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Molecular and physiological adaptation to prolonged drought stress in the leaves of two Andean potato genotypes

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Abstract. Responses to prolonged drought and recovery from drought of two South American potato (*Solanum tuberosum* L. ssp. *andigena* (Juz & Buk) Hawkes) landraces, Sullu and Ccompis were compared under field conditions. Physiological and biomass measurements, yield analysis, the results of hybridisation to a potato microarray platform (44 000 probes) and metabolite profiling were used to characterise responses to water deficit. Drought affected shoot and root biomass negatively in Ccompis but not in Sullu, whereas both genotypes maintained tuber yield under water stress. Ccompis showed stronger reduction in maximum quantum yield under stress than Sullu, and less decrease in stomatal resistance. Genes associated with PSII functions were activated during recovery in Sullu only. Evidence for sucrose accumulation in Sullu only during maximum stress and recovery was observed, in addition to increases in cell wall biosynthesis. A depression in the abundance of plastid superoxide dismutase transcripts was observed under maximum stress in Ccompis. Both sucrose and the regulatory molecule trehalose accumulated in the leaves of Sullu only. In contrast, in Ccompis, the raffinose oligosaccharide family pathway was activated, whereas low levels of sucrose and minor stress-mediated changes in trehalose were observed. Proline, and expression of the associated genes, rose in both genotypes under drought, with a 3-fold higher increase in Sullu than in Ccompis. The results demonstrate the presence of distinct molecular and biochemical drought responses in the two potato landraces leading to yield maintenance but differential biomass accumulation in vegetative tissues.

Additional keywords: *Solanum tuberosum*, ssp. *andigena*, metabolomics, osmoprotectants, transcriptomics, trehalose.

Introduction

Demand for potato, the fourth most important crop worldwide, as a food source and industrial raw material is increasing. Many of the world's poorest households in several regions, in particular South America, will increasingly depend on this crop for subsistence and income generation. Also, in many of these regions drought is a major threat to productivity and food security. Drought is a primary abiotic stress that not only reduces yield, but also negatively affects product quality and decreases reliance on predictions about harvestable biomass from year-to-year. Severe water deficit impacts on physiological processes such as photosynthesis, growth, and the subsequent metabolism and partitioning of carbohydrates that are crucial for realising the yield potential as well as affecting product quality.

Plants perceive and respond to drought stress by altering regulatory circuits in transcription and protein expression dynamics in ways that impose changes on biochemical pathways, and then alter physiological and developmental processes. Physiologically, the inevitable decline in tissue water during drought initiates pathways that tend to advance root growth, while stomatal resistance increases as a water-saving measure that then negatively affects CO_2-fixation and the photosynthetic machinery. In potato, drought has been found to differentially alter dry matter partitioning among cultivars (Jefferies 1993), and the ability to generate greater

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aboveground biomass has been correlated with superior drought tolerance (Schittenhelm *et al*. 2006). Extensively studied mechanisms of plant molecular responses to drought stress include water uptake and retention, differential growth maintenance, and minimising damage or the necessity of repair (Ingram and Bartels 1996; Seki *et al*. 2002; Rabbani *et al*. 2003; Rizhsky *et al*. 2004).

Plant drought tolerance is tightly linked both physiologically and biochemically to the sensing of oxidative load and redox-dependent signalling (Geigenberger *et al*. 2005) that affects changes in stress hormones and triggers signalling and defence pathways (Liu *et al*. 1998; Rizhsky *et al*. 2002; Rook and Bevan 2003; Bray 2004; Bartels and Sunkar 2005; Mittler 2006; Wong *et al*. 2006; Seki *et al*. 2007). In photosynthetic tissues, carbohydrate metabolism responds to water deficit with changes in carbohydrate partitioning (Geigenberger *et al*. 2004), involving sucrose phosphate synthase, modulation of starch biosynthesis, and redox sensing as key elements (Quick *et al*. 1989). Genes and proteins are activated that are often associated with the biosynthesis of protective compounds and osmolytes, such as sucrose, other (complex) sugars or sugar alcohols, or proline and other N-containing compounds such as citrulline (Uno *et al*. 2000; Yokota *et al*. 2002; Zhu 2002; Himmelbach *et al*. 2003; Oono *et al*. 2003; Verslues and Zhu 2005; Rook *et al*. 2006; Watkinson *et al*. 2006; Yamaguchi-Shinozaki and Shinozaki 2006). Although the biochemical nature of the osmolyte is species- or family-specific, accumulation pathways in general include a redox-dependent component in which trehalose 6-phosphate appears to play an intermediary signalling function connecting changes in redox homeostasis with metabolic adjustments (Garg *et al*. 2002; Avonce *et al*. 2004; Kolbe *et al*. 2005).

Previously, resistance to drought stress in the leaves of Andean potato accessions was correlated with specific patterns of gene expression involving genes encoding antioxidant, flavonoid, and carbohydrate metabolism, using relatively small cDNA microarrays (Watkinson *et al*. 2006; Schafleitner *et al*. 2007). Here, we employ a novel potato-specific transcript platform, composed of 44 000 features deposited as oligonucleotides, to analyse drought and drought recovery responses in leaves of two *Solanum tuberosum* L. ssp. *andigena* (Juz & Buk) Hawkes genotypes grown in the field at high altitude. We place the transcript response in the context of physiological parameters and metabolite measurements.

Materials and methods

Plant material and culture conditions

Two field plots (5×25 m), located at the CIP field station La Victoria in Huancayo (Peru) at 3200 m above sea level, were prepared with humic soil (pH 4) and equipped with nets and roofs made of transparent plastic. Plastic foil barriers prevented uncontrolled water inflow from the sides as well as from below, resulting in a soil depth of 50 cm.

Sprouted seed potatoes of the clones Sullu and Ccompis were sown on 14 October 2005 in blocks of five plants in a randomised complete block design with four replications. The blocks cultivated with Ccompis and Sullu were separated by blocks planted with the potato varieties SA2563 (*Solanum tuberosum* L. ssp. *andigena* (Juz & Buk) Hawkes), Cceccorani and Puca Pishgush (*Solanum stenotonum* (Juz. and Bukasov) ssp. *goniocalyx*) and Perricholi (*Solanum tuberosum* L ssp. *tuberosum* × *S. tuberosum* ssp. *andigena*). The distances between rows was 1 m and spaces between plants in a row was 30 cm.

The plots were fertilised with 100 : 160 : 120 kg ha^{-1} N : P : K before planting and with 100 kg ha^{-1} nitrogen 30 days after planting. Fungicide and insecticide sprays (Dithane M-45 [Mancoceb 80%], Dow AgroSciences de Columbia S.A., Soledad Atlantico, Columbia; Antracol WP 70 [Proponeb 70%], Bayer CropScience S.A., Santa Fe de Bogota, Columbia; Acrobat MZ [Dimetomorf 9% + Mancozeb 60%], BASF Química Columbiana S.A., Bogota, Columbia; Fitoraz WP 76 (Propineb 70% + Cymoxanil 6%], Bayer CropScience S. A.; Hortiquim 50 EC [Permetrin 50 g L^{-1}], Hockley International, Stockport, UK; Arribo [Cypermetrin], FMC Corporation, Philadelphia, PA, USA) were applied according to suppliers' recommendations.

In the drought plot, irrigation was stopped on day 45 after planting (28 November 2005) and plants were exposed to drought for 59 days, until 26 January 2006. The control plot was irrigated throughout the growing period and the soil water potential was kept between 0 and −0.02 MPa.

Soil water content

Soil water potentials between 0 and −0.2 MPa were determined tensiometrically (Watermark; Campbell Scientific, Logan, UT, USA). In parallel and below this soil water potential, soil water content was determined as gram of water per gram of soil for a soil profile from 0 to 50 cm depth for each experimental block, and values were averaged over the experimental plots.

Relative leaf water content

Relative water content (RWC) was tested weekly after drought onset according to Tourneux *et al*. (2003) using the 3rd and 5th leave of three replicate plants of each block.

Yield analysis

Biomass distribution was determined on the three central plants of three blocks of each clone 133 days after planting, on 15 February 2006. Dry mass of the plant material was measured after oven drying at 60°C for 3 days. Tuber yield was determined after the final harvest, 145 days after planting, on 8 March 2006.

Stomatal conductance

Stomatal conductance was measured using an AP4 porometer (Delta-T Devices, Cambridge, UK) between 0900 and 1000 hours every second day on the petiole of the third fully expanded leaf of the main stem of the plants. Separate measurements were done on three plants of each of the three repeated blocks in both treatments and the resulting values of each week were averaged for each treatment. Additionally, diurnal time course studies of stomatal behaviour were performed on days 30 and 52 after drought onset, and 27 days after recuperation irrigation at 0600, 0900, 1200 and 1500 hours on the third leaf of each of three replicate plants of three replicate plots.

Chlorophyll fluorescence

Chlorophyll fluorescence measurements were done on the same leaves and same time points as the stomatal conductance analysis using a Hansatech plant efficiency analyser (Hansatech, King's Lynn, Norfolk, UK) using a dark adaptation time of 30 min. F_v/F_M as well as F_0 was determined as described by Maxwell and Johnson (2000).

Sampling of leaves for gene expression and metabolite analysis

Samples were taken from two biological replications for both drought-exposed and control plants of each clone. The third fully-expanded leaf of the main stem of the three central plants of a block were pooled and shock frozen in liquid nitrogen for each sample, and used for gene expression and metabolite analysis. Total RNA was extracted from leaves by the TRIZOL method (Invitrogen, Carlsbad, CA, USA), precipitated and lyophilised. For metabolite analysis, the shock frozen material was lyophilised.

Complementary DNA for real-time PCR was synthesised from 3 μg total RNA with superscript III reverse transcriptase (Invotrogen) using 200 ng random hexamer primers and 50 min synthesis time at 50°C. PCR primers were designed based on tentative consensus sequences of TIGR StGI (see Table S1 in the accessory publication available form the online version of *Functional Plant Biology*) using the Vector NTI software (Informax, Invitrogen). Real-time PCR was performed with 50 ng cDNA using DyNAmo SYBR-Green qPCR Kit (Finnzymes) in 10 μL reaction volumes on a Chromo 4 Four-Colour Real-Time System (MJ-research), with 0.25 μM primer end concentration and the following cycling steps: initial denaturation for 2 min at 94°C, followed by 40 cycles with 15 s 94°C, 20 s 55°C and 20 s at 72°C and 10 min terminal elongation at 72°C. Relative quantification of transcript abundance in treated and control plants was done according to Pfaffl *et al*. (2002) using the potato Cytochrome *b* oxidase gene (TIGR Id TC116542) as internal standard to correct for different amounts of RNA input for cDNA synthesis (Weller *et al*. 2000). At least three technical repeats per biological repeat were analysed. Standard curves for real-time PCR amplification for all primers have been established using 10-fold dilutions of purified PCR fragments in concentrations between 10 pg and 0.1 fg. The curves obtained allowed the determination of PCR efficiency according to Pfaffl *et al*. (2002).

Metabolite profiling

Polar phase extractions from 10−15 mg of dried potato leaves were derivatised (Fiehn *et al*. 2000; Roessner *et al*. 2000). Sample material (1−2 μL) of sample was injected with an 8 : 1 split ratio and analysed on an HP5890 gas chromatograph equipped with a HP5973 mass selective detector (Agilent Inc., Palo Alto, CA, USA). Gas chromatography was conducted with a 30 m SPB-50 column with a 0.25 mm internal diamter and 0.25 μm film thickness (Supelco, Belfonte, CA, USA). The injection temperature was 230°C and the interface was 250°C. The carrier gas was helium set at a constant flow rate of 1 mL min^{-1}. The temperature program was: 70°C for 5 min, then 5°C min^{-1} up to 310°C, and 310°C for 10 min. Spectra were evaluated according to Lozovaya *et al*. (2006).

Datasets contained five replicates per sample and were statistically analysed by *t*-test and 1-way ANOVA using the algorithm incorporated into Microsoft Excel 2002 (Microsoft Corporation, Seattle, WA, USA). Differences were determined to be statistically significant at $P < 0.05$. Standard errors were calculated for all replicate samples. Additionally, ANOVA and Fisher's l.s.d. were performed on metabolites for which complete data were available.

RNA isolation

RNA was isolated from ~2 g of leaf tissue, each sample being obtained from the leaves of three plants as described above, using a phenol-based method retrieved from the TIGR website (http://www.tigr.org/tdb/potato/microarray_SOPs.shtml, accessed 3 May 2007).

Microarray hybridisations

RNA from leaves at the point of maximum stress (25 days after water withheld) were hybridised on custom printed *in situ* synthesised 60-mer oligonucleotide microarrays containing 44 000 genes (Agilent Technologies, Santa Clara, CA). Reagents were purchased from Agilent; hybridisation was followed according to the manufacturer's instructions.

Analysis of microarray data

Data were analysed by the method utilised in previous work, (Watkinson *et al*. 2003; Li *et al*. 2006*a*; Sioson *et al*. 2006). After normalisation by Lowess and quantitation, the sensitivity of individual genes to the experimental treatments is estimated using a two-stage statistical analysis (Wolfinger *et al*. 2001). The first stage removes global effects while the second estimates the interaction between individual genes and experimental treatments. A significant effect of the treatment was set at the 95% confidence level.

Identification of Arabidopsis *orthologues of potato sequences*

As a first step, the potato oligonucleotides (60-mers) sequences present on the array were compared with the cDNA sequences present in the Sol Genomics Network (SGN; http://www.sgn.cornell.edu/, accessed 7 February 2007) and in the TIGR potato database (STGI release 11 data; http://www.tigr.org/tdb/potato/, accessed 18 September 2006) using standalone NCBI-BLAST v2.2.14 (Altschul *et al*. 1997). The top cDNA sequence hit of at least a 50 base-pair match to a potato oligonucleotide was considered to be the representative gene for that oligonucleotide. Sequences corresponding to the spotted oligonucleotides were used to identify putative orthologues in *Arabidopsis thaliana* (L.) Heynh. (AGI; TIGR6 release) using blastx. Only AGI hits with e-values of at least 1e-10 were considered. In addition, only cases of unambiguous matching of one AGI number to one potato oligonucleotide were used for further analysis of gene expression.

Results

Physiological responses and biomass partitioning

Soil water content (SWC) during the trial in the irrigated plots remained between 40 and 47% and decreased in the drought stress plot to 36% during the first 17 days of withholding water, and reached 21% by day 60. Fifteen days after re-irrigating (15 DR), the SWC increased to 34 and 35% at harvest, on day 145 after planting [100 days after onset of drought (DD), 40 DR].

Total biomass production was higher in Ccompis than in Sullu under both drought and control conditions (Table 1). Lower shoot and root biomass were observed in unstressed Sullu compared with Ccompis (94 *v.* 244 mg g^{-1} fresh wt for shoot and 8.5 *v.* 17.5 for root). Under drought stress, both shoot and root biomass were substantially lower in drought stressed Ccompis plants than in the corresponding controls (135 *v.* 234 and 12 *v.* 17, respectively), whereas drought had little effect on shoot or root biomass in the more resistant Sullu. Harvest index was higher in Sullu than in Ccompis, i.e. the smaller Sullu plants partitioned relatively more biomass to tubers. Neither tuber number nor dry weight was significantly influenced by drought in either line. Both lines maintained their relative leaf water content under drought, a slight decrease observed in both lines was statistically insignificant.

Significant differences in weekly means of maximum quantum yield of PSII between irrigated and drought exposed plants measured during the morning appeared under drought in Ccompis only, and only after 30 days of drought, suggesting progressive drought-related photoinhibition in the leaves of this line. This was not observed in Sullu under drought stress, where differences in weekly means of chlorophyll fluorescence were similar in drought and control plants (Fig. 1). However, in daily time course measurements, a decrease in maximum quantum yield appeared in both clones late under drought, when during noon and afternoon maximum quantum yield dropped significantly in Ccompis to 94–95% of control values, and reached in Sullu values of 82–86% of control plants. Six days after re-irrigation, maximum quantum yield in drought-exposed Sullu plants had recovered completely, and Ccompis maximum quantum yield during noon and afternoon remained at low drought values, i.e. showed no recovery.

Weekly means of stomatal resistance increased in both clones from week 2 after drought onset with higher mean resistance in Sullu than in Ccompis (Fig. 2), and declined to low levels during recovery. Irrigated plants of both clones showed similar diurnal changes of stomatal behaviour. In contrast, plants exposed to

Table 1. Effects of 60 days of drought stress on biomass partitioning in two Andean potato genotypes, Ccompis and Sullu

Plant part	Genotype	Drought (g dry weight)	s.e.	Control (g dry weight)	s.e.	Loss (%)
Tuber	Ccompis	69	31	77	38	13
	Sullu	81	40	94	32	14
Leaf	Ccompis	85	27	124	61	31
	Sullu	47	22	58	26	19
Shoot	Ccompis	135	49	245	90	45
	Sullu	100	58	95	46	0
Root	Ccompis	12	4	18	8	31
	Sullu	9	4	9	6	0
Total biomass	Ccompis	301	94	463	178	35
	Sullu	237	108	255	102	7
Harvest index	Ccompis	0.23	–	0.17	–	–
	Sullu	0.34	–	0.37	–	–

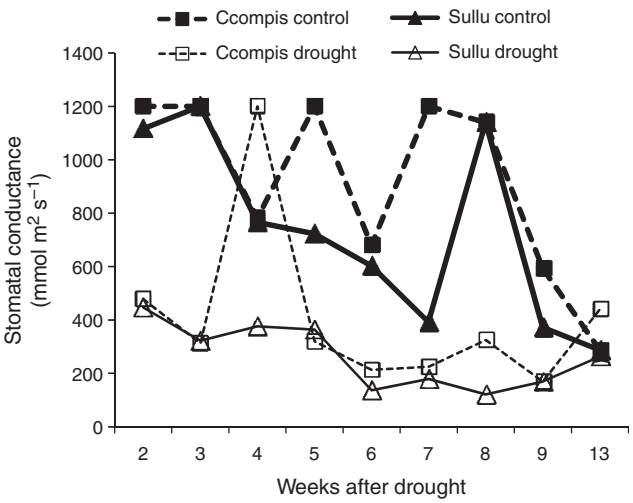

Fig. 1. Weekly means of stomatal conductance in two Andean potato genotypes, Ccompis and Sullu, under drought and control conditions. Weeks 2–8: after drought onset. Weeks 9 and 13: after recuperation irrigation. Differences of stomatal resistance between drought and control treatment were significant for Ccompis at $P = 0.05$ in weeks 2, 3 and 8 and at $P = 0.01$ in weeks 5, 6, 7 after drought and in week 1 after recovery. In Sullu, differences between treatments were significant at $P = 0.05$ in weeks in week 4, 6 and 8 and at $P = 0.01$ in week 3.

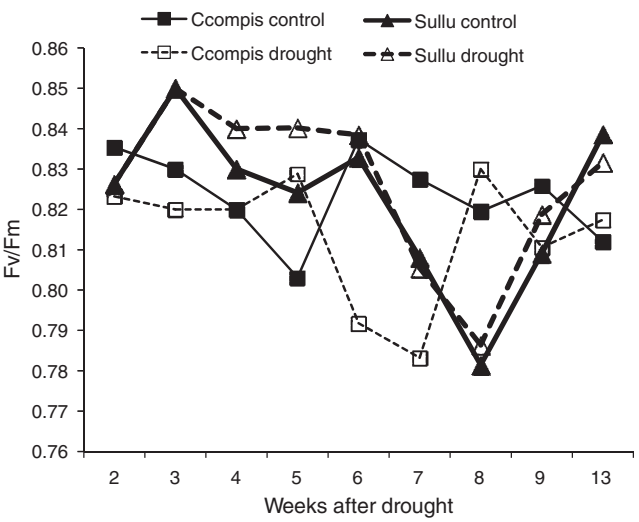

Fig. 2. Weekly means of maximum quantum yield of photosynthesis in Ccompis and Sullu under drought and control conditions. Weeks 2–8: after drought onset. Weeks 9 and 13: after recuperation irrigation. Differences of quantum yield between drought and control treatment were significant for Ccompis at $P = 0.05$ in week 7 after drought.

drought conditions exhibited line-specific diurnal patterns, with the stomata of Sullu closing around 1000 h and opening again in the afternoon, whereas stomatal resistance remained high in Ccompis throughout.

Under irrigated conditions, stomatal resistance was negatively correlated with biomass production and yield in both clones ($r^2 = 0.9116$). This correlation disappeared under drought, indicating that stomatal aperture was no longer a factor contributing to tuber yield. There was no correlation between maximum quantum yield of chlorophyll fluorescence and yield or biomass under irrigated or drought conditions in this long-term drought experiment in the field.

Metabolites

Metabolite profiles by GC−MS of leaves of drought exposed and control plants revealed highly clone-dependent patterns (Tables 2−7, S2, S3, S4). Metabolite values generally showed statistical significance at the 95% confidence levels, with the exception of alanine and valine values among the amino acids, and glucose level in stressed plants were more variable.

For Ccompis, the data showed a clear separation between the profiles of drought-stressed and well watered plants throughout the experiment. In contrast, profiles of drought-exposed and irrigated plants of clone Sullu were similar early under drought (day 45 DD; days after onset of drought) and during recovery (day 75 DD), but not under long-lasting drought (day 60 DD), when differences between drought-exposed and control plants were particularly pronounced.

Under drought, organic acids, amino acids, sugar, sugar alcohols and amines accumulated in leaf tissue in a clone dependent manner. Generally, Sullu showed earlier and significantly greater solute accumulation than Ccompis, with the exception of fructose. In both lines, solute accumulation was greatest on day 60 after drought (AD), the time point corresponding to maximum drought stress. Sullu was able to sustain metabolite pools under drought stress much longer than Ccompis, a measure of resistance to the altered condition, whereas metabolites in Ccompis were profoundly affected by drought and significantly reduced.

Organic acids

The organic acid levels in both clones are presented in Tables 2 and 3. Under well watered conditions, levels of caffeic acid, citric acid, fumaric acid, pyruvic acid, shikimic acid and threonic acid were significantly higher in Sullu, and Ccompis contained significantly more malic acid, quinic acid and succinic acid. Drought caused an increase of total concentrations of organic acids in Sullu (ascorbic acid, fumaric and shikimic acid were down in Sullu under drought) and a decrease in Ccompis (pyruvic, quinic and shikimic acid increased in Ccompis), the latter because of a significant drop in malic acid amount accompanied by significant decreases in fumaric acid and threonic acid. In contrast, significant increases in malic acid and citric acid were mainly responsible for the increases in organic acid concentration in Sullu under drought. Drought led to increases in citric acid, quinic acid and pyruvic acid levels in both clones. Ccompis also accumulated significant levels of isoascorbic acid, oxalic acid and hydrobenzoic acid, which were not detectable in Sullu, and exhibited significantly elevated concentrations of ascorbic acid and caffeic acid as well as decreased levels of succinic acid in comparison to control plants.

Sugars and sugar alcohols

In ~50% of sugars measured, Sullu had significanlty higher content than in Ccompis, regardless of the sampling time point

Table 2. Effects of drought stress on organic acid levels in the leaves of two Andean potato genotypes, Sullu and Ccompis

Data were analysed between treatments and between two Andean potato genotypes and are shown as mean ± s.e. * in treated samples indicates statistically significant difference between drought-stressed and control samples ($P \leq 0.05$). * in control samples indicates that the metabolite could not be determined in drought-stressed sample. † in Sullu indicates statistically significant difference between Sullu and Ccompis at that stage/growth condition ($P \leq 0.05$). † in Ccompis means the metabolite could not be determined in Sullu. DW, dry weight; n.d., not determined

Organic acids	DW basis	45 days control		45 days drought		60 days control		60 days drought	
		Sullu	Ccompis	Sullu	Ccompis	Sullu	Ccompis	Sullu	Ccompis
a-Ketoglutaric acid	$\mu g\ g^{-1}$	487 ± 35†	63 ± 3*	306 ± 20*†	n.d.	474 ± 2†	51 ± 4*	96 ± 11*	n.d.
Ascorbic acid	$\mu g\ g^{-1}$	148 ± 17†	25 ± 1	181 ± 25†	38 ± 1*	319 ± 7†	28 ± 2	293 ± 19	94.7 ± 11*
Caffeic acid	$\mu g\ g^{-1}$	602 ± 1†	392 ± 49	600 ± 24	511 ± 27	1086 ± 234†	441 ± 17	1213 ± 146	806 ± 64*
Citric acid	$mg\ g^{-1}$	6.52 ± 0.56†	3.64 ± 0.05	7.56 ± 0.49†	3.31 ± 0.15	3.89 ± 0.32†	1.63 ± 0.12	8.11 ± 0.65*	7.83 ± 0.47*
Fumaric acid	$mg\ g^{-1}$	2.59 ± 0.03†	1.10 ± 0.07	1.88 ± 0.12*†	0.50 ± 0.03*	1.05 ± 0.10	1.10 ± 0.26	0.44 ± 0.04*	0.52 ± 0.04*
Isocitric acid	$\mu g\ g^{-1}$	911 ± 25†	n.d.	1010 ± 98	n.d.	1153 ± 37†	n.d.	1294.7 ± 55	n.d.
Isoascorbic acid	$\mu g\ g^{-1}$	n.d.	n.d.	n.d.	23 ± 1*†	n.d.	n.d.	n.d.	38.0 ± 1*†
Malic acid	$mg\ g^{-1}$	17 ± 1†	1.9 ± 0.6	20.7 ± 1.3	9.37 ± 0.62*	18.79 ± 0.41†	3.50 ± 0.26	25.4 ± 1.4*	21.3 ± 1.30*
Malonic acid	$\mu g\ g^{-1}$	12 ± 1	8683 ± 834	20 ± 0*†	12725 ± 504	18 ± 3†	8500 ± 1847	34 ± 1	11961 ± 436
Oxalic acid	$\mu g\ g^{-1}$	103 ± 6*	59 ± 5	n.d.	85 ± 2*†	142 ± 9*†	68 ± 3	n.d.	145 ± 11*†
p-Hydroxybenzoic acid	$\mu g\ g^{-1}$	n.d.	n.d.	n.d.	87 ± 5*†	n.d.	n.d.	n.d.	109 ± 5*†
Pyruvic acid	$\mu g\ g^{-1}$	493 ± 59†	38 ± 3	572 ± 28†	67 ± 5*	412 ± 49†	38 ± 2	731.8 ± 55*	76 ± 3*
Quinic acid	$mg\ g^{-1}$	1.93 ± 0.09	1.91 ± 0.15	1.87 ± 0.15	1.83 ± 0.15	1.51 ± 0.86†	2.23 ± 0.15	1.65 ± 0.86	3.71 ± 0.07*
Salicylic acid	$\mu g\ g^{-1}$	n.d.	n.d.	n.d.	12 ± 0*†	n.d.	n.d.	n.d.	33 ± 1*
Shikimic acid	$\mu g\ g^{-1}$	812 ± 53†	527 ± 35	698 ± 30	572 ± 40	768.3 ± 14†	535 ± 21	807.6 ± 49	689 ± 9*
Succinic acid	$mg\ g^{-1}$	1.02 ± 0.07	1.47 ± 0.17	1.10 ± 0.06	1.23 ± 0.06	0.87 ± 0.38†	1.70 ± 0.65	730.5 ± 65	0.98 ± 0.12*
Threonic acid	$mg\ g^{-1}$	2.5 ± 0.2†	0.80 ± 0.09	2.14 ± 0.14†	0.45 ± 0.02*	1.67 ± 0.62†	0.82 ± 0.06	0.37 ± 0.03*	0.73 ± 0.11
Total	$mg\ g^{-1}$	35.3 ± 1.4	37.8 ± 1.1	38.6 ± 1.4	30.8 ± 0.8	32.2 ± 0.6	52.2 ± 3.2	41.1 ± 1.6	49.1 ± 1.4

Table 3. Organic acid levels in the leaves of two Andean potato genotypes, Sullu and Ccompis, upon recovery after drought stress

Data were analysed between treatments and between two Andean potato genotypes and are shown as mean ± s.e. * in treated samples indicates statistically significant difference between drought-stressed and control samples ($P \leq 0.05$). * in control samples indicates that the metabolite could not be determined in drought-stressed sample. † in Sullu indicates statistically significant difference between Sullu and Ccompis at that stage/growth condition ($P \leq 0.05$). † in Ccompis means the metabolite could not be determined in Sullu. DW, dry weight; n.d., not determined

Organic acids	DW basis	75 days control		60 days drought, 15 recovery	
		Sullu	Ccompis	Sullu	Ccompis
a-Ketoglutaric acid	$\mu g\,g^{-1}$	584 ± 44†	80 ± 3	766 ± 60†	11 ± 0*
Ascorbic acid	$\mu g\,g^{-1}$	268 ± 25†	32 ± 1	117 ± 12*†	150 ± 9*
Caffeic acid	$\mu g\,g^{-1}$	452 ± 41	588 ± 42	722 ± 45*†	1445 ± 124*
Citric acid	$mg\,g^{-1}$	5.49 ± 0.18†	1.82 ± 0.07	14.83 ± 0.88*†	5.35 ± 0.36*
Fumaric acid	$mg\,g^{-1}$	1.23 ± 0.13	0.91 ± 0.04	1.52 ± 0.12†	0.38 ± 0.04*
Isocitric acid	$\mu g\,g^{-1}$	862 ± 9†	n.d.	930 ± 63†	n.d.
Isoascorbic acid	$\mu g\,g^{-1}$	n.d.	n.d.	n.d.	n.d.
Malic acid	$mg\,g^{-1}$	21.8 ± 0.8†	34.4 ± 0.3	21.3 ± 0.9†	28.5 ± 2.4
Malonic acid	$\mu g\,g^{-1}$	14 ± 1†	22394 ± 482	28.5 ± 2*†	10979.8 ± 1419*
Oxalic acid	$\mu g\,g^{-1}$	189 ± 18†	39 ± 2	91 ± 5*	99 ± 7*†
p-Hydroxybenzoic acid	$\mu g\,g^{-1}$	n.d.	n.d.	n.d.	n.d.
Pyruvic acid	$\mu g\,g^{-1}$	651 ± 71†	67 ± 6	74 ± 38†	76 ± 3
Quinic acid	$mg\,g^{-1}$	1.79 ± 0.04†	2.69 ± 0.14	4.28 ± 0.26*	4.29 ± 0.04*
Salicylic acid	$\mu g\,g^{-1}$	n.d.	n.d.	n.d.	n.d.
Shikimic acid	$\mu g\,g^{-1}$	841 ± 42†	561 ± 28	802 ± 37†	485 ± 35
Succinic acid	$mg\,g^{-1}$	0.82 ± 0.08†	2.21 ± 0.13	1.13 ± 0.06	0.92 ± 0.12*
Threonic acid	$mg\,g^{-1}$	1.44 ± 0.03†	1.14 ± 0.08	1.37 ± 0.09†	0.08 ± 0.15
Total	$mg\,g^{-1}$	36.4 ± 0.8	67.0 ± 3.1	48.7 ± 1.3	53.55 ± 2.8

Table 4. Effects of drought stress on sugar levels in two Andean potato genotypes, Sullu and Ccompis

Data were analysed between treatments and between two Andean potato genotypes and are shown as mean ± s.e. * in treated samples indicates statistically significant difference between drought-stressed and control samples ($P \leq 0.05$). * in control samples indicates that the metabolite could not be determined in drought-stressed sample. † in Sullu indicates statistically significant difference between Sullu and Ccompis at that stage/growth condition ($P \leq 0.05$). † in Ccompis means the metabolite could not be determined in Sullu. DW, dry weight; n.d., not determined

Sugars	DW basis	45 days control		45 days drought		60 days control		60 days drought	
		Sullu	Ccompis	Sullu	Ccompis	Sullu	Ccompis	Sullu	Ccompis
Arabinose	$\mu g\,g^{-1}$	60 ± 3†	n.d.	71 ± 6	98 ± 8*	54 ± 3†	n.d.	116 ± 1*	137 ± 11*
Fructose	$mg\,g^{-1}$	8.9 ± 0.7	10.7 ± 0.7	9.0 ± 0.3	13.3 ± 1.5	9.2 ± 0.3†	12.8 ± 0.6	11.9 ± 0.4*†	19.9 ± 1.7*
Galactose	$mg\,g^{-1}$	2.6 ± 0.3	2.1 ± 0.3	5.6 ± 0.4*†	2.9 ± 0.2	2.3 ± 0.3	2.5 ± 0.1	6.5 ± 0.1*†	4.2 ± 0.7*
Glucose	$mg\,g^{-1}$	12.1 ± 0.5†	8.4 ± 1.1	13.3 ± 0.3*	12.7 ± 1.2	11.3 ± 0.7	11.9 ± 0.4	15.3 ± 0.3*	12.8 ± 1.5
Glucose-6-P	$\mu g\,g^{-1}$	408 ± 4†	63 ± 5	313 ± 20†	111 ± 9*	385 ± 27†	135 ± 7	308 ± 9	297 ± 62*
Isomaltose	$\mu g\,g^{-1}$	186 ± 3†	n.d.	241 ± 25	266 ± 23*	211 ± 9†	n.d.	387 ± 41*	339 ± 2*
Lyxose	$\mu g\,g^{-1}$	n.d.	378 ± 21†	n.d.	718 ± 11*†	n.d.	429 ± 27†	n.d.	652 ± 62*†
Maltose	$\mu g\,g^{-1}$	125 ± 14†	343 ± 20	366 ± 30*	408 ± 25	369 ± 23	326 ± 25	884 ± 38*	674 ± 89*
Melezitose	$\mu g\,g^{-1}$	507 ± 55†	162 ± 4	566 ± 17†	173 ± 7	637 ± 67†	114 ± 9	364 ± 27*	396 ± 40*
Melibiose	$\mu g\,g^{-1}$	402 ± 24†	227 ± 67	1006 ± 54*†	211 ± 3	1338 ± 107†	178 ± 16	4585 ± 502*†	1129 ± 36*
Raffinose	$\mu g\,g^{-1}$	428 ± 20†	n.d.	433 ± 23	379 ± 43*	482 ± 33†	n.d.	525 ± 44†	1528 ± 91*
Rhamnose	$\mu g\,g^{-1}$	74 ± 1†	180 ± 4	76 ± 14	89 ± 4*	69 ± 6†	148 ± 11	83 ± 8	117 ± 7
Ribose	$\mu g\,g^{-1}$	476 ± 21†	760 ± 78	380 ± 39	427 ± 49*	372 ± 26†	705 ± 57	407 ± 24†	807 ± 142
Sorbose	$mg\,g^{-1}$	5.6 ± 0.4†	2.7 ± 0.2	5.8 ± 0.5†	4.3 ± 0.2*	6.0 ± 0.2†	3.6 ± 0.1	7.2 ± 0.1†	9.0 ± 0.7*
Sucrose	$mg\,g^{-1}$	20.7 ± 1.7†	0.4 ± 0.0	21.4 ± 1.6†	0.9 ± 0.0*	21.7 ± 1.4†	2.2 ± 0.1	42.0 ± 1.2*†	6.9 ± 1.4*
Trehalose	$\mu g\,g^{-1}$	579 ± 12†	210 ± 9	1004 ± 25*†	359 ± 10*	679 ± 29†	200 ± 21	1480 ± 84*†	385 ± 16*
Xylose	$\mu g\,g^{-1}$	341 ± 38	279 ± 23	523 ± 6*†	308 ± 5	533 ± 52†	256 ± 11	546 ± 32†	358 ± 10*
Total	$mg\,g^{-1}$	53.4 ± 1.9	27.0 ± 1.4	60.5 ± 1.7	37.6 ± 2.0	55.6 ± 1.7	35.4 ± 0.7	92.5 ± 1.4	59.7 ± 2.9

and treatment (Tables 4 and 5). Nevertheless, relative increases in sugar concentration in comparison to control plants were greater in Ccompis than in Sullu. The largest increase in total sugar concentration in comparison to control plants appeared on day 60 AD. Ccompis contained constitutively higher concentrations of fructose, glucose, lyxose, maltose and ribose, while in Sullu very high sucrose levels were observed. In Sullu, the largest share of sugar accumulation was due to an increase of sucrose in leaves,

Table 5. Sugar levels in the leaves of two Andean potato genotypes, Sullu and Ccompis, upon recovery after drought stress
Data were analysed between treatments and between two Andean potato genotypes and are shown as mean ± s.e. * in treated samples indicates statistically significant difference between drought-stressed and control samples ($P \leq 0.05$). * in control samples indicates that the metabolite could not be determined in drought-stressed sample. † in Sullu indicates statistically significant difference between Sullu and Ccompis at that stage/growth condition ($P \leq 0.05$). † in Ccompis means the metabolite could not be determined in Sullu. DW, dry weight; n.d., not determined

Sugars	DW basis	75 days control		60 days drought, 15 recovery	
		Sullu	Ccompis	Sullu	Ccompis
Arabinose	$\mu g\,g^{-1}$	72 ± 2†	n.d.	260 ± 11*†	143 ± 4*
Fructose	$mg\,g^{-1}$	17.1 ± 1.2†	21.5 ± 0.8	16.1 ± 1.3†	29.4 ± 1.0*
Galactose	$mg\,g^{-1}$	8.3 ± 0.2†	4.3 ± 0.3	6.0 ± 0.1*	7.1 ± 0.5*
Glucose	$mg\,g^{-1}$	12.9 ± 1.0†	21.2 ± 1.9	17.8 ± 0.8*	19.2 ± 2.7
Glucose-6-P	$\mu g\,g^{-1}$	224 ± 12†	352 ± 17	314 ± 37	314 ± 6
Isomaltose	$\mu g\,g^{-1}$	248 ± 14†	n.d.	318 ± 33†	489 ± 13
Lyxose	$\mu g\,g^{-1}$	n.d.	341 ± 22†	n.d.	661 ± 33*†
Maltose	$\mu g\,g^{-1}$	330 ± 43*	549 ± 11	365 ± 15*	652 ± 48
Melezitose	$\mu g\,g^{-1}$	510 ± 53†	1218 ± 95	679 ± 30†	268 ± 45*
Melibiose	$\mu g\,g^{-1}$	637 ± 63†	461 ± 24	465 ± 46†	354 ± 2*
Raffinose	$\mu g\,g^{-1}$	455 ± 21†	n.d.	561 ± 59†	915 ± 74*
Rhamnose	$\mu g\,g^{-1}$	85 ± 7†	144 ± 13	82 ± 7†	180 ± 3
Ribose	$\mu g\,g^{-1}$	384 ± 24†	674 ± 43	306 ± 20†	568 ± 35
Sorbose	$mg\,g^{-1}$	6.2 ± 0.3†	8.0 ± 0.1	6.0 ± 0.3†	8.9 ± 0.5
Sucrose	$mg\,g^{-1}$	26.5 ± 1.0†	2.8 ± 0.2	41.7 ± 1.9*†	6.5 ± 0.4*
Trehalose	$\mu g\,g^{-1}$	500 ± 51†	250 ± 9	885 ± 89*†	381 ± 38*
Xylose	$\mu g\,g^{-1}$	587 ± 25†	337 ± 7	533 ± 25†	333 ± 7
Total	$mg\,g^{-1}$	75.1 ± 1.9	62.2 ± 2.1	92.4 ± 2.5	76.3 ± 3.0

Table 6. Effects of drought stress on amino acid levels in two Andean potato genotypes, Sullu and Ccompis
Data were analysed between treatments and between two Andean potato genotypes and are shown as mean ± s.e. * in treated samples indicates statistically significant difference between drought-stressed and control samples ($P \leq 0.05$). * in control samples indicates that the metabolite could not be determined in drought-stressed sample. † in Sullu indicates statistically significant difference between Sullu and Ccompis at that stage/growth condition ($P \leq 0.05$). † in Ccompis means the metabolite could not be determined in Sullu. DW, dry weight; n.d., not determined

Amino acid	DW basis	45 days control		45 days drought		60 days control		60 days drought	
		Sullu	Ccompis	Sullu	Ccompis	Sullu	Ccompis	Sullu	Ccompis
Alanine	$\mu g\,g^{-1}$	117 ± 15	123 ± 7	112 ± 9	166 ± 27	112 ± 1	111 ± 14	191 ± 5*	185 ± 6*
Asparagine	$mg\,g^{-1}$	0.5 ± 0.0	0.9 ± 0.2	0.5 ± 0.0†	1.3 ± 0.1	0.4 ± 0.0†	1.1 ± 0.2	0.6 ± 0.0*†	1.3 ± 0.2
Aspartic acid	$mg\,g^{-1}$	1.06 ± 0.06	0.69 ± 0.02	0.94 ± 0.07	1.21 ± 0.10*	0.77 ± 0.05	0.74 ± 0.02	1.56 ± 0.13*	1.70 ± 0.07*
b-Alanine	$\mu g\,g^{-1}$	n.d.	26 ± 2†	n.d.	42 ± 3*†	n.d.	31 ± 1†	n.d.	47 ± 3†
Cysteine	$\mu g\,g^{-1}$	n.d.	1251 ± 93	n.d.	279 ± 7*†	n.d.	1801 ± 102†	n.d.	261 ± 14*†
GABA	$mg\,g^{-1}$	2.0 ± 0.2†	1.3 ± 0.0	2.6 ± 0.1	2.3 ± 0.1*	2.1 ± 0.1	1.6 ± 0.2	2.9 ± 0.1*	3.1 ± 0.2*
Glutamic acid	$\mu g\,g^{-1}$	n.d.	135 ± 15*†	785 ± 67*†	n.d.	n.d.	147 ± 11*†	2349 ± 128*†	n.d.
Glycine	$\mu g\,g^{-1}$	185 ± 17	45 ± 4	209 ± 18	170 ± 9*	195 ± 21†	56 ± 1	329 ± 17*	450 ± 39*
Isoleucine	$\mu g\,g^{-1}$	5 ± 0†	n.d.	5 ± 0†	10 ± 1*	6 ± 0†	n.d.	52 ± 4*†	20 ± 2*
Leucine	$\mu g\,g^{-1}$	53 ± 3†	n.d.	45 ± 3†	110 ± 5*	85 ± 2†	n.d.	388 ± 30*	317 ± 9*
Lysine	$\mu g\,g^{-1}$	457 ± 35	373 ± 30	434 ± 24†	312 ± 21	416 ± 28†	599 ± 51	894 ± 73*†	356 ± 12*
Phenylalanine	$\mu g\,g^{-1}$	69 ± 5	48 ± 2	61 ± 3	85 ± 4*	53 ± 4	53 ± 3	640 ± 29*†	100 ± 5*
Proline	$\mu g\,g^{-1}$	n.d.	n.d.	3266 ± 261*†	184 ± 14*	n.d.	n.d.	4961 ± 302*†	1398 ± 55*
Pyroglutamic acid	$\mu g\,g^{-1}$	167 ± 7†	n.d.	160 ± 9†	81 ± 5*	123 ± 21†	n.d.	316 ± 42*†	71 ± 10*
Serine	$\mu g\,g^{-1}$	1055 ± 71†	127 ± 8	1140 ± 63†	129 ± 60	819 ± 49†	113 ± 20	1056 ± 65†	187 ± 23
Threonine	$\mu g\,g^{-1}$	53 ± 4†	n.d.	218 ± 19*†	65 ± 1*	179 ± 16†	n.d.	207 ± 21†	115 ± 9*
Tryptophane	$\mu g\,g^{-1}$	119 ± 7†	36 ± 3*	125 ± 14†	n.d.	138 ± 20*†	42 ± 3*	n.d.	n.d.
Tyrosine	$\mu g\,g^{-1}$	139 ± 38†	54 ± 2*	128 ± 7†	n.d.	127 ± 14†	78 ± 6*	204 ± 6*†	n.d.
Valine	$\mu g\,g^{-1}$	197 ± 13	191 ± 3	162 ± 7†	221 ± 9	191 ± 10	195 ± 14	866 ± 13*	826 ± 124*
Total	$mg\,g^{-1}$	6.2 ± 0.2	5.3 ± 0.2	10.9 ± 0.3	6.8 ± 0.2	5.7 ± 0.1	6.7 ± 0.3	17.5 ± 0.4	10.4 ± 0.4

while in Ccompis augmentation of glucose and fructose was prevalent early under drought (day 30 AD) and some sucrose accumulation took place on day 60 AD. Further clone-dependent differences concerned glucose-6-phosphate and arabinose, which accumulated early under drought (day 30 AD) in Ccompis and later (day 60 AD) in Sullu. Additionally, Ccompis showed a rise in

Table 7. Amino acid levels in the leaves of two Andean potato genotypes, Sullu and Ccompis, upon recovery after drought stress
Data were analysed between treatments and between two Andean potato genotypes and are shown as mean ± s.e. * in treated samples indicates statistically significant difference between drought-stressed and control samples ($P \leq 0.05$). * in control samples indicates that the metabolite could not be determined in drought-stressed sample. † in Sullu indicates statistically significant difference between Sullu and Ccompis at that stage/growth condition ($P \leq 0.05$). † in Ccompis means the metabolite could not be determined in Sullu. DW, dry weight; n.d., not determined

Amino acid	DW basis	75 days control		60 days drought, 15 recovery	
		Sullu	Ccompis	Sullu	Ccompis
Alanine	$\mu g\,g^{-1}$	103 ± 9	136 ± 12	160 ± 17†	388 ± 21*
Asparagine	$\mu g\,g^{-1}$	0 ± 0	835 ± 154	0 ± 0	1499 ± 63*†
Aspartic acid	$\mu g\,g^{-1}$	667 ± 40	813 ± 64	757 ± 48	953 ± 61
b-Alanine	$\mu g\,g^{-1}$	n.d.	31 ± 2†	n.d.	40 ± 2†
Cysteine	$\mu g\,g^{-1}$	n.d.	1721 ± 90†	n.d.	257 ± 23*†
GABA	$mg\,g^{-1}$	1.9 ± 0.2†	2.7 ± 0.1	2.9 ± 0.5	3.2 ± 0.1*
Glutamic acid	$\mu g\,g^{-1}$	n.d.	109 ± 8*†	n.d.	n.d.
Glycine	$\mu g\,g^{-1}$	216 ± 13	266 ± 53	345 ± 50	238 ± 46
Isoleucine	$\mu g\,g^{-1}$	0 ± 0	n.d.	0 ± 0	n.d.
Leucine	$\mu g\,g^{-1}$	0 ± 0	n.d.	0 ± 0	n.d.
Lysine	$\mu g\,g^{-1}$	0 ± 0	373 ± 32†	0 ± 0	330 ± 27†
Phenylalanine	$\mu g\,g^{-1}$	24 ± 5†	64 ± 6	17 ± 1†	85 ± 2
Proline	$\mu g\,g^{-1}$	252 ± 0	n.d.	318 ± 18†	543 ± 30*
Pyroglutamic acid	$\mu g\,g^{-1}$	517 ± 68†	n.d.	413 ± 28†	182 ± 14*
Serine	$\mu g\,g^{-1}$	453 ± 41†	102 ± 9	793 ± 47*†	142 ± 5
Threonine	$\mu g\,g^{-1}$	79 ± 4†	n.d.	79 ± 7†	207 ± 18*
Tryptophane	$\mu g\,g^{-1}$	87 ± 2*	73 ± 7	n.d.	26 ± 2*
Tyrosine	$\mu g\,g^{-1}$	169 ± 12	152 ± 14	218 ± 1†	12 ± 0*
Valine	$\mu g\,g^{-1}$	104 ± 7	70 ± 6	23 ± 3*	14 ± 2*
Total	$mg\,g^{-1}$	4.5 ± 0.2	7.4 ± 0.2	6.0 ± 0.5	8.1 ± 0.1

isomaltose, lyxose, and melezitose concentrations, which all remained unchanged in Sullu. Ribose and xylose concentrations did not increase under drought in either clone.

Raffinose family oligosaccharides

Overall, the raffinose family of oligosaccharides (RFO) pathway was responsive to drought stress in Ccompis, with little change observed in Sullu. Ccompis showed a rise in raffinose from a not detectable level to 1528 μg g dry weight^{-1} (DW) under long-term drought, whereas there was little effect of drought on raffinose in Sullu (525 *v.* 482 $\mu g\,g^{-1}$ DW; treated *v.* control values) (Table 4). Melibiose levels showed a similar pattern with Ccompis showing a 6-fold higher level at maximum stress, whereas there was 3.5 fold change in Sullu at the same time point. In contrast, levels of melibiose at the recovery point were lower in drought-stressed than in control samples of Ccompis (354 *v.* 465 $\mu g\,g^{-1}$ DW; treated *v.* control), and a significant difference was seen between control and treatment in Sullu at recovery (637 *v.* 465 $\mu g\,g^{-1}$ DW) (Table 5). Drought caused a 4-fold increase in galactinol levels (3243 to 13 859 $\mu g\,g^{-1}$ DW) in Ccompis, with a less significant change in Sullu (8755 to 12 909 $\mu g\,g^{-1}$ DW), although neither levels at maximum stress nor during recovery differed greatly between the clones in the treated samples. Inositol levels increased 2-fold in Ccompis and 3-fold in Sullu at the maximum stress time point. At the recovery point, inositol levels were similar under both conditions in Ccompis and Sullu.

Amino acids and other N-containing compounds

Total amino acid contents were similar in unchallenged plants of both clones for the entire 60 days period, but strongly diverged under drought (Tables 6 and 7). Constitutive asparagine, β-alanine, cysteine and glycine levels were higher in Ccompis, and Sullu contained higher levels of serine, threonine, tryptophan, tyrosine and valine. Under drought, amino acid levels increased to a greater extent in Sullu than in Ccompis, but changes in amino acid concentrations generally happened earlier in Ccompis than in Sullu. Divergent accumulation patterns were observed with lysine, which decreased in Ccompis, but increased in Sullu.

Constitutive levels of amines such as ethanolamine and putrescine were higher in Sullu than in Ccompis. Drought caused additional accumulation of these compounds resulting in comparable ethanolamine amounts in both lines, and putrescine remained lower in Ccompis than in Sullu. Phosphoric acid, present in much higher concentrations in untreated Ccompis than in Sullu, decreased under drought in Ccompis but increased in Sullu.

Metabolites in known drought-responsive and -regulatory pathways

Several metabolites have long been associated with plant drought responses, in particular proline, trehalose, and sucrose. In both clones, but especially in Sullu, high proline levels were measured in drought-exposed plants. Trehalose accumulated early under drought (day 30 AD) in Ccompis and late (day 60 AD) in Sullu (Tables 4 and 5). Trehalose levels in Sullu at maximum stress were 4-fold higher than in Ccompis (1480 *v.* 385, respectively) with differences still remaining after a 15 day recovery. Similarly divergent accumulation patterns were observed with γ-aminobutyric acid (GABA) and glutamic acid, which

decreased in Ccompis, but increased in Sullu. Control levels of ascorbic acid were higher in Sullu than in Ccompis for both drought time points (Sullu, 148 and 319 $\mu g\ g^{-1}$ DW for the 30 and 60 day control time points, Ccompis, 25 and 28 $\mu g\ g^{-1}$ DW, respectively). Exposure to drought resulted in 3-fold increase in ascorbate levels in Ccompis at the 60 day time point (95 *v*. 28; stressed *v*. control), but a corresponding increase was not observed in Sullu at the maximum stress time point. Despite a 3-fold increase in ascorbate in Ccompis the difference was only significant at 0.05 and not at 0.01 but the differences in Sullu were significant lower in the stressed samples at 0.01. At the recovery time point, ascorbate levels in the treated samples from Ccompis were higher again (150 *v*. 32; stressed *v*. control), whereas, in Sullu, ascorbate levels were actually lower in the samples that had been subjected to drought stress than they were in the corresponding controls (118 *v*. 268; stressed *v*. control).

Gene expression

Gene expression changes in control, drought-exposed and re-watered plants of Ccompis and Sullu were measured by microarray analysis at two times: after 60 days of water deficit and 15 days after re-watering. The number of genes that responded under drought and recovery conditions is shown in Table 8. The numbers that responded after 60 days of drought did not differ greatly between the lines (536 induced in Sullu only, 605 in Ccompis only, 645 repressed in Sullu only, 779 in Ccompis only). However, under recovery conditions, the number of uniquely responsive genes in Sullu was greater than that observed in Ccompis (1819 induced in Sullu, and 467 in Ccompis, 1388 repressed in Sullu, and 271 repressed in Ccompis). Smaller numbers of genes that responded in both lines were observed under drought conditions (118 and 179 induced and represssed in both lines, respectively). Compared with the total number of responsive genes, those commonly responsive were greater in number under recovery conditions (315 induced and 160 repressed, respectively).

The microarray gene expression data were validated by real-time PCR measurement of transcript abundance for selected genes at two time points, maximum stress and recovery in both clones (Table 9). From the data points obtained, 17/20 correlated between microarray analysis and real-time PCR control.

Table 8. Effect of 60 days of drought and 15 days of recovery on gene expression in Sullu and Ccompis

Values are represented as the ratio of treated to control for each time point. All reported values were determined to be significant at the 95% level (see Materials and methods). In addition, at 60 days after drought, 118 genes were induced in both lines, whereas 179 genes were repressed. At the recovery point, 315 genes were induced in both lines, whereas 160 genes were repressed in common

Line	60 days drought		60 days drought + 15 days recovery	
	Induced	Repressed	Induced	Repressed
Ccompis	605	779	467	271
Sullu	536	645	1819	1318

Photosynthesis-related genes and carbon flow

The majority of drought-responsive genes encoding thylakoid-associated processes were upregulated during recovery in Sullu, with no response was seen at either time point in Ccompis. In particular, genes encoding PSII proteins were upregulated in Sullu during recovery, whereas no PSII genes responded in Ccompis. In contrast, PSI-associated genes, and also chloroplast envelope protein genes, responded with increased transcript abundance in both genotypes during recovery (Fig. 3).

Genes encoding the large subunit of rubisco, thioredoxin-responsive plastidic glyceraldehyde-3-phosphate dehydrogenase and fructose-1,6-bisphosphatase genes, and enzymes of photosynthetic carbon fixation, were upregulated during recovery in Sullu, with little change in expression in Ccompis at either the drought or recovery time points (Fig. 4). An isoamylase gene, associated with starch degradation, was upregulated at the maximum stress point in Ccompis. There was downregulation of sucrose synthase in Sullu, suggesting a buildup of sucrose in Sullu. A gene encoding the glucose-6-phosphate/phosphate translocator2 was downregulated in Sullu at the recovery time point, with no change observed in Ccompis. A gene encoding a chloroplast envelope phosphate transporter was upregulated in Sullu at the recovery time point, with no response observed in Ccompis, and a gene associated with the chloroplast phosphate translocator was downregulated in Ccompis at the point of maximum stress. Three genes which act to regulate rates of sucrose breakdown, SNF1-related protein kinases, responded, with an increase in expression of one of the genes at the point of maximum stress in Ccompis, and a decrease in all three genes at the recovery point in Sullu.

Table 9. Results of real-time (RT) PCR amplification of selected genes in comparison to microarray gene expression analysis results

Amplification and statistical analysis were carried out as in the Materials and mehods. The values given in column RT represent *n*-fold induction/repression compared to untreated control plants measured by RT gene expression assessment. In the POCI column, + indicates induced genes, − repressed genes and 1 unchanged genes. nd, not determined; POCI, potato oligoID; TC, TIGR ID; SGN, ID from Solanaceae database

	POCI	TC	SGN	Ccompis day 60		Ccompis recup		Sullu day 60		Sullu recup	
				RT	POCI	RT	POCI	RT	POCI	RT	POCI
Peroxiredoxin	MICRO.318.C6_1224	141787	U274344	−1.5	1	−1.4	1	2.6	1	2.6	+
SOD	MICRO.1819.C6_21	139039	U271296	−9	−	−1.5	1	1.36	0	2.9	0
LTP	MICRO.2125.C2_536	145196	U268923	80.1	+	2.1	1	−6.6	+	−2.7	−
SNF-1	MICRO.13181.C1_878	132860	U274703	1.5	1	−2.1	1	1	nd	−1.6	−
SuSy	MICRO.1765.C1_1320	135042	U273939	28	+	3.3	+	−2.1	+	−1.2	−

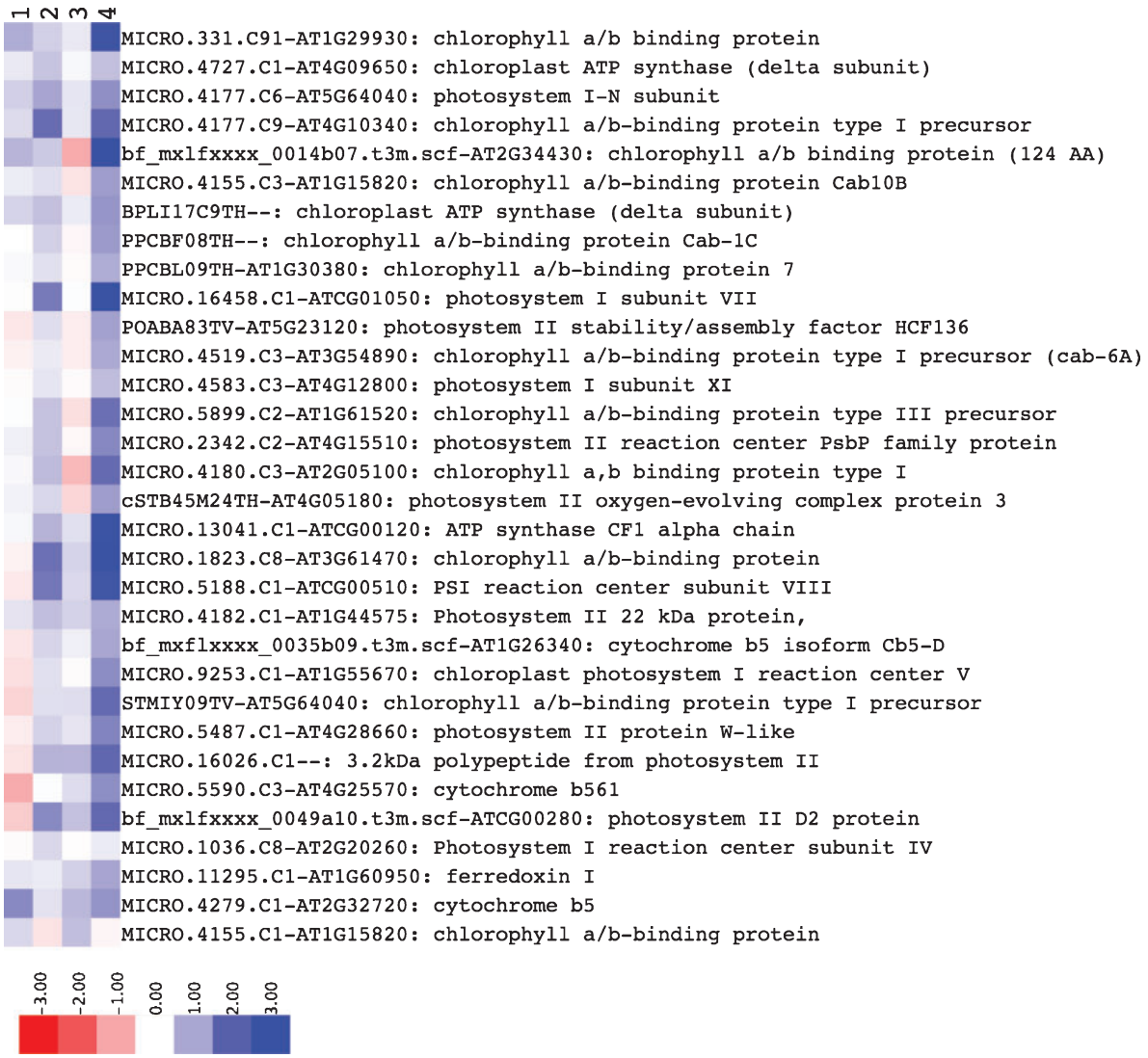

Fig. 3. Effects of 60 days of drought stress and recovery from drought on expression of thylakoid-associated genes in the leaves of two Andean potato genotypes, Sullu and Ccompis. Values shown were obtained from RNA isolated from leaves after 60 days of drought, and 15 days after re-watering. RNA isolation, and hybridisation were conducted as in Materials and methods. Statistical analysis (cut off set at 95% confidence) was conducted as described in Li *et al*. (2006*a*, 2006*b*), and matching, where possible, of potato oligonucleotides to putative *Arabidopsis* orthologues was conducted as described in Materials and methods. In cases where a putative *Arabidopsis* orthologue was identified, the AGI number is included in the annotation.

The responses of genes associated with cell wall biosynthesis are shown in Fig. 5. As in the case for the other groups of functional genes examined, the greatest number of responses was observed at the recovery time point for Sullu. Among the genes that responded positively in Sullu recovery was the COBRA gene, associated with cell plate formation in *Arabidopsis*.

The responses of genes associated with mitochondrial function are shown in Fig. 6. During recovery in Sullu, seven mitochondrial carrier proteins involved in solute transport across the mitochondrial membrane were upregulated. Additionally, a gene annotated as a mitochondrial energy transfer protein but probably encoding a plastid-located adenine nucleotide transporter (Leroch *et al*. 2005) functioning in purine export from plastids as well as a mitochondrial basic amino acid transporter involved in arginine−ornithine exchange were induced. Further activated mitochondrial genes in recovering Sullu encode two ubiquinol-cytochrome *c* reductases, two glycine hydroxymethyltransferases, an aminomethyltransferase precursor and an epsilon subunit of mitochondrial F1-ATPase.

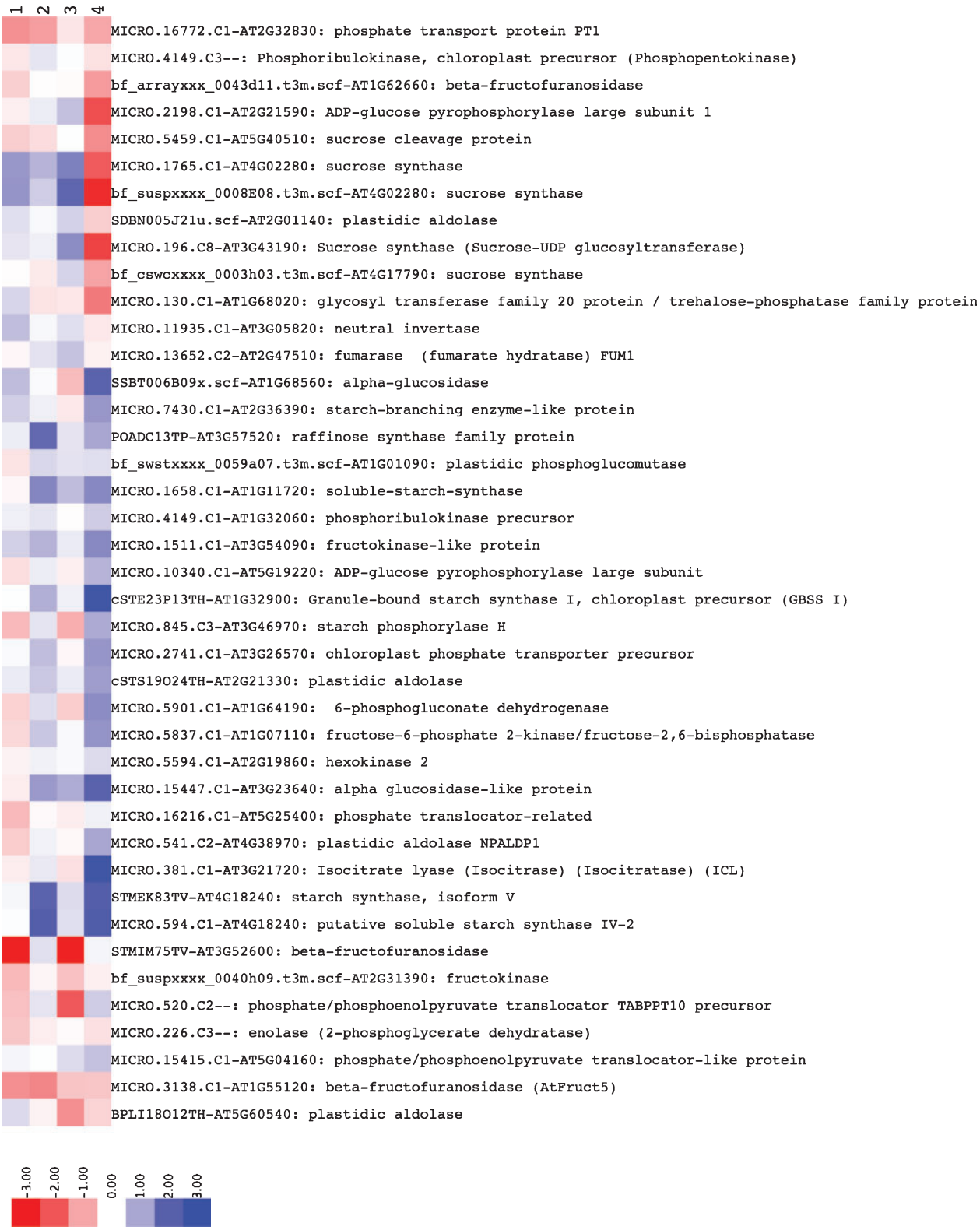

Fig. 4. Effects of 60 days of drought stress and recovery from drought on carbohydrate metabolism-associated genes in the leaves of two Andean potato genotypes, Sullu and Ccompis. Methods and abbreviations as in Fig. 3.

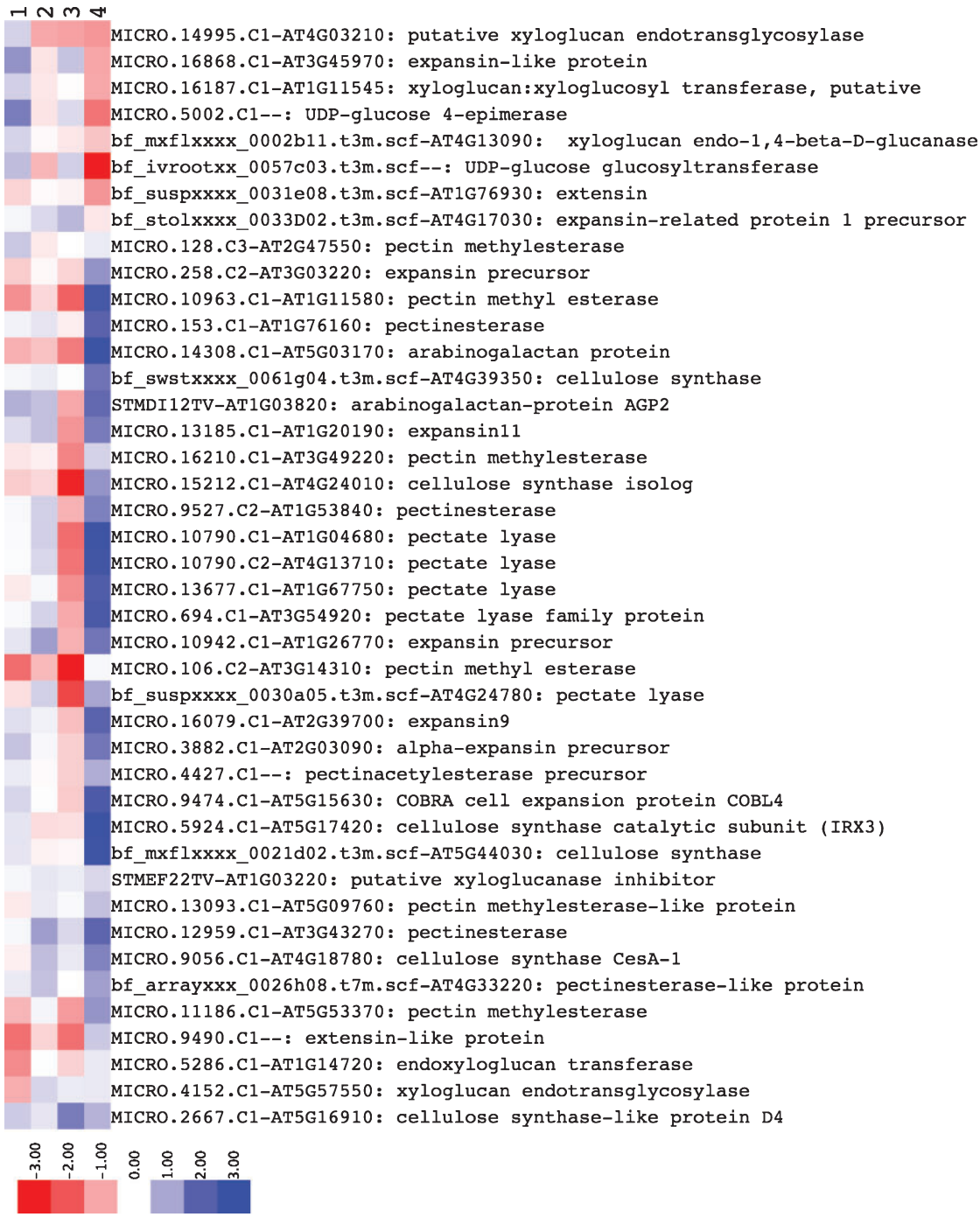

Fig. 5. Effects of 60 days of drought stress and recovery from drought on cell wall-associated genes in the leaves of Sullu and Ccompis, two Andean potato genotypes. Methods and abbreviations as in Fig. 3.

Downregulated mitochondrial genes in recovering Sullu comprised a phosphate translocator gene, an alternative oxidase gene and five mitochondrial amino acid metabolism genes (3-methyl-2-oxobutanoate dehydrogenase, isovaleryl-CoA dehydrogenase, two branched-chain α keto-acid dehydrogenase genes, and acetylornithine aminotransferase). A mitochondrial carrier protein gene, annotated as small calcium-binding mitochondrial carrier 2 protein gene was

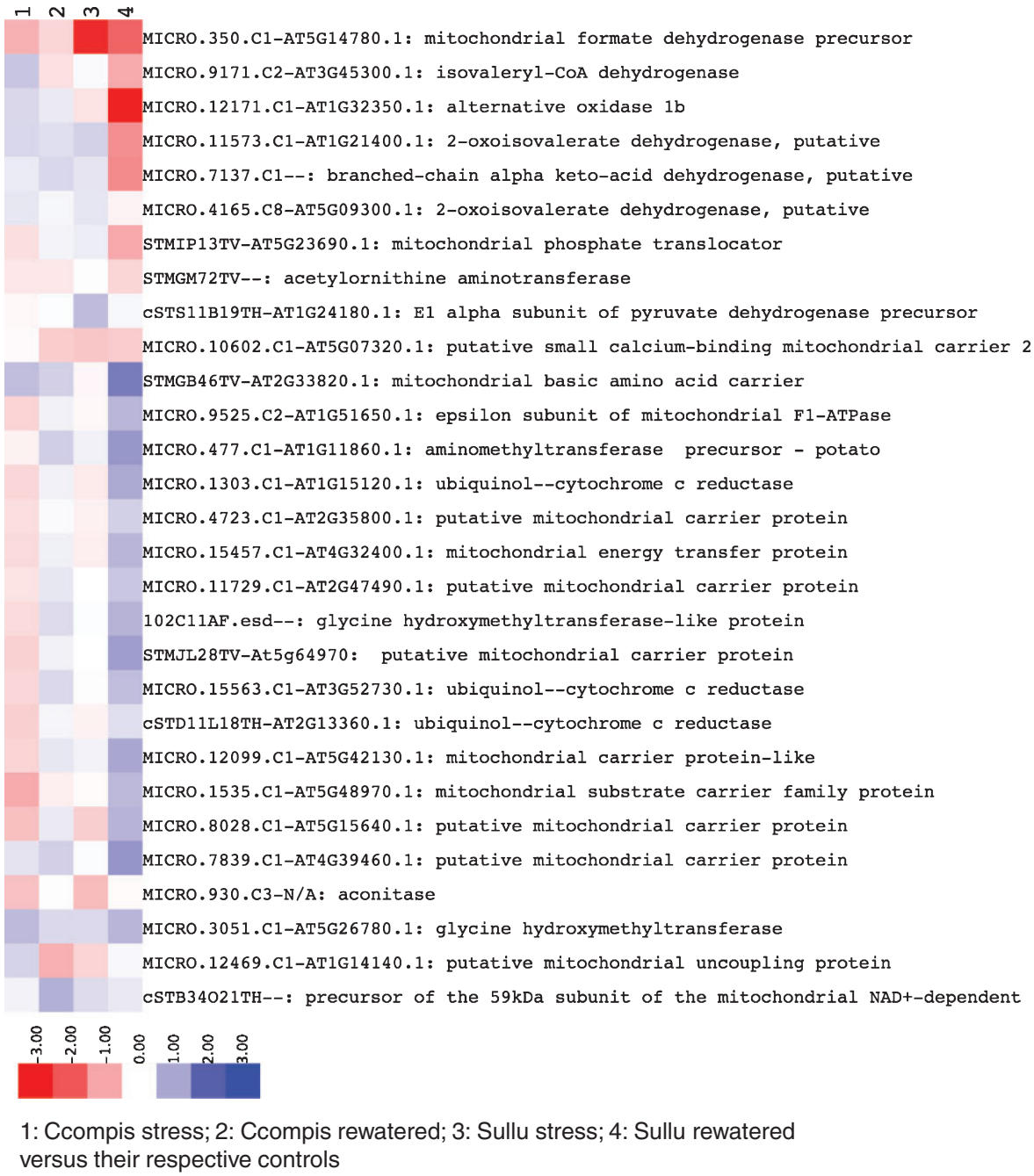

Fig. 6. Effects of 60 days of drought stress and recovery from drought on genes associated with mitochondrial function in the leaves of two Andean potato genotypes, Sullu and Ccompis. Methods and abbreviations as in Fig. 3.

downregulated in recovering plants of both clones and also in stressed Sullu plants. A mitochondrial formate dehydrogenase was found repressed in Sullu under stress and during recovery.

Responses of redox-responsive genes

The greatest number of different responses among redox-responsive genes between the lines was observed for genes whose products localise to the chloroplast (Fig. 7). Ccompis showed downregulation of all SOD genes whose products are chloroplast-localised. This included both copper chaperone genes, associated with Cu/Zn SOD activity, and those encoding FeSODs, which are also located in the plastid. In contrast, several glutaredoxin-encoding genes encoding proteins with other subcellular locations, were upregulated during recovery in both genotypes. Genes encoding glutathione-associated enzymes were downregulated during recovery in Sullu, and unresponsive in Ccompis. A group of

Fig. 7. Effects of 60 days of drought stress and recovery from drought on ROS-responsive genes in the leaves of Sullu and Ccompis, two Andean potato genotypes. Methods and abbreviations as in Fig. 3.

four thioredoxin-related genes, all of whose products are targeted to the chloroplast, were induced during recovery only in Sullu. In addition, a cytosolic Cu/Zn SOD was induced in Sullu, but was unresponsive in Ccompis. Four redox-responsive regulatory genes responded positively in Sullu recovery only.

Responses of chaperones

Six members of the family of heat shock transcription factors (HSFs) (Fig. 8) showed statistically significant differential responses. One of them, a homologue to AT4G36990 (HSFB1) in *Arabidopsis* and homologous to tomato HSFB1, is highly induced at maximum stress in Ccompis only. Two homologues to *Arabidopsis* HSFs AT2G41690 (HsfB3) and AT3G51910 (HSFA7A) are highly induced during recovery in Sullu, the time point during which a peak of gene expression was observed.

During recovery, different chaperones were induced in Sullu and Ccompis (Fig. 8). Two HSP90s, one located in the chloroplast (AT2G04030) and one in the endomembrane system (AT5G56010), respectively, were induced in both lines, however, induction was slightly more pronounced in Sullu. Together with the chloroplastic HSP90, a chloroplastic HSP70 (AT2G32120) was induced in Ccompis. In contrast, in

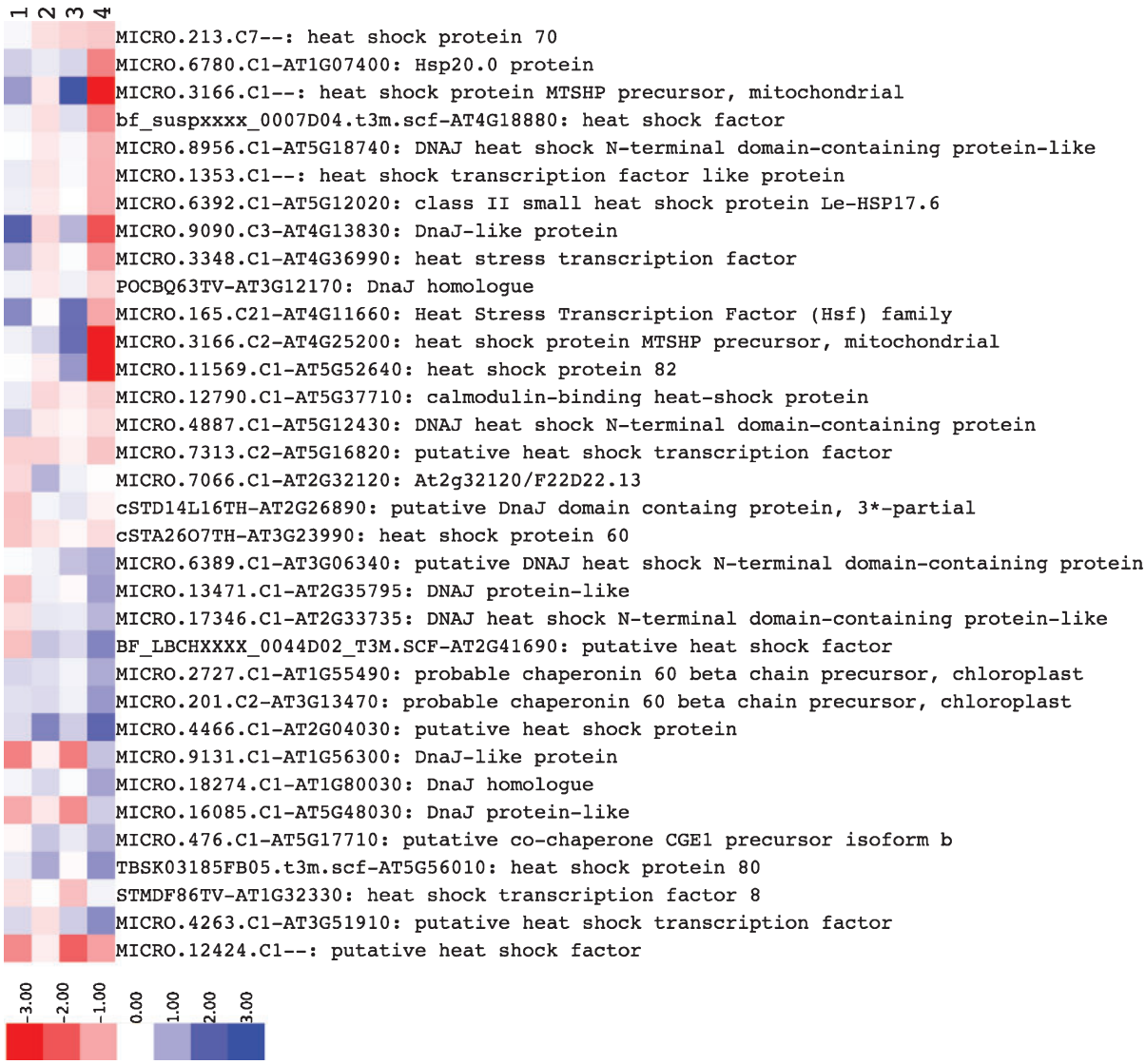

Fig. 8. Effects of 60 days of drought stress and recovery from drought on chaperone gene expression in the leaves of two Andean potato genotypes, Sullu and Ccompis. Methods and abbreviations as in Fig. 3.

Sullu homologues to chaperonin-60 β subunits (At1g55490, At3g13470) and a DnaJ homologue (At1g80030) whose products are localised in the chloroplast, were highly induced.

Discussion

The results represent novel information from a field study involving long-term drought stress, conducted on two closely related Andean potato landraces with integration of datasets that recorded physiological, metabolite and gene expression responses. The physiological responses to water deficit differed significantly between the two lines, with line Sullu maintaining photosynthesis activity in contrast to Ccompis (Figs 1, 2). Also, during recovery from severe stress, Sullu regained physiological homeostasis but Ccompis did not.

These physiological stress response differences are mirrored by distinct transcript profile differences and supported by metabolite profiles. Studies that investigated several species for drought-relevant changes in transcript populations have increased recently and consensus, if not in transcript number and identity, then at least in functional categories of genes affected, is emerging (Sreenivasulu *et al.* 2007). The categories of downregulated transcripts include growth and development in a wide sense, and photosynthesis, transporters, and gene regulation, *sensu strictu*. Stress defenses, whether avoidance, tolerance or resistance, are included in such downregulated functions (Gadjev *et al.* 2006; Mohammadi

et al. 2007), but most emphasis has been focussed on upregulated functions (Mittler *et al*. 2004; Rizhsky *et al*. 2004; Zhang *et al*. 2005). Among those, ABA-dependent and independent functions in the regulation of transcription, synthesis of water-conserving or water-retaining proteins, signal transduction pathways, especially those involving reactive oxygen species (ROS)-generating, ROS-signalling or ROS-detoxifying pathways, have attracted much attention (Papp *et al*. 2004; Verslues and Zhu 2005; Zhang *et al*. 2005; Dietz *et al*. 2006; Gadjev *et al*. 2006; Rosado *et al*. 2006; Skopelitis *et al*. 2006; Ma and Bohnert 2007; Mohammadi *et al*. 2007). Our analysis adds not only a study of effects of prolonged drought stress in the field, but also a comparative analysis of closely-related yet differently reacting lines. The juxtaposition of these lines, under field conditions, aided understanding to a significant degree. These lines of Andean potatoes, several with outstanding stress tolerance, could provide germplasms for the generally more drought-sensitive *S. tuberosum.*

The effect of drought stress on total metabolite pools could be recognised earlier in Ccompis than in Sullu, which, apparently, engaged different or superior countermeasures. The nearly 7-fold higher sucrose levels in Sullu at the time of maximum stress, compared with Ccompis, may be reflective of its superior capacity to maintain osmolytes for protection. However, during the 60-day exposure to drought Ccompis accumulated sucrose to a larger degree than Sullu, which contained much higher sucrose amounts than Ccompis in the absence of stress. This suggested a constitutively superior ability to withstand prolonged drought stress on the part of Sullu, as has been observed for gene expression profiles in other comparisons of stress tolerance among ecotypes of *Arabidopsis* and a close relative (Inan *et al*. 2004; Taji *et al*. 2004; Li *et al*. 2006*b*) and accessions of Andean potato (Watkinson *et al*. 2006). In fact, the repression of β-fructofuranosidase at day 60 of drought in both lines suggested increased sucrose biosynthesis during maximum stress.

The disaccharide trehalose, which is thought to play a role in hexokinase-dependent signalling, has been linked to drought tolerance (Avonce *et al*. 2004), and to the induction of stress-responsive genes (Bae *et al*. 2005). Trehalose accumulated in drought stressed Sullu leaves at the point of maximum stress but not in Ccompis. A trehalose-6-phosphate phosphatase gene, the particular *Arabidopsis* homologue of which has been identified as possessing only phosphatase activity (Eastmond *et al*. 2003), was repressed at the 60 day drought time point in Sullu only, suggesting a buildup of trehalose-6-phosphate, which may have an intermediary signalling function connecting changes in redox homeostasis with metabolic adjustments, in particular, coordinating respiratory and photosynthetic activities (Garg *et al*. 2002; Schluepmann *et al*. 2004), and post-translationally regulating starch biosynthesis through thioredoxin (Kolbe *et al*. 2005).

The respective responses of antioxidant genes in Sullu and Ccompis under severe drought stress and recovery show great differences between the clones, and may be one of the bases for better recovery of the photosynthetic machinery in Sullu. Seven genes encoding chloroplast-localised SODs, both Cu/Zn and Fe-containing proteins, were repressed in Ccompis, but not in Sullu, at the 60 day drought stress time point, suggesting a persistence of the superoxide anion in the chloroplasts of Ccompis, with the potential that the extremely toxic hydroxyl radicals via the Fenton reaction might increase. Further, no genes encoding plastid-localised antioxidant proteins were induced in Ccompis at the 60 day drought stress. This is a relatively novel observation. The downregulation, as opposed to induction, of antioxidant genes or enzymes under abiotic stress is not often observed although several recent reports document this effect for SOD responses to stress at the transcript and enzyme levels (Slesak *et al*. 2003; Jithesh *et al*. 2006). The cells of Sullu, in contrast, may have been better protected against ROS, since a cytosolic Cu/Zn SOD was induced in Sullu at the 60 day drought time point, in addition to two plastid-localised thioredoxin-related genes.

Metabolites in the pathway leading to RFO, galactinol and inositol, appeared to have increased under drought stress in both lines to a similar degree, but raffinose accumulation was observed only in Ccompis. RFO pathway stimulation, frequently observed under short-term drought conditions (Taji *et al*. 2002) distinguishes the drought response of both clones. Since melibiose, a breakdown product of raffinose, was higher in Sullu than in Ccompis at both drought stress time points, flux through the RFO pathway may have been accelerated in Sullu, although the levels of raffinose precursors were also elevated. The RFO pathway is associated with resistance to temperature stress and responses to drought stress (Kaplan *et al*. 2004).

In contrast, proline levels rose higher under drought stress in Sullu than in Ccompis, suggesting the possibility that the RFO pathway in the field did not contribute significantly to protection in leaves but that the accumulated proline may have afforded some protection to leaf cell function in Sullu, as has been commonly reported (for a review see Kavi Kishor *et al*. 2005). Recent data also suggest that proline may exert an antioxidative rather than an osmoprotective effect (Molinari *et al*. 2007). That being the case, Sullu leaf cells may be better protected against ROS than Ccompis through the action of proline, as well as through their superior SOD activities.

During recovery, Sullu shows many more genes regulated in functions that indicate a functional, active plastid in terms of C-fixation, carbon conversion into other carbohydrates, and export of carbon than in Ccompis. For example, Sullu resumes C-fixation in recovery but seems to defer or downregulate sucrose degradation. The repression of three SNF-1 related kinases was observed during recovery only in Sullu. These kinases regulate starch biosynthesis through post-translational thioredoxin-mediated redox activation of ADP-glucose pyrophosphorylase (Tiessen *et al*. 2003), suggesting that sucrose buildup continued or resumed in the more drought resistant landrace, which showed significantly higher sucrose levels than Ccompis under non-stress conditions. Plastid-localised thioredoxins were induced in Sullu during recovery but not in Ccompis, also attesting to increased photosynthetic carbon fixation. The increased expression of the regulatory hexokinase-2 transcript (Rolland and Sheen 2005; Sheen 2005) in Sullu but not in Ccompis during recovery suggested the continued induction of stress-mediated sugar signalling pathways. The decreased expression of sucrose synthase in Sullu, but not in Ccompis, during recovery also suggested sucrose buildup at that point. During recovery, Sullu shows a comparable induction of transcripts in functions that lead to N-transport, N-assimilation, ammonium utilisation including

NH4-transfer that is not approximated by Ccompis. Interesting is that a glutamine synthetase is downregulated after 60 days of stress in Ccompis.

Transcripts for mitochondrial proteins were found differentially regulated predominantly in Sullu during recovery. Activation of mitochondrial carrier proteins and ubiquinol−cytochrome *c* reductase, also known as a processing peptidase, a bifunctional enzyme involved in electron transport as well as protein import into mitochondria (Emmermann and Schmitz 1993) and of an epsilon subunit of mitochondrial F1-ATPase during recovery pinpoint increased mitochondrial electron transport and ATP synthesis. An orthologue to the basic amino acid carrier BAC1 of *A. thaliana* facilitating the import of arginine and the export of ornithine from mitochondria (Hoyos *et al*. 2003) also was found upregulated in recovering Sullu. Transcript accumulation of glycine hydroxymethyl transferase and aminomethyltransferase, a component of the glycine decarboxylation complex, may be due to increased photorespiration in recovering Sullu. Important in photorespiratory dissipation of light energy (Noctor *et al*. 2002), the glycine decarboxylase complex and glycine hydroxymethyl transferase cooperate to salvage photorespiratory glycine for regeneration of C3 units that can re-enter the Calvin cycle, while stress tends to repress transcripts for this pathway (Oliver 1994; Hourton-Cabassa *et al*. 1998). However, no downregulation of, for example, glycine hydroxymethyl transferase was observed in our experiment in either Sullu or Ccompis.

The observed changes in amino acid accumulation were accompanied by repression of four mitochondrial amino acid catabolism and an arginine biosynthesis gene. Mitochondrial formate dehydrogenase transcripts are known to accumulate under drought stress (Hourton-Cabassa *et al*. 1998). In our experiment, we did not measure accumulation of this transcript in stressed plants, on the contrary, in Sullu this gene appeared repressed in drought stressed and recovering plants. Repression of formate dehydrogenase was shown to lead to high proline accumulation in potato under drought stress via an un-known mechanism (Ambard-Bretteville *et al*. 2003). In Sullu, both proline accumulation and formate dehydrogenase repression under stress and recovery were observed. A mitochondrial uncoupling protein and an alternative oxidase gene were repressed in Ccompis and Sullu respectively during recovery. Both genes dissipate the proton gradient formed through respiration in mitochondria without the synthesis of ATP to balance the cellular energy level in response to stress protecting plant cells against oxidative stress (Borecky *et al*. 2006).

The chaperone genes induced in Sullu during recovery, were genes whose location is mostly predicted to localise the chloroplast (HSP90, two chaperonins 60, HSP40 and a GRPe) might be involved in the folding of new proteins being translocated to the chloroplast, since this line already resumed control photosynthesis levels.

Similar to the responses observed with the chaperones, Sullu showed maintenance of transcripts for auxin-induced proteins during stress to a greater extent and in more of the genes in this category than Ccompis (data not shown). During recovery from drought stress, Sullu induced expression of a larger number of transcripts leading to or resuming auxin transport, auxin-binding and specifying auxin-responsive proteins compared with Ccompis, supporting Sullu's predisposition to recover more readily than Ccompis. ABA-responsive genes showed little response to the prolonged drought stress, in contast to its central role in response to brief exposure (data not shown). Several Myb and bZIP transcription factor (TF) genes were differently affected in Sullu than in Ccompis (data not shown), but further evidence for the functional roles of those particular TFs in potato is needed before the gene expression data can be evaluated.

It seems that Sullu owes its relatively higher drought tolerance to a constitutively higher and more strongly increased sucrose content, proline accumulation, increases in trehalose levels, induction of chloroplast-localised chaperones, and more flexible signalling and ROS scavenging capacities. These responses are manifested in a superior capacity to protect the photosynthetic machinery for a prolonged period of drought stress in the field, and to induce the expression of photosynthetic genes and those associated with carbon and nitrogen metabolism during the full recovery of photosynthesis after re-watering. Such differences between the lines appear to be associated with the ability of Sullu to maintain metabolic homeostasis under drought stress, and to recover from the stress faster than Ccompis.

We compared the metabolite data reported here with those obtained in a 27 day greenhouse drought study conducted on Sullu (Vasquez-Robinet *et al.* 2008). Although duration, light intensities and night temperatures distinguished the greenhouse and field experiments, parallels emerged. In the two sets of plants, values obtained for the 45 day and 60 day time points in the field and the 27 day time point in the greenhouse indicated that proline, a putative osmoprotectant, and GABA, a stress-signalling molecule, behaved identically in the direction of the change induced by drought in Sullu. (Table S5), albeit both proline and GABA levels were overall much higher in the field samples. Sucrose levels were high in the Sullu controls in both sets of data, with a significant increase occurring only at the 60 day time point in the field, suggesting that that degree of stress experienced over time was necessary for drought to stimulate sucrose accumulation. Trehalose, also a mediator of stress-related responses, increased in the field only under the longer drought conditions in Sullu, again pointing to the greater degree of stress, possibly amplified by age, providing the signal for the metabolic adjustment.

Significantly, there was no clear evidence for drought-dependent accumulation of raffinose in Sullu in either experiment, in contrast to Ccompis in the field. Apparently, Ccompis induces the RFO pathway, indicated by galactinol accumulation, that would channel raffinose into this pathway. Sucrose and proline may act as major osmoprotectants in Sullu under drought stress, rather than raffinose.

Levels of the antioxidant ascorbate were unaffected by drought stress in Sullu in both experiments. This is in contrast to the gene expression data for the field trial, obtained only for the 60 day time point, where chloroplast-associated redox genes, such as thioredoxins and SODs, were upregulated preferentially in Sullu. The greenhouse gene expression data for Sullu, collected

at day 27 of drought, suggest upregulation of a chloroplast-localised thioredoxin gene product and also of genes associated with the glutathione pathway. Since the major concentration of glutathione is in photosynthesising plastids, the result may indicate a need for additional antioxidant protection for the chloroplast. It seems that antioxidant protection in Sullu under both conditions relies more on activation of ROS scavenging processes based in the chloroplast, than on induction of ascorbate biosynthesis, an essential and central antioxidant process outside the chloroplast.

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