome P450s and the associated expression pattern of the potato clones in Andigena. Potato and Arabidopsis sequence data was acquired as in this figure. Labeling for the expression table is as in Fig. 1.

changes in candidate genes. The genes targeted were, isoflavonoid reductase, leucocyanidin dioxygenase, chalcone isomerase, and anthocyanin 3-*O*-glycosyltransferase. In general, the real-time PCR data matches the microarray data (Table 5). However, in some instances, it is not possible to make that determination as the data for the microarray is NA due to poor signal, excessive signal or high background. For example, with A3OGT, the data for VTSAO1 matches for the first cycle of stress but, because the array data is NA for the second cycle in tubers it is not possible to compare that result with the real-time PCR identified positive change in transcript level. With CHI in tubers of VTSA03 after the first cycle, the microarray indicates no change in transcript level, whereas the real-time data shows a substantial induction of transcript levels. Transcript analysis of isoflavone reductase using real-time PCR also shows good similarity with the microarray results. Since adapted lines may have higher constitutive expression of defense genes, we compared the

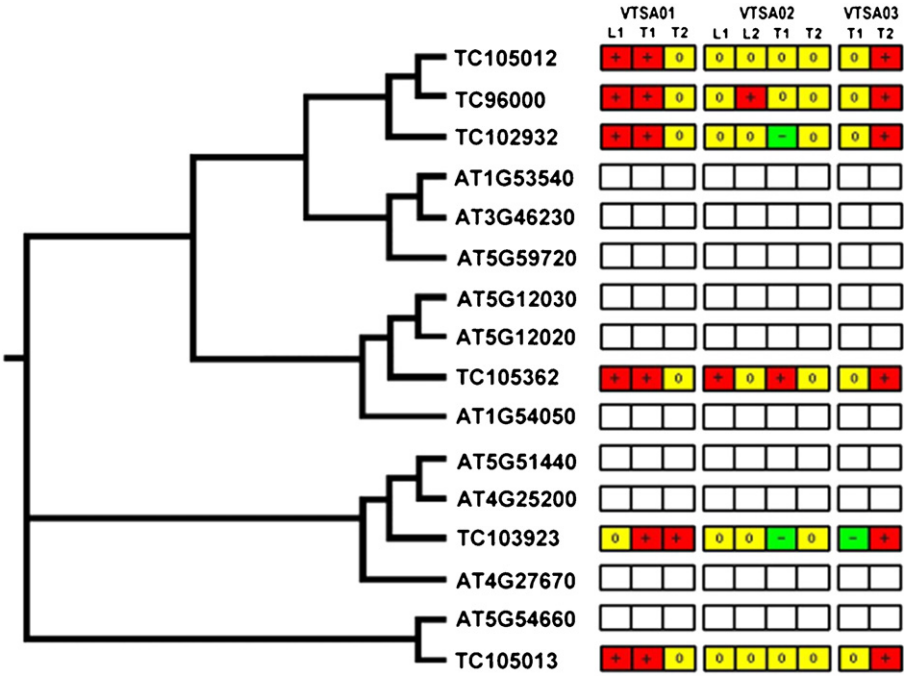

Fig. 6. Phylogenetic tree of *S. tuberosum* and Arabidopsis small heat shock protein genes and the associated expression pattern of the *S. tuberosum* clones in Andigena. Potato and Arabidopsis sequence data was acquired as in Fig. 5. Sequence alignments were performed as in this figure and expression profile is labeled as in Fig. 1.

			VTSA01			VTSA02				VTSA03	
Clone ID	Contig	Annotation	L1	T1	T2	L1	L2	T1	T2	T1	T2
STMCD17	9017	peptidyl-prolyl cis-trans isomerase-like	0	0	-	+	0	+	0	0	0
STMCJ85	9017	peptidyl-prolyl cis-trans isomerase-like	0	-	NA	+	0	+	0	0	0
STMCP26	20833	peptidylprolyl isomerase (EC 5.2.1.8)	-	-	0	0	0	+	0	0	0
STMCR57	20833	peptidylprolyl isomerase (EC 5.2.1.8)	-	-	-	0	0	+	0	0	0
STMCX46	20786	peptidylprolyl isomerase (EC 5.2.1.8)	+	+	+	+	0	-	+	0	+
STMDB64	20786	peptidylprolyl isomerase (EC 5.2.1.8)	+	+	+	+	0	-	+	0	+
STMDE47	20786	peptidylprolyl isomerase (EC 5.2.1.8)	+	+	+	+	0	-	+	+	+
STMGH64	20786	peptidylprolyl isomerase (EC 5.2.1.8)	+	0	0	+	+	0	+	-	0
STMGY96	20786	peptidylprolyl isomerase (EC 5.2.1.8)	+	+	+	0	0	-	+	-	0

Fig. 7. Expression profiles of ESTs encoding peptidyl–prolyl *cis–trans* isomerases. ESTs encoding peptidyl–prolyl *cis–trans* isomerases were grouped and their expression profiles are represented here. Figure is labeled as in Fig. 1.

expression of these gene among control plants of different accessions. It can be seen in Table 6 that in VTSA01 (adapted accession) the transcript levels of the analyzed genes are much higher compared to their expression in the two other accessions, suggesting that VTSA01 has a higher constitutive level of expression of genes associated with stress tolerance.

4. Discussion

4.1. Photosynthetic recovery from stress identifies acclimated and adapted accessions of Andigena potato

Potatoes selected in the Andes by indigenous peoples represent a valuable source of germplasm because they are able

Table 5
Real-time PCR results of one selected gene shown by microarray analysis to have altered transcript levels during drought stress

	VTSA01 (adapted)			VTSA02 (intermediate)				VTSA03 (acclimated)	
	L1	T1	T2	L1	L2	T1	T2	T1	T2
A3OGT	0.93 ± 0.16	0.12 ± 031	11.5 ± 0.28	0.49 ± 0.21	0.99 ± 0.1	2.29 ± 0.22	115.6 ± 0.28	N/A	N/A
CHI	1.28 ± 0.25	N/A	0.11 ± 0.29	0.14 ± 0.27	1.29 ± 0.19	N/A	0.14 ± 0.31	6.26 ± 0.26	N/A
IFR	0.92 ± 0.24	1.5 ± 0.38	1.38 ± 0.3	0.02 ± 0.27	0.54 ± 0.21	0.36 ± 0.24	0.19 ± 0.29	0.99 ± 0.26	1.98 ± 0.27
LDOX	0.76 ± 0.24	N/A	0.11 ± 0.33	0.089 ± 0.28	0.3 ± 0.15	0.12 ± 0.26	0.006 ± 0.33	6.2 ± 0.3	0.85 ± 0.26

Anthocyanodin 3-*O*-glycosyltransferase (A3OGT), chalcone isomerase (CHI), isoflavone reductase (IFR) and leucocyanidin reductase (LDOX) expression was analyzed by real-time PCR using SYBR green technology. Accessions are indicated. Organ and cycle numbers are as in Table 4. Values represent estimated fold change ± standard error.

Table 6
Differences in gene expression between accessions of *Andigena* using real-time PCR

Comparison	Organ	A3OGT		CHI		IFR	
		Control	Stressed	Control	Stressed	Control	Stressed
01 vs. 02	L1	0.2135	553.21	3.94	0.01	5.0767	553.21
01 vs. 02	T1	36.752	88.824	0.36	2.58	1.1315	88.824
01 vs. 02	T2	0.0227	0.0691	924.86	0.07	1512.5	0.069
01 vs. 03	T1	1824.6	5.0291	0.005	0.25	0.005	5.0291
01 vs. 03	T2	0.3546	0.0018	1018.75	70.29	3960.5	0.002
02 vs. 03	T1	49.645	0.82	0.01	0.31	0.004	0.82
02 vs. 03	T2	15.643	0.0255	1.10	1016.95	2.619	0.026

Levels of A3OGT, CHI, and IFR expression for stressed and control plants were compared across accessions. 01 vs. 02 (VTSA01 vs. VTSA02), 01 vs. 03 (VTSA01 vs. VTSA03), 02 vs. 03 (VTSA02 vs. VTSA03). Organ and cycle numbers are as in Table 4.

to withstand high light, drought and cold stress, suggesting the presence of superior genetic mechanisms for stress tolerance. Such lines show superior performance under conditions that challenge cultivated varieties that are normally grown in less stressful environments. We have analyzed three accessions of Andigena for drought tolerance or susceptibility based on their ability to recover photosynthetic capability upon rewatering after water deficit. After one cycle of stress, all plants had lost photosynthetic capability with rates approaching zero. After rewatering, only plants within accession VTSA01 regained full photosynthetic capability and this accession also maintained the ability to recover photosynthesis after a second cycle of stress. We define the ability for full photosynthetic recovery with no prior drought exposure as adaptation (see [6]).

Accession VTSA03 did not show photosynthetic recovery after the first cycle of stress though it did recover photosynthetic capability to 50% of controls after a second cycle of stress also. We define the ability to recover photosynthesis subsequent to an initial drought exposure as acclimation.

A third accession (VTSA02) had 40% recovery after one cycle of stress and 50% recovery after the second cycle of stress. This accession does not appear to be fully adapted, though it shows some stress resistance after the first cycle of stress. We conclude that this represents an intermediate type of stress resistance between what we define as acclimation and adaptation. This would suggest that the diversity inherent in Andigena will represent a spectrum of drought resistant phenotypes from adapted genotypes, to acclimated genotypes and genotypes with no stress resistance or susceptibility.

4.2. A suggested specific role for ROS related metallothioneins (but not SOD, and APX) in accessions of Andigena

Many stresses result in the increased production of reactive oxygen species, ROS [1]. While these activated forms of oxygen also function as signals, protection from excess ROS is a necessity for plants to maintain metabolic homeostasis. It is expected, and has been shown, that genes associated with antioxidants are up regulated in response to drought stress [31,32]. Our results show that a group of metallothionein genes was induced under stress conditions. In contrast, the more widely studied antioxidant genes, such as the APXs and SODs did not respond to drought stress in this experiment. Metallothioneins have been implicated in metal homeostasis, ROS detoxification and in the maintenance of cellular redox balance [33,34]. The antioxidant activity of metallothioneins appears to be independent of that of the NADPH/Ascorbate/glutathione pathway. In fact, they have been shown to compensate for SOD deficiency in mammalian cells [35]. In Arabidopsis, a metallothionein has been linked to senescence and to induced cell death [36]. Other data suggest that metallothioneins are up regulated in response to compounds that induce oxidative stress [37], suggesting that it is an ROS scavenger. It has been shown that genes related to anti-oxidants are induced upon exposure to a mild dose of H_2O_2 and this protects plants from subsequent highlight induced oxidative stress [38]. Expression of metallothionein correlates with adaptive or acclimatory ability (Fig. 1, Table 3) such that in the adapted accession (VTSA01) metallothionein is induced after both cycles of stress whereas in the acclimated accession (VTSA03) metallothioneins are induced only when acclimation occurs after the second cycle of stress. The expression pattern of metallothioneins thus correlates with the acquisition of drought tolerance.

4.3. A possible role for flavonoid metabolism in adaptation and acclimation

Genes encoding enzymes of the flavonoid pathway are, in general, coordinately induced in VTSA02. Given the nature of the genes that are induced, the data are consistent with increases in the main pathway of flavonoid production that leads to the production of anthocyanins (flavanone 3′-hydroxylase which forms dihydrokaempferol, which, in turn, is metabolized by dihydroflavanol reductase to proanthocyanadin). Several of the anthocyanin genes that show induction are involved in side chain modification of the core anthocyanidin suggesting that specific anthocyanins are being produced. Anthocyanins may be involved in protection from excess excitation energy in leaves, as has been shown in Arabidopsis [39] or could serve as antioxidants [40]. If the anthocyanins are acting as anti-oxidants, our results suggest that VTSA02 has a different

response to oxidative stress than either VTSA01 or VTSA03, though, based on its phenotype between acclimation and adaptation, this response is no less effective. Furthermore, it can be seen that isoflavone reductase, which represents a significant branch point in the flavonoid pathway, is induced in tubers of both VTSA01 and VTSA03 after the second cycle of stress. Therefore, the results indicate an array of phenotypic differences relating to drought tolerance and, in addition, reveal different molecular mechanisms among the accessions to cope with drought.

As we did not see an expected correlation between the expression of genes of flavonoid metabolism and adaptation/acclimation to drought, we compared the basal levels of expression of flavonoid genes between different accessions using real-time PCR. The results showed that there was a higher constitutive level of expression of several genes associated with flavonoid production in the adapted accession, VTSA01 (Table 6). It would appear that genes associated with flavonoid production are constitutively expressed at a higher level in the adapted accession (VTSA01) compared to accessions that did not show adaptation, providing stress protection at the onset of stress conditions. High constitutive expression may constitute part of the adaptive mechanism as has been shown for Arabidopsis [14,15], and pea [41].

4.4. Genes associated with chromatin remodeling are correlated with adaptation or acclimation

The coordinate expression of genes related to a particular phenotype are often controlled by transcriptional regulators that act at different levels. For example, histone deacetylases could be considered master regulators as their activity is believed to alter specific regions of chromatin and thus modulate transcription [42]. The CBF regulon, is under transcriptional control of the DREB/CF transcription factors, but recent data shows that histone modification is crucial to the expression of COR genes (Pavangadkar and Thomashow, unpublished). The DREB/CF transcription factors are activated upon initial perception of the stress and thus direct transcription of their targets, allowing them to be functional for subsequent stress exposure: the process of acclimation. Here, several histone deacetylases were induced in the adapted accession (VTSA01) and the purely acclimated accession (VTSA03). HDACs are believed to function in silencing transcription as the removal of acetyl groups allows the nucleosomes to pack more tightly. The correlative expression of HDACs with drought tolerance phenotypes suggests that gene silencing is important to both adaptive and acclimatory responses.

4.5. Transcription factors correlate with adaptation to drought stress

Both GATA type IV and C3H motif zinc-finger transcriptional regulators showed responses upon recovery from drought stress. The GATA family has been implicated in light-regulated gene expression and in the regulation of development and pattern formation [43], although the precise function of many GATA transcription factors has not been elucidated. The GATA protein identified here shows induction in tubers after the first cycle of stress in tubers of VTSA01 (at the time of adaptation) and VTSA02 after the second cycle of stress (at the time of acclimation). The same gene was repressed in tubers of the acclimated accession (VTSA03) after the first cycle of stress (prior to acclimation). The correlation of the expression of this GATA-TF with the timing of adaptation and acclimation suggests that it may regulate genes that are necessary to these different stress responses.

In addition, several bZIP and MYB TFs are regulated under drought stress in the accessions (Figs. 2 and 3). Responses of some bZIP proteins to drought has been reported before [7,8], most notably an ABA responsive protein that is part of the ABA-dependent, drought-stress signaling cascade. This gene shows induction in leaves of VTSA01 (adapted) and VTSA02 (adapted/acclimated) in the first cycle of stress and in tubers of VTSA03 (acclimated) after the second cycle of stress suggesting an ABA-dependent response pathway in potato drought stress tolerance that is correlated with the acquisition of tolerance. It is interesting to note that an RD22-like gene (RD22 is a MYB-driven, ABA responsive, drought related gene from Arabidopsis) is repressed in nearly all tissues and accessions, suggesting that its role in potato may not be drought related or that the true drought-responsive potato homolog of RD22 is not present on the array. Again, differences in expression among the accessions suggest untapped genetic variability in the Andigena germplasm.

4.6. Hormone-associated effects

Cytochrome P450s (CYP) represent a multi-faceted, sequence-divergent, family of genes with functions in diverse biosynthetic pathways. CYPS, which form a large gene family in Arabidopsis, catalyze oxidation reactions, many of which have been associated with stress. Potato ESTs aligning with Arabidopsis CYPs associated with indole glucosinolate showed induction in VTSA01 (adapted) leaves and VTSA02 (intermediate). In addition to their role in biotic stress defense processes, the action of the CYP associated with glucosinolate production may be to shuttle the auxin precursor away from auxin and thus prevent auxin accumulation [44]. CYPs associated with brassinolide metabolism are also induced in leaves of VTSA02. Auxin and brassinolide have overlapping function in terms of cell division, however, it has been demonstrated that the mechanism for promoting cell division is distinct for the two compounds [45]. Brassinolides have been implicated in stress responses [46,47] suggesting that their mode of action is stress-dependent. Taken together, the data presented here suggests that adaptive/acclimatory processes in leaves necessary to overcome water stress include a cell cycle regulatory process induced by brassinolide and separate from auxin. This process is not apparent in tubers of the acclimated line (VTSA03), again pointing to a myriad of phenotypic responses to drought in Andean potato.

4.7. Chaperones

We previously demonstrated that induction of specific HSP chaperone genes was associated with photosynthetic recovery after drought stress in pine [6]. In potato, ESTs encoding the sHSPs were induced in the adapted accession at the time of adaptation (first cycle) and in the acclimated accession at the time of acclimation (second cycle; Fig. 7). The sHSPs were shown to be induced during the desiccation stage of seed development and under heat stress [48,49]. In vitro studies have shown that sHSPs can bind selectively to non-native proteins, prevent their aggregation and maintain them in a competent state for refolding by other chaperones [50]. Cytosolic Arabidopsis homologs of these potato proteins (At5g120202, AtHSP17.6-II; At5g12030, AtHSP17.6A) showed induction under osmotic stress and one (At5g12030) was reported to enhance drought tolerance in Arabidopsis [51]. Taken together the results suggest that sHSPs are an important component of adaptive and acclimatory responses in Andigena.

The chaperone family of peptidyl–prolyl *cis–trans* isomerases (PPiase) shows differential regulation across the accessions. In all three accessions, the FKBP type of PPiases (ROF1) are induced specifically either at the time of adaptation or acclimation (Fig. 7). An FKBP type PPiase from Arabidopsis is associated with heat stress [52], suggesting that the chaperonin activity of these genes is crucial to stress tolerance. A homolog of the ROC7 cyclophilin is only expressed in tubers of the intermediate accession (VTSA02) at the time where plants showed partial adaptation. This cyclophilin is ER localized [53] suggesting that it plays a role in proper folding of proteins in the secretory pathway. Its expression in the intermediate accession suggests that it plays a unique role in the adaptive response of this accession.

5. Conclusions

We studied the recovery of photosynthesis after drought stress on three accessions of Andigena and were able to identify one accession that showed adaptation, one accession that showed acclimation and one that showed a phenotype that had features in common with adaptation and acclimation. Using the TIGR microarray platform to analyze changes in gene transcript levels, we found that specific patterns of gene expression were correlated with the resistance phenotypes. Induction of metallothioneins constitutes an anti-oxidant response that correlates with the acquisition of stress tolerance at both adaptation and acclimation. These genes are preferentially induced over those normally associated with anti-oxidant response. In an intermediate accession, induction of genes of the flavonoid pathway indicates a separate anti-oxidant type response. However, in the adapted accession, the genes encoding flavonoid biosynthetic enzymes have a greater constitutive level of expression and this basal level of expression correlates with adaptive-type drought tolerance. Induction of genes encoding HDACs also correlates with the acquisition of stress tolerance suggesting that gene silencing is important to adaptation and acclimation to drought stress. Finally, induction of the HSP 90s correlates with drought resistance. In all, the data show that, though potato has some common pathways for drought tolerance, unique pathways exist across a range of genotypically divergent accessions.

Acknowledgements

We wish to thank Drs. P. Li and V. Poroyko (UIUC) for discussions and help with data analyses. We also thank Shrinivasrao P. Mane for help generating figures. Support in part has been provided by the US National Science Foundation (DBI 0223905, EIA-0103660, and EIA-0219322 to HJB, RG, CV-R, JIW, LSH), by the Centro Internacional de la Papa to LH and RG, and by institutional support from VT and UIUC for RG, CV-R, HJB and LH.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.plantsci.2006.07.010.

References

[1] R.G. Alscher, J.L. Donahue, C.L. Cramer, Reactive oxygen species and antioxidants: relationships in green cells, Physiol. Plantarum 100 (1997) 224–233.
[2] Q. Liu, M. Kasuga, Y. Sakuma, H. Abe, S. Miura, K. Yamaguchi-Shinozaki, K. Shinozaki, Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, in Arabidopsis, Plant Cell 10 (1998) 1391–1406.
[3] M. Seki, M. Narusaka, H. Abe, M. Kasuga, K. Yamaguchi-Shinozaki, P. Carninci, Y. Hayashizaki, K. Shinozaki, Monitoring the expression patterns of 1300 Arabidopsis genes under drought and cold stresses by using a full-length cDNA microarray, Plant Cell 13 (2001) 61–72.
[4] Z.N. Oztürk, V. Talame, M. Deyholos, C.B. Michalowski, D.W. Galbraith, N. Gozukirmizi, R. Tuberosa, H.J. Bohnert, Monitoring large-scale changes in transcript abundance in drought- and salt-stressed barley, Plant Mol. Biol. 48 (2002) 551–573.
[5] L.S. Heath, N. Ramakrishnan, R.R. Sederoff, R.W. Whetten, B.I. Chevone, C.I. Struble, V.Y. Jouenne, D. Chen, L. van Zyl, R. Grene, Studying the functional genomics of stress responses in loblolly pine with the Expresso microarray experiment management system, Comput. Funct. Genom. 3 (2002) 226–243.
[6] J.I. Watkinson, A.A. Sioson, C. Vasquez-Robinet, M. Shukla, D. Kumar, M. Elis, L.S. Heath, N. Ramakrishnan, B. Chevone, L.T. Watson, L. van Zyl, U. Egertsdotter, R.R. Sederoff, R. Grene, Photosynthetic acclimation is reflected in specific patterns of gene expression in drought-stressed loblolly pine, Plant Physiol. 133 (2003) 1702–1716.
[7] Y. Uno, T. Furihata, H. Abe, R. Yoshida, K. Shinozaki, K. Yamaguchi-Shinozaki, Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 11632–11637.
[8] J. Kang, H. Choi, M. Im, S.Y. Kim, Arabidopsis basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling, Plant Cell 14 (2002) 343–357.
[9] H. Abe, T. Urao, T. Ito, M. Seki, K. Shinozaki, K. Yamaguchi-Shinozaki, Arabidopsis AtMyc2 (bHLH) and AtMyb2 (MYB) function as transcriptional activators in abscisic acid signaling, Plant Cell 15 (2003) 63–78.
[10] H. Sakamoto, K. Maruyama, Y. Sakuma, T. Meshi, M. Iwabuchi, K. Shinozaki, K. Yamaguchi-Shinozaki, Arabidopsis Cys2/His-type

zinc-finger proteins function as transcription repressors under drought, cold, and high-salinity stress conditions, Plant Physiol. 136 (2004) 2734–2746.
[11] D. Cook, S. Fowler, O. Fiehn, M.F. Thomashow, A prominent role for the CBF cold response pathway in configuring the low-temperature metabolome of Arabidopsis, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 15243–15248.
[12] S. Fowler, M.F. Thomashow, Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway, Plant Cell 14 (2002) 1675–1690.
[13] X. Zhang, S.G. Fowler, H. Cheng, Y. Lou, S.Y. Rhee, E.J. Stockinger, M.F. Thomashow, Freezing-sensitive tomato has a functional CBF cold response pathway, but a CBF regulon that differs from that of freezing-tolerant Arabidopsis, Plant J. 39 (2004) 905–919.
[14] G. Inan, Q. Zhang, P. Li, Z. Wang, Z. Cao, H. Zhang, C. Zhang, T.M. Quist, S.M. Goodwin, J. Zhu, H. Shi, B. Damsz, T. Charbaji, Q. Gong, S. Ma, M. Fredricksen, D.W. Galbraith, M.A. Jenks, D. Rhodes, P.M. Hasegawa, H.J. Bohnert, R.J. Joly, R.A. Bressan, J.K. Zhu, Salt cress. A halophyte and cryophyte Arabidopsis relative model system and its applicability to molecular genetic growth and development of extremophiles, Plant Physiol. 135 (2004) 1718–1737.
[15] T. Taji, M. Seki, M. Satou, T. Sakurai, M. Kobayashi, K. Ishiyama, Y. Narusaka, M. Narusaka, J.K. Zhu, K. Shinozaki, Comparative genomics in salt tolerance between Arabidopsis and Arabidopsis-related halophyte salt cress using Arabidopsis microarray, Plant Physiol. 135 (2004) 1697–1709.
[16] R. Weisz, J. Kaminski, S. Smilowitz, Water-deficit effects on potato leaf growth and transpiration—utilizing fraction extractable soil-water for comparison with other crops, Am. Potato J 71 (1994) 829–840.
[17] P.M.K. Deblonde, J.F. Ledent, Effects of moderate drought on green leaf number, stem height, leaf length and tuber yield of potato cultivars, Eur. J. Agron. 14 (2001) 31–41.
[18] P.S. Basu, A. Sharma, I.D. Garg, N.P. Sukumaran, Tuber sink modifies photosynthetic response in potato under water stress, Environ. Exp. Bot. 42 (1999) 25–39.
[19] L. Dalla Costa, G. Delle Vedov, G. Gianquinto, R. Giovanardi, A. Peressotti, Yield, water use efficiency and nitrogen uptake in potato: influence of drought stress, Potato Res. 40 (1997) 19–34.
[20] D. Levy, Osmotic potential of potatoes subjected to a single cycle of water deficit, Potato Res. 35 (1992) 17–24.
[21] R.D. Wolfinger, G. Gibson, E.D. Wolfinger, L. Bennet, H. Hamadeh, P. Bushel, C. Afshari, R.S. Paules, Assessing gene significance from cDNA microarray expression data via mixed models, Comput. Biol. 8 (2001) 625–637.
[22] W. Jin, M.R. Riley, R.D. Wolfinger, K.P. White, G. Passador-Gurgel, G. Gibson, The contributions of sex, genotype and age to transcriptional variance in *Drosophila melanogaster*, Nat. Genet. 29 (2001) 389–395.
[23] C. Stasolla, L. van Zyl, U. Egertsdotter, D. Craig, W. Liu, R.R. Sederoff, The effects of polyethylene glycol on gene expression of developing white spruce somatic embryos, Plant Physiol. 131 (2003) 49–60.
[24] A.A. Sioson, S.P. Mane, P. Li, W. Sha, L.S. Heath, H.J. Bohnert, R. Grene, The statistics of identifying differentially expressed genes in Expresso and TM4: a comparison, BMC Bioinform. 7 (2006), doi:10.1186/1471-2105-7-215.
[25] S. Dzeroski, N. Lavrac (Eds.), Relational Data Mining, Springer, Berlin, 2001.
[26] A. Sioson, J.I. Watkinson, C. Vasquez-Robinet, M. Ellis, M. Shukla, D. Kumar, N. Ramakrishna, L.S. Heath, R. Grene, B.I. Chevone, K. Kafadar, L.T. Watson, Expresso and chips: creating a next generation microarray experiment management system, in: Proceedings of the Next Generation Software Workshop, 17th International Parallel and Distributed Processing Symposium, Nice France, IEEE Computer Society, Los Angeles, CA, 2003.
[27] M. Stonebraker, D. Kemnitz, The POSTGRES next-generation database management system, Commun. ACM 34 (1991) 78–92.
[28] S. Muggleton, Scientific knowledge discovery using inductive logic programming, Commun. ACM 42 (1999) 42–46.
[29] S.K. Kurdistani, S. Tavazoie, M. Grunstein, Mapping global histone acetylation patterns to gene expression, Cell 117 (2004) 721–733.
[30] F. Eymery, P. Rey, Immunocytolocalization of CDSP32 and CDSP34, two chloroplastic drought-induced stress proteins in *Solanum tuberosum* plants, Plant Physiol. Biochem. 37 (1999) 305–312.
[31] P. Bueno, A. Piqueras, J. Kurepa, A. Savoure, N. Verbruggen, M. Van Montagu, D. Inze, Expression of antioxidant enzymes in response to abscisic acid and high osmoticum in tobacco BY-2 cell cultures, Plant Sci. 138 (1998) 27–34.
[32] N. Smirnoff, Plant resistance to environmental stress, Curr. Opin. Biotechnol. 9 (1998) 214–219.
[33] P. Coyle, J.C. Philcox, L.C. Carey, A.M. Rofe, Metallothionein: the multipurpose protein, Cell. Mol. Life Sci. 59 (2002) 627–647.
[34] S.E. Theocharis, A.P. Margeli, A. Koutselinis, Metallothionein: a multifunctional protein from toxicity to cancer, Int. J. Biol. Marker. 18 (2003) 162–169.
[35] K. Ghoshal, S. Majumder, Z. Li, T.M. Bray, S.T. Jacob, Transcriptional induction of metallothionein-I and -II genes in the livers of Cu, Zn-superoxide dismutase knockout mice, Biochem. Biophys. Res. Commun. 264 (1999) 735–742.
[36] A. Butt, C. Mousley, K. Morris, J. Beynon, C. Can, E. Holub, J.T. Greenberg, V. Buchanan-Wollaston, Differential expression of a senescence-enhanced metallothionein gene in Arabidopsis in response to isolates of *Perenospera parasitica* and *Pseudomonas syringae*, Plant J. 16 (1998) 209–221.
[37] S. Navabpour, K. Morris, R. Allen, E. Harrison, S.A.-H. Mackerness, V. Buchanon-Wollaston, Expression of senescence-enhanced genes in response to oxidative stress, J. Exp. Bot. 54 (2003) 2285–2292.
[38] S. Karpinski, H. Reynolds, B. Karpinska, G. Wingsle, G. Creissen, P. Mullineauz, Systemic signaling and acclimation in response to excess excitation energy in Arabidopsis, Science 284 (1999) 654–657.
[39] M. Havaux, K. Kloppstech, The protective functions of carotenoid and flavonoid pigments against visible radiation at chilling temperature investigated in Arabidopsis *npq* and *tt* mutants, Planta 213 (2001) 953–966.
[40] K.S. Gould, J. McKelvie, K.R. Markham, Do anthocyanins function as antioxidants in leaves? Imaging of H_2O_2 in red and green leaves after mechanical injury, Plant Cell Environ. 25 (2002) 1261–1269.
[41] J.L. Donahue, C.M. Okpodu, C.L. Cramer, E.A. Grabau, R.G. Alscher, Responses of antioxidants to paraquat in pea leaves, Plant Physiol. 113 (1997) 249–257.
[42] C. Zhou, H. Labbe, S. Sridha, L. Tian, M. Latoszek-Green, Z. Yang, D. Brown, B. Miki, K. Wu, Expression and function of HD2-type histone deacetylases in Arabidopsis development, Plant J. 38 (2004) 715–724.
[43] J.C. Reyes, M.I. Muro-Pastor, F.J. Florencio, The GATA family of transcription factors in Arabidopsis and rice, Plant Physiol. 134 (2004) 1718–1732.
[44] S. Bak, F.E. Tax, K.A. Feldman, D.W. Galbraith, R. Feyerweisen, CYP83B1, a cytochrome p450 at the metabolic branch point in auxin and indole glucosinolate biosynthesis in Arabidopsis, Plant Physiol. 113 (2001) 101–112.
[45] Y. Miyazawa, N. Nakajima, T. Abe, A. Sakai, S. Fujioka, S. Kawano, T. Kuroiwa, S. Yoshida, Activation of cell proliferation by brassinolide application in tobacco BY-2 cells: effects of brassinolide on cell proliferation, cell-cycle related gene expression and organeller DNA contents, J. Exp. Bot. 54 (2003) 2669–2678.
[46] H. Savenstrand, M. Brosche, A. Strid, Ultraviolet-B signaling: Arabidopsis brassinosteroid mutants are defective in UV-B regulated defence gene expression, Plant Physiol. Biochem. 42 (2004) 687–694.
[47] G.Z. Yang, J.A. Shen, J. Yasaki, M. Ishikawa, S. Shimatani, N. Kishimoto, H. Matsumoto, S. Komatsu, Microarray analysis of brassinosteroid- and giberrelin-regulated gene expression in rice seedlings, Mol. Genet. Genom. 271 (2004) 468–478.
[48] W. Sun, M. Van Montague, N. Verbruggen, Small heat shock proteins and stress tolerance in plants, Biochim. Biophys. Acta 1577 (2002) 1–9.
[49] E. Basha, G.J. Lee, L.A. Breci, A.C. Hausrath, N.R. Buan, K.E. Giese, E. Vierling, The identity of proteins associated with a small heat

shock protein during heat stress in vivo indicates that these chaperones protect a wide range of cellular functions, J. Biol. Chem. 279 (2004) 7566–7575.

[50] J.L. Garrett, E. Vierling, A small heat shock protein cooperates with heat shock protein 70 systems to reactivate a heat-denatured protein, Plant Physiol. 122 (2000) 189–198.

[51] W. Sun, C. Bernard, B. van de Cotte, M. Van Montague, N. Verbruggen, At-HSP17.6A, encoding a small heat shock protein in Arabidopsis, can enhance osmotolerance upon overexpression, Plant J. 27 (2001) 407–415.

[52] I. Kurek, K. Aviezer, N. Erel, E. Herman, A. Brieman, The wheat peptidyl–prolyl *cis–trans* isomerase FKBP77 is heat induced and developmentally regulated, Plant Physiol. 119 (1999) 693–704.

[53] K. Jackson, D. Soll, Mutations in a new Arabidopsis cyclophilin disrupt its interaction with protein phosphatase 2A, Mol. Genet. Genom. 262 (1999) 830–838.