Supplementary material for:

ASCORBATE PEROXIDASE 1 Plays a Key Role in the response of *Arabidopsis thaliana* to Stress Combination.

Proteomic analysis of plants subjected to drought, heat stress, or a combination of drought and heat stress.

Protein extraction. Plant tissue (400 mg) was flash-frozen and grounded in liquid nitrogen. Grounded tissue was re-suspended in 1 ml of buffer containing 50 mM Tris-HCl (pH-8); 0.5 M sucrose; 1 mM $MgCl_2$; 10 mM EDTA; 10 mM EGTA; 5 mM DTT and protease inhibitor cocktail for plant cell and tissue extracts (Sigma, St Louis; MO). Extracts were centrifuged in a bench top centrifuge (1,500 x g, 1 min) to remove cell debris and then centrifuged at 150,000 x g for 30 min. The supernatant was collected and proteins were precipitated by adding TCA (25% final concentration), incubating for 10 min on ice and centrifugation (18,000 x g, 10 min). Pellets were washed twice with cold acetone, air dried for 5 min, re-suspended in 260 µl DeStreak Rehydration Solution (GE Healthcare, Piscataway; NJ), gently vortexed for 90 min, and sonicated in a water bath sonicator for 10 min. All samples were spun at 16,000 x g for 10 minutes and supernatants were recovered. The protein content of the supernatants was determined using the EZQ protein assay (Invitrogen, Carlsbad; CA). Extracts were diluted so that they were normalized to the lowest protein concentration.

Two-dimensional gel electrophoresis. All reagents and solutions for 2-D electrophoresis, IPG strips, and SDS polyacrylamide gels were obtained from Bio-Rad (Hercules, CA), except as noted. 195 µl of each extract was loaded onto a 3-10L 11 cm IPG strip by overnight passive rehydration. Isoelectric focusing was carried out on a Bio-Rad Protean IEF cell using a program as follows: 250 V, linear ramp for 20 minutes; 8000 V, linear ramp for 2 hours 30 minutes; and 8000 V for a total of 20,000 Vhr (all steps with a maximum current of 50 µA per gel). Strips were stored at –80^{o}C, then thawed and incubated twice for 10 minutes each in 8M urea, 2% SDS, 0.05 M Tris-HCl, pH 8.8, 20% glycerol. The first incubation contained 2% DTT and the second contained 2.5% iodoacetamide. The strips were then layered on 4-20% Criterion Tris-HCl gradient gels and embedded in place with 0.5% agarose, along with BenchMark Protein Ladder molecular weight markers (Invitrogen, Carlsbad; CA). Electrophoresis was performed at a constant current of 200 mA until the dye front ran off the gel. Gels were stained overnight with Bio-Safe Coomassie or Sypro Ruby (BioRad, Hercules; CA).

Imaging, comparison of and sampling of protein spots. Stained gels were imaged on a VersaDoc imager; images of gels were compared using PDQuest version 8.0.1 software and spots were cut using the EXQuest spot cutter (all from BioRad, Hercules; CA). For quantification, all 12 gels (3 repeats each of control untreated, drought-stressed, heat-stressed, or drought and heat stress combination), were used in one match set and normalized to total intensity of all valid spots. Protein spots that either increased or decreased by at least two fold, in at least two of the three biological replications, were then cut from the gels and analyzed by MALDI-TOF-TOF. The detection threshold for our analysis was, on average, 0.005% of total protein loaded. Protein spots that either increased or decreased in intensity by at least two fold, in at least two of the three biological replications, were then cut from the gels and analyzed by MALDI-TOF-TOF

Protein digestion and mass spectrometry. Spots were digested using Investigator^{TM} Proprep^{TM} (Genomic Solutions, Ann Arbor; MI), we used a previously described protocol (1) with some modifications. Samples were washed twice with 25mM ammonium Bicarbonate

(ABC) and 100% acetonitrile, reduced and alkylated using 10 mM DTT and 100 mM Iodoacetamide and incubated with 75ng Trypsin for 6 hrs at 37^{o} C (trypsin was dissolved in 200 µl of 0.3% formic acid (aq) immediately prior to initiation of the digestion protocol).

Samples were prepared and spotted onto a MALDI (Matrix Assisted laser Desorption Ionization) target with ZipTipu-C18 (Millipore, Billerica; MA). Samples were aspirated and dispensed 3 times and eluted with conditioning solution (70% ACN, 0.2% formic acid) containing 5mg/ml MALDI matrix (α-Cyano-4-hydroxycinnamic acid) and 10mM ammonium phosphate, 0.5µl were spotted onto the MALDI target.

Mass Spectrometry. All mass spectrometric data was collected using an ABI 4700 MALDI TOF/TOF (Applied Biosystems, Foster City; CA). The data was acquired in reflector mode from a mass range of 700 – 4000 Daltons and 1250 laser shots were averaged for each mass spectrum. Each sample was internally calibrated if both the 842.51 and 2211.10 ions from trypsin autolysis were present. If both ions were not found we used the instrument's default calibration. The eight most intense ions from the MS analysis, which were not on the exclusion list, were subjected to MS/MS. For MS/MS analysis the mass range was 70 to precursor ion with a precursor window of -1 to 3 Daltons with an average 5000 laser shots for each spectrum. The data were stored in an Oracle database.

Database Search. The data was extracted from the Oracle database and a peak list was created by GPS Explorer software (Applied Biosystems, Foster City; CA) from the raw data generated from the ABI 4700. This peak list was based on signal to noise filtering and an exclusion list and included de-isotoping. The resulting file was then searched by Mascot (Matrix Science, Boston; MA) against an *Arabidopsis thaliana* database. A tolerance of 20 ppm was used if the sample was internally calibrated and 200 ppm tolerance if the default calibration was applied. Protein identification was validated by the following criteria: greater than 20 ppm mass accuracy on all MS ions and all ions in at least two MS/MS spectra, which were not modified, had to be accounted for.

Legends for Tables:

Supplementary Table 1. List of proteins identified by 2D-gels in plants subjected to drought (D), heat (H), or a combination of drought and heat stress (D+H) as described in the experimental procedure section of the manuscript. Proteins, with their corresponding spot numbers, were grouped based on their response to the various stresses. The protein score, confidence (Conf.) and peptide number (Pep.) are based on the GPS Mascot analysis. Proteins are identified by their SwissPort ID and the corresponding gene-annotation (downloaded from TAIR (www.arabidopsis.org)). Localization (Local.) of the protein was determined by the TAIR and PSORT predictions (www.arabidopsis.org and http://psort.ims.u-tokyo.ac.jp/form.html respectively). Localizations includes chloroplasts (chl), mitochondria (mit), cytoplasm (cyt), various membranal systems (mem), nucleus (nuc), endoplasmic reticulum (er), extra cellular proteins (ex), peroxizomes (per), microbody (mic), cell wall (wall) and unknown (unk). Experimental details are described above. Abbreviations: NC, no change in protein accumulation; DN, decrease in protein accumulation; UP, increase in protein accumulation.

Supplementary Table 2. Proteins that specifically accumulate in plants during a combination of drought and heat stress. Proteins are listed with their corresponding spot numbers. Log fold change of the corresponding transcript is shown based on ATH1 experiments reported previously (2). Other details as in Supplementary table 1.

REFERECES:

1. Rosenfeld, J., Capdevielle, J., Guillemot, J. C., and Ferrara, P. (1992) *Anal Biochem* **203**, 173-179
2. Rizhsky, L., Liang, H., Shuman, J., Shulaev, V., Davletova, S., and Mittler, R. (2004) *Plant Physiol* **134**, 1683-1696

Supplementary Table 1. List of proteins identified by 2D-gels in plants subjected to drought (D), heat (H), or a combination of drought and heat stress (D+H).

spot number	gene	SwissPort	score	conf.	# of peptides	local.	protein
Drought-NC; Heat-NC; Drought+Heat-UP							
107	At4g39730	O65660	93	99	5	chl	lipid-associated family protein
108	At4g39730	O65660	180	100	2	chl	lipid-associated family protein
202	At5g37780	P25854	137	100	6	unk	calmodulin-1/4 (CAM1)
708	AtCg00490	O03042	431	100	18	chl	Rubisco LSU
709	AtCg00490	O03042	503	100	24	chl	Rubisco LSU
1101	**At1g32470**	Q9LQL0	**204**	**100**	**5**	**mit.**	**glycine cleavage system H protein**
1101	**At2g22170**	Q9SIE7	**142**	**100**	**6**	**cyt.**	**lipid-associated family protein,**
1601	At5g10760	Q9LEW3	149	100	8	mem	aspartyl protease family protein
1707	At3g54050	P25851	251	100	11	chl	Fructose-1,6-bisphosphatase
1904	AT4g24280	Q9STW6	424	100	18	chl	HSP70
2004	**At1g67040**	Q9FZH7	**231**	**100**	**6**	**cyt.**	**expressed protein**
2004	**At5g38420**	P10797	**240**	**100**	**7**	**chl.**	**Rubisco Small subunit chain B2**
2301	At3g04790	Q9S726	161	100	5	chl	ribose 5-phosphate isomerase-related
2304	At3g23600	Q2V3T4	93	99	7	cyt	dienelactone hydrolase
2605	At3g12780	Q9LD57	75	96	8	mit	phosphoglycerate kinase
2801	At2g28000	P21238	467	100	26	chl	Cpn60
2808	AtCg00120	P56757	753	100	24	chl.	ATP synthase alpha chain
3002	At5g38420	P10797	377	100	11	chl.	Rubisco Small subunit chain B2
3108	At2g21660	Q03250	409	100	11	nuc.	Glycine-rich RNA binding protein GRP7
3203	**At3g14210**	Q9LJG3	**75**	**95**	**5**	**er**	**Lipase/acylhydrolase; myrosinase-associated protein**
3203	**At5g16450**	Q9FFE0	**106**	**100**	**7**	**er**	**dimethylmenaquinone methyltransferas**
3204	At2g43560	O22870	229	100	9	chl	Probable FKBP-type peptidyl-prolyl cis-trans isomerase
3205	At1g06680	Q42029	265	100	9	chl	Oxygen-evolving enhancer protein 2 (OEE2/PsbP)
3209	**At3g25770**	Q9LS02	**326**	**100**	**10**	**chl.**	**AOC3 allene oxide cyclase**
3209	**At5g20630**	P94072	**138**	**100**	**2**	**ext. cell.**	**GLP3 germin-like protein 3**
3709	AtCg00480	P19366	513	100	18	chl.	ATP synthase beta subunit
3803	AT5g26000	P37702	137	100	13	mem	glycosyl hydrolase family 1 protein
3804	At1g55490	P21240	247	100	15	chl.	CPN-60
3806	At5g26000	P37702	427	100	21	mem	glycosyl hydrolase family 1 protein
4007	At1g67090	P10795	219	100	7	chl	Rubisco SSU 1A
4305	**At1g07890**	Q05431	**307**	**100**	**10**	**cyt.**	**APX1**
4305	**At1g09130**	Q8L770	**84**	**99.4**	**7**	**chl.**	**ClpR3**
4307	At1g07890	Q05431	152	100	9	cyt.	APX1
4307	**At3g01500**	P27140	**312**	**100**	**12**	**chl.**	**CA1 (CARBONIC ANHYDRASE 1)**
4504	**At1g75280**	P52577	**180**	**100**	**13**	**cyt.**	**isoflavone reductase**
4504	**At2g43750**	P47999	**257**	**100**	**13**	**chl.**	**cysteine synthase**
4504	**At3g47520**	Q9SN86	**110**	**100**	**10**	**chl.**	**NAD-malate dehydrogenase**
4509	At3g47520	Q9SN86	249	100	14	chl	NAD-dependent malate dehydrogenase

4605	**At4g23600**	Q9SUR6	**241**	**100**	**18**	**mem**	**CORI3 cystathionine beta-lyase**
4802	At3g16850	Q94C86	77	97	5	mem	glycoside hydrolase family 28 protein / polygalacturonase (pectinase)
4805	**At3g16850**	Q94C86	**82**	**99**	**8**	**mem**	**glycoside hydrolase family 28 protein / polygalacturonase (pectinase)**
4805	**At4g34200**	O49485	**205**	**100**	**18**	**chl**	**D-3-phosphoglycerate dehydrogenase / 3-PGDH**
4809	At5g11670	Q9LYG3	227	100	16	cyt.	AtNADP-ME2 (malic enzyme 2)
5506	At1g53240	Q9ZP06	751	100	14	mit.	NAD dependent malate dehydrogenase
5702	At1g23310	Q2V4L7	594	100	29	cyt.	GGT1 (alanine-2 oxoglutarate aminotransferase 1)
6003	At1g67090	P10795	323	100	8	chl	Rubisco SSU 1A
6008	At5g20620	P59263	431	100	10	cyt	polyubiquitin (UBQ4)
6102	At3g17020	Q9LSP5	250	100	11	mem	universal stress protein (USP) family protein, similar to ENOD18
6511	At1g13440	Q56WJ4	248	100	9	cyt	glyceraldehyde 3-phosphate dehydrogenase, cytosolic
6602	At1g13440	Q56WJ4	464	100	17	mit.	Glyceraldehyde 3-phosphate dehydrogenase, cytosolic
6604	At3g14210	Q9LJG3	146	100	12	mem	myrosinase-associated protein
7405	At3g12500	P19171	194	100	5	mem.	Class I chitinase (Fragment)
8304	At3g11780	Q9SF20	98	99	3	unk	MD-2-related lipid recognition domain-containing protein
Drought-UP; Heat-DN; Drought+Heat-UP							
401	At4g24770	Q04836	368	100	10	chl	cp31 RNA-binding protein RNP-T
402	At4g24770	Q04836	288	100	10	chl	cp31 RNA-binding protein RNP-T
Drought-UP; Heat-NC; Drought+Heat-UP							
505	At1g47128	P43297	132	100	4	mem	cysteine proteinase (RD21A) / thiol protease
1102	At2g35370	P25855	356	100	7	mit.	GDCH glycine decarboxylase complex H protein
1801	At5g53140	Q94AT1	119	100	8	mit	Protein phosphatase 2C-like
2505	At3g55800	P46283	309	100	19	chl	sedoheptulose-1,7-bisphosphatase (SBPase)
3102	**At3g52960**	Q949U7	**358**	**100**	**7**	**chl.**	**peroxiredoxin type 2**
3102	**At5g53490**	P81760	**81**	**98.8**	**5**	**chl.**	**thylakoid lumenal 17.4 kDa protein**
3601	AtCg00480	P19366	167	100	10	chl	ATPB (ATPase β-subunit)
4603	**At2g39730**	P10896	**296**	**100**	**11**	**chl**	**Rubisco activase**
4603	**At3g52180**	Q2V2SO	**240**	**100**	**15**	**chl**	**plant-specific protein tyrosine phosphatase (PTP)**
5307	At3g01500	P27140	221	100	11	chl	carbonic anhydrase 1 (CA1)
7212	At4g11650	P50700	158	100	7	cyt.	osmotin-like protein (OSM34)
Drought-NC; Heat-UP; Drought+Heat-UP							
206	At1g75950	Q39255	107	100	3	unk	SKP1-like (SCF E3 ubiquitin ligase)
309	At4g24770	Q04836	159	100	8	chl	cp31 RNA-binding protein RNP-T
2204	At4g25200	Q96331	383	100	9	mit.	mitochondrial small heat shock protein (HSP23.6-M)
2207	At3g46230	P19036	159	100	8	cyt.	HSP 17.4
2403	At5g66570	P23321	408	100	18	chl	photosystem II oxygen-evolving complex protein 1 (OEE1/PsbO)
2802	At3g09440	O65719	535	100	30	cyt	heat shock cognate 70 kDa protein 3 (HSC70-3)
3301	At1g06680	Q42029	604	100	9	chl.	PsbP (OE23)
3303	At3g01500	P27140	89	99	5	chl	carbonic anhydrase 1 (CA1)

4201	At3g62030	P34791	642	100	13	chl	Peptidyl-prolyl cis-trans isomerase (CYP20-3)
5302	**At1g67090**	P10795	**106**	**99**	**8**	**chl**	**Rubisco SSU 1A**
5302	**At4g09010**	P82281	**320**	**100**	**12**	**chl**	**APX4**
5508	At1g53240	Q9ZP06	385	100	7	mit	malate dehydrogenase (NAD)
6205	At4g38740	P34790	307	100	11	cyt	cytosolic cyclophilin (ROC1)
6505	At3g26650	P25856	82	99	5	chl	glyceraldehyde 3-phosphate dehydrogenase A
6506	At3g26650	P25856	240	100	14	chl	glyceraldehyde 3-phosphate dehydrogenase A
7610	At5g11420	Q9LYE7	215	100	12	mem	expressed protein
7802	At1g20620	Q42547	157	100	15	per	CAT3 (catalase)

Drought-UP; Heat-UP; Drought+Heat-UP

406	**At1g47128**	P43297	**94**	**99**	**6**	**mem**	**cysteine proteinase (RD21A) / thiol protease**
406	**At4g02450**	Q8L7U4	**86**	**99**	**6**	**unk**	**glycine-rich protein**
1009	At2g21660	Q03250	173	100	6	nuc	ATGRP7 (Glycine-rich RNA-binding protein 7) (same as 3107)
1411	At5g09650	Q9LXC9	363	100	15	chl	inorganic pyrophosphatase
1503	At1g23820	Q9ZUB3	219	100	11	mem	SPDS1 Spermidine synthase
2905	At5g36210	Q9FG66	81	99	8	chl	expressed protein
3201	At3g62030	P34791	81	98.8	2	chl.	peptidyl-prolyl cis-trans isomerase
3309	At3g55440	P48491	71	89.5	4	cyt.	Triosephosphate isomerase
3503	At2g21330	Q9SJU4	279	100	8	chl.	fructose-bisphosphate aldolase
3506	**At2g21330**	Q9SJU4	**189**	**100**	**10**	**chl.**	**fructose-bisphosphate aldolase**
3506	**At5g23120**	O82660	**101**	**100**	**8**	**chl.**	**photosystem II stability/assembly factor, chloroplast (HCF136)**
3506	**At5g50850**	Q38799	**93**	**99.9**	**8**	**mit.**	**pyruvate dehydrogenase E1 component beta subunit**
3507	At1g79550	Q9SAJ4	376	100	15	cyt	phosphoglycerate kinase
3604	At1g32060	P25697	431	100	12	chl.	phosphoribulokinase (PRK) / phosphopentokinase
3702	**At5g19770**	P20363	**97**	**100**	**3**	**cyt.**	**Tubulin alpha-3/alpha-5 chain**
3702	**AtC00480**	P19366	**342**	**100**	**10**	**chl.**	**ATP synthase beta subunit**
3807	At1g55490	P21240	329	100	21	chl	Cpn60 (GroEL)
4002	At1g67090	P10795	296	100	9	chl.	Rubisco Small subunit chain 1A
4501	At4g38970	Q944G9	464	100	15	chl.	fructose-bisphosphate aldolase
5909	**At3g14210**	Q9LJG3	**80**	**98**	**12**	**mit**	**glycine dehydrogenase**
5909	**At4g35830**	Q42560	**125**	**100**	**16**	**cyt**	**Aconitate hydratase**
6502	At3g04120	P25858	139	100	9	cyt	Glyceraldehyde 3-phosphate dehydrogenase
6708	At1g78850	Q9ZVA4	262	100	13	mem.	curculin-like (mannose-binding) lectin family protein
7402	At3g16530	Q9LK72	228	100	10	mem.	Lectin like protein

Drought-DN; Heat-NC; Drought+Heat-DN

1704	At3g54050	P25851	299	100	13	chl	Fructose-1,6-bisphosphatase
2603	**At2g39730**	P10896	**122**	**100**	**5**	**chl.**	**ribulose bisphosphate carboxylase/oxygenase activase**
2603	**At3g12780**	Q9LD57	**124**	**100**	**6**	**mem.**	**phosphoglycerate kinase**
6508	At1g13440	Q56WJ4	515	100	18	cyt	glyceraldehyde 3-phosphate dehydrogenase, cytosolic
7404	At1g76150	Q8VYI3	152	100	11	cyt.	maoC-like dehydratase domain-containing protein

7508	At5g09660	Q9ZP05	455	100	13	micro	malate dehydrogenase (NAD)
7707	At3g02360	Q9FWA3	542	100	17	mem.	6-phosphogluconate dehydrogenase
7901	At1g78570	Q9SYM5	372	100	25	unk	NAD-dependent epimerase/dehydratase f (dTDP-glucose 4,6-dehydratase)
Drought-UP; Heat-UP; Drought+Heat-DN							
2307	**At3g23600**	Q9LUG8	**73**	**93**	**7**	**cyt**	**dienelactone hydrolase**
2307	**At5g02790**	Q9LZ06	**152**	**100**	**13**	**unk**	**In2-1 protein (glutathaion S-transferase)**
Drought-NC; Heat-NC; Drought +Heat-DN							
2904	At3g19170	Q9LJL3	411	100	32	chl/mit	Zinc metalloprotease pitrilysin subfamily A
4703	AtCg00490	O03042	562	100	12	chl.	Rubisco large chain
5906	At5g17920	O50008	547	100	25	cyt	ATCIMS (methionine synthase)
5907	At5g17920	O50008	542	100	28	cyt	ATCIMS (methionine synthase) (same as 5906)
6601	**At3g52880**	Q9LFA3	**140**	**99**	**12**	**per**	**monodehydroascorbate reductase**
7505	At2g36460	Q9SJQ9	254	100	13	cyt	fructose bisphosphate aldolase
Drought-DN; Heat-DN; Drought+Heat-DN							
105	At4g23670	Q9SUR0	381	100	9	cyt.	major latex protein-related
301	At3g12390	Q9LHG9	80	98.6	5	cyt.	nascent polypeptide associated complex alpha chain protein,
403	At4g24770	Q04836	255	100	9	chl.	31 kDa ribonucleoprotein
504	At1g30230	P48006	708	100	12	cyt.	elongation factor 1-beta / EF-1-beta
1503	At1g23820	Q9ZUB3	263	100	6	cyt.	spermidine synthase 1 (SPDSYN1) / putrescine aminopropyltransferase 1
1701	AtCg00490	O03042	502	100	14	chl.	Rubisco large chain
3107	At2g21660	Q03250	184	100	8	nuc	ATGRP7 (Glycine-rich RNA-binding protein 7)
4906	At4g26970	Q94A28	305	100	24	mit	aconitate hydratase
4907	AtCg00490	O03042	498	100	20	chl	Rubisco LSU
5301	**At1g67090**	P10795	**119**	**100**	**10**	**chl**	**Rubisco SSU 1A**
5301	**At5g02240**	Q94EG6	**278**	**100**	**13**	**mem**	**expressed protein**
5805	**At3g11830**	Q9SF16	**138**	**100**	**14**	**cyt.**	**chaperonin similar to T-complex protein 1, eta subunit**
5805	**At4g00570**	Q8L7K9	**286**	**100**	**18**	**mit.**	**Malate oxidoreductase**
6304	At2g30860	O80852	727	100	15	cyt.	Glutathione S-transferase
6308	At2g30860	O80852	87	99.7	2	cyt.	Glutathione S-transferase
6503	At2g01140	Q9ZU52	254	100	8	chl./mit.	fructose-bisphosphate aldolase
6504	At3g26650	P25856	99	99.9	9	chl	glyceraldehyde 3-phosphate dehydrogenase A
6716	At3g54400	Q9M2U7	102	100	5	mem.	Nucleoid DNA-binding-like protein
7203	At5g10860	Q9LEV3	351	100	13	mit.	CBS domain-containing protein
7304	At1g56450	Q7DLR9	128	100	4	cyt.	20S proteasome beta subunit (PBG1)
7609	At3g08030	Q9SFB1	411	100	18	unk	expressed protein
7611	At5g11420	Q9LYE7	310	100	16	mem	expressed protein
8001	**At1g03600**	Q9LR64	**82**	**99**	**4**	**chl**	**photosystem II family protein**
8001	**At4g05180**	Q41932	**168**	**100**	**9**	**chl**	**16 kDa Oxygen-evolving protein (OEE3)**
8610	At1g29670	Q9C7N4	288	100	13	mem	GDSL-motif lipase/hydrolase

8612	AT3g14420	Q9LRR9	375	100	20	unk	glycolate oxidase
9801	AtCg00490	O03042	616	23	24	chl	Rubisco LSU
Drought-UP; Heat-UP; Drought+Heat-NC							
2306	At1g06680	Q42029	289	100	7	chl	Oxygen-evolving enhancer protein 2 (OEE2/PsbP)
6702	AtCg00490	O03042	124	100	7	chl	Rubisco LSU
Drought-DN; Heat-UP; Drought+Heat-UP							
6103	At5g59720	P19037	136	100	9	unk	Heat shock protein 18
Drought-NC; Heat-DN; Drought+Heat-DN							
8108	At4g05180	Q41932	483	100	16	chl	Oxygen-evolving enhancer protein 3 (OEE3/PsbQ)
8402	**At4g30270**	P24806	**123**	**100**	**8**	**cell wall**	**MERI5B endo-xyloglucan transferase**
8402	**At4g30270**	P24806	**409**	**100**	**13**	**cyt.**	**MERI5B endo-xyloglucan transferase**
Drought-DN; Heat-UP; Drought+Heat-DN							
411	At4g24770	Q04836	385	100	13	chl	cp31 RNA-binding protein RNP-T
9108	At1g67090	P10795	359	100	10	chl	Rubisco SSU 1A
Drought-UP; Heat-NC; Drought+Heat-NC							
1405	At5g38480	P42644	138	100	5	cyt.	14-3-3-like protein GF14 psi (General regulatory factor 3)
2706	AtCg00490	O03042	85	99.6	3	chl.	Rubisco large chain
3105	**At2g28190**	O78310	**383**	**100**	**6**	**chl**	**Superoxide dismutase [Cu-Zn]**
3105	**At3g62030**	P34791	**212**	**100**	**12**	**chl**	**Peptidyl-prolyl cis-trans isomerase (CYP20-3)**
4302	**At2g30870**	P42761	**106**	**99**	**9**	**cyt**	**glutathione transferase**
4302	**At3g01500**	P27140	**329**	**100**	**12**	**chl**	**carbonic anhydrase 1 (CA1)**
4507	**At1g53240**	Q9ZP06	**226**	**100**	**7**	**mit**	**malate dehydrogenase (NAD)**
4507	**At4g14880**	P47998	**130**	**100**	**7**	**cyt**	**cysteine synthase**
Drought-NC; Heat-UP;Drought+Heat-NC							
6809	At5g17920	O50008	203	100	11	cyt.	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase
9601	**At1g29670**	Q9C7N4	**318**	**100**	**13**	**mem**	**GDSL-motif lipase/hydrolase**
9601	**At3g14420**	Q9LRR9	**113**	**100**	**11**	**unk**	**glycolate oxidase**

Supplementary Table 2. Proteins that specifically accumulate in plants during a combination of drought and heat stress.

Spot	Gene	Score	Conf.	Pep.	Local.	Protein	array fold change (log)
107/108	At4g39730	93	99	5	chl	lipid-associated family protein	0.85
202	At5g37780	137	100	6	unk	calmodulin-1/4 (CAM1)	0.75
1101	At1g32470	204	100	5	mit	glycine cleavage system H protein	-0.5
1101	At2g22170	142	100	6	cyt	lipid-associated family protein,	-2.4
1601	At5g10760	149	100	8	mem	aspartyl protease family protein	-2.2
1707	At3g54050	251	100	11	chl	Fructose-1,6-bisphosphatase	-0.4
1904	At4g24280	424	100	18	chl	HSP70	2.10
2004	At1g67040	231	100	6	cyt	expressed protein	NC
2301	At3g04790	161	100	5	chl	ribose 5-phosphate isomerase-related	-1.8
2304	At3g23600	93	99	7	cyt	dienelactone hydrolase	-0.7
2605	At3g12780	75	96	8	mit	phosphoglycerate kinase	-0.3
2801	At2g28000	467	100	26	chl	Cpn60	1.50
2808	AtCg00120	753	100	24	chl	ATP synthase α chain	-2.7
3108	At2g21660	409	100	11	nuc	Glycine-rich RNA binding protein ATGRP7	0.95
3203	At3g14210	75	95	5	er	Lipase/acylhydrolase; myrosinase-associated protein	NC
3203	At5g16450	106	100	7	er	dimethylmenaquinone methyltransferas	-1.0
3204	At2g43560	229	100	9	chl	Probable FKBP-type peptidyl-prolyl cis-trans isomerase	-1.8
3205	At1g06680	265	100	9	chl	Oxygen-evolving enhancer protein 2 (OEE2/PsbP/OE23)	NC
3209	At3g25770	326	100	10	chl	allene oxide cyclase AOC3	NC
3209	At5g20630	138	100	2	ext	germin-like protein 3 GLP3	-2.5
3709	AtCg00480	513	100	18	chl	ATP synthase β-subunit	NC
3803	At5g26000	137	100	13	mem	glycosyl hydrolase family 1 protein	NC
3804	At1g55490	247	100	15	chl	CPN60	1.25
3806	At5g26000	427	100	21	mem	glycosyl hydrolase family 1 protein	-0.4
4305	At1g07890	307	100	10	cyt	ascorbate peroxidase 1 APX1	0.60
4305	At1g09130	84	99.367	7	chl	ClpR3	0.45
4307	At1g07890	152	100	9	cyt	ascorbate peroxidase 1 APX1	0.60
4504	At1g75280	180	100	13	cyt	isoflavone reductase	0.85
4504	At2g43750	257	100	13	chl	cysteine synthase	NC
4504	At3g47520	110	99.999	10	chl	NAD-dependent malate dehydrogenase	NC

4509	At3g47520	249	100	14	chl	NAD-dependent malate dehydrogenase	NC
4605	At4g23600	241	100	18	mem	Cystine lyase CORI3	0.80
4802	At3g16850	77	97	5	mem	glycoside hydrolase family 28 protein / polygalacturonase (pectinase)	-1.5
4805	At3g16850	82	99	8	mem	glycoside hydrolase family 28 protein / polygalacturonase (pectinase)	-1.5
4805	At4g34200	205	100	18	chl	D-3-phosphoglycerate dehydrogenase 3-PGDH	NC
4809	At5g11670	227	100	16	cyt	malic enzyme 2 NADP-ME2	-1.0
5307	At3g01500	312	100	12	chl	carbonic anhydrase 1 CA1	-0.85
5702	At1g23310	594	100	29	cyt	glutamate:glyoxylate aminotransferase 1 GGT1	0.85
6008	At5g20620	431	100	10	cyt	polyubiquitin (UBQ4)	1.80
6102	At3g17020	250	100	11	mem	universal stress protein (USP), similar to ENOD18 (*Vicia faba*)	0.60
6511	At1g13440	248	100	9	cyt/mit	glyceraldehyde 3-phosphate dehydrogenase	NC
6602	At1g13440	464	100	17	cyt/mit	glyceraldehyde 3-phosphate dehydrogenase	NC
6604	At3g14210	146	100	12	mem	myrosinase-associated protein	NC
7405	At3g12500	194	100	5	mem	Class I chitinase (Fragment)	NC
8304	At3g11780	98	99	3	unk	MD-2-related lipid recognition domain-containing protein	-0.75

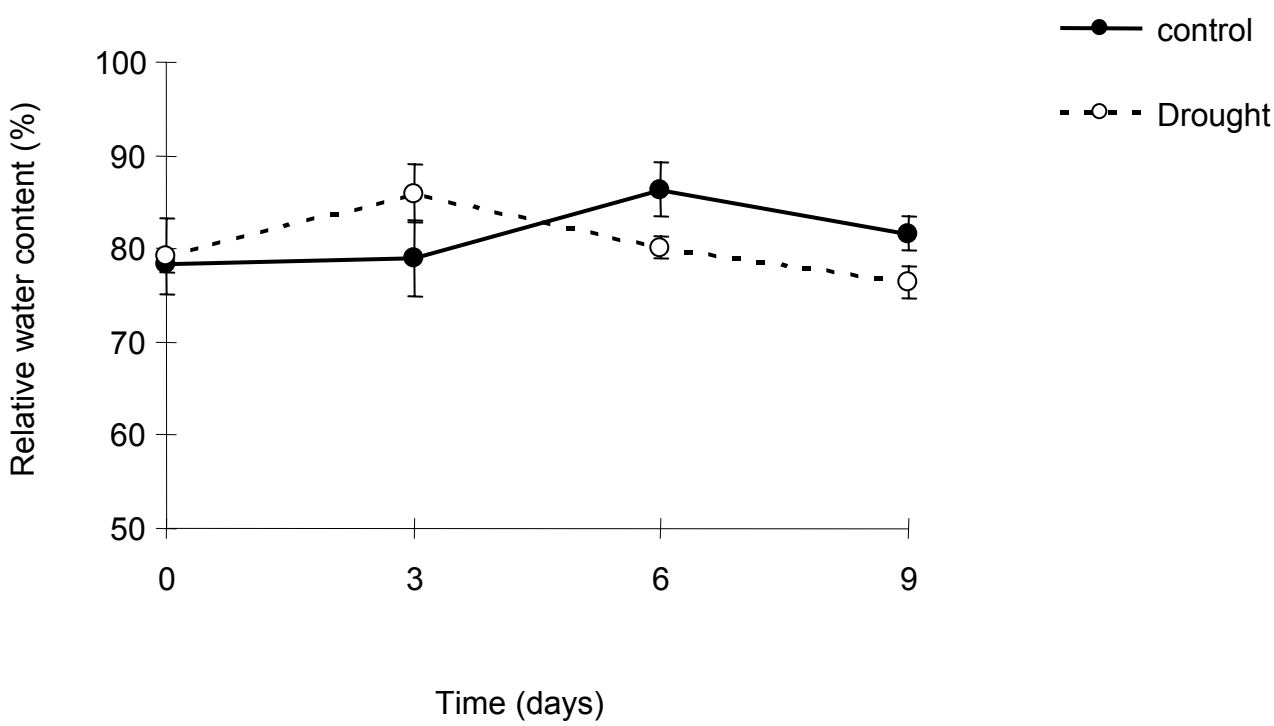

Figure S1. Relative water content of Arabidopsis leaves after water withholding

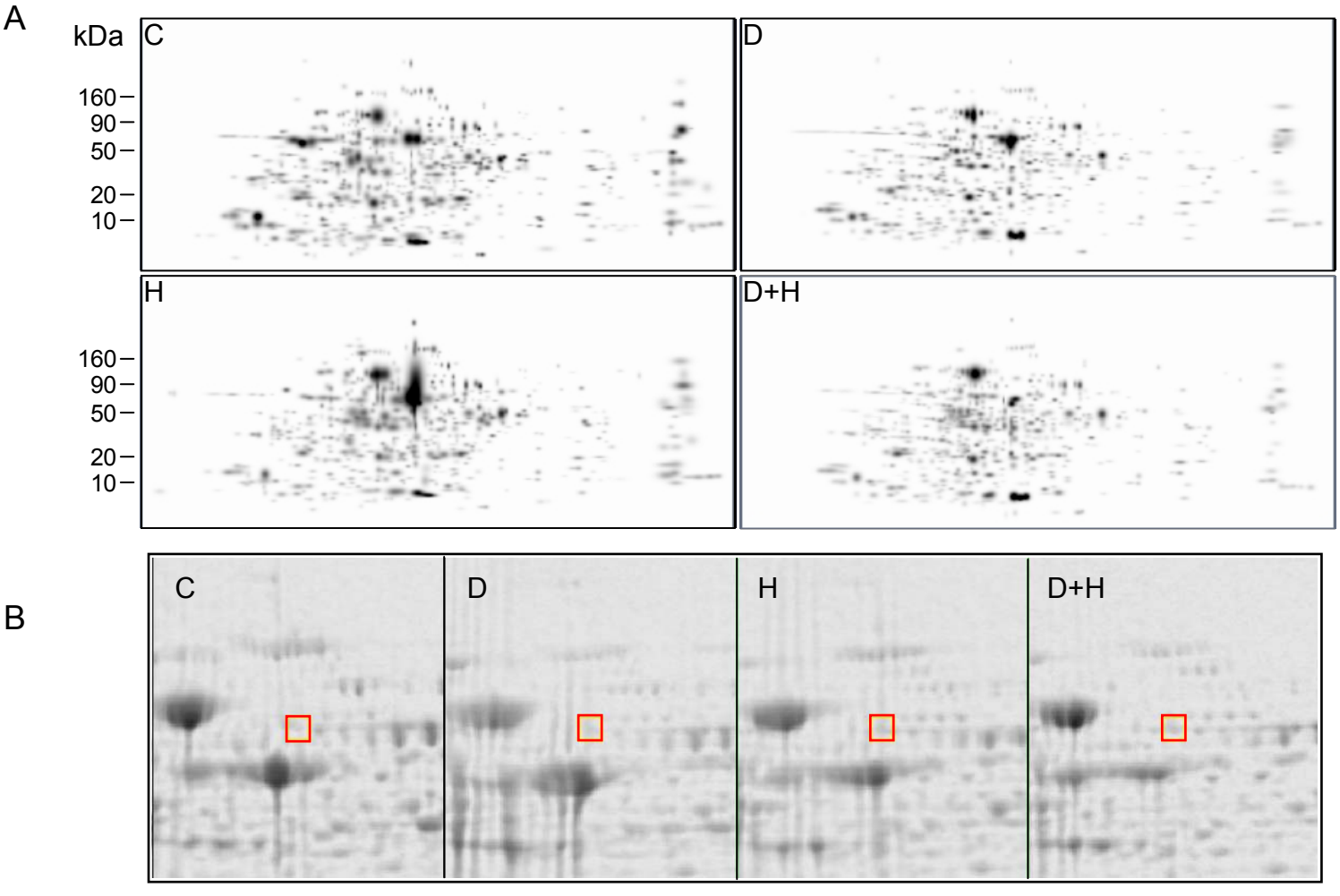

Figure S2. A. Two-dimensional gels of *Arabidopsis* soluble proteins obtained from control (C), drought-stressed (D), heat-stressed (H), or plants subjected to a combination of drought and heat stress (D+H). Proteins were first separated on a pH 3-10 IEF strip and then ran on a 4-20% SDS-PAGE. Images presented are gels created *in silico* by matching three replicate gels for each treatment. B. Magnification of two-dimensional gels depicting spot number 4809 containing ME2. Gels shown are one replication out of three for control, drought, heat or drought and heat combination gels.

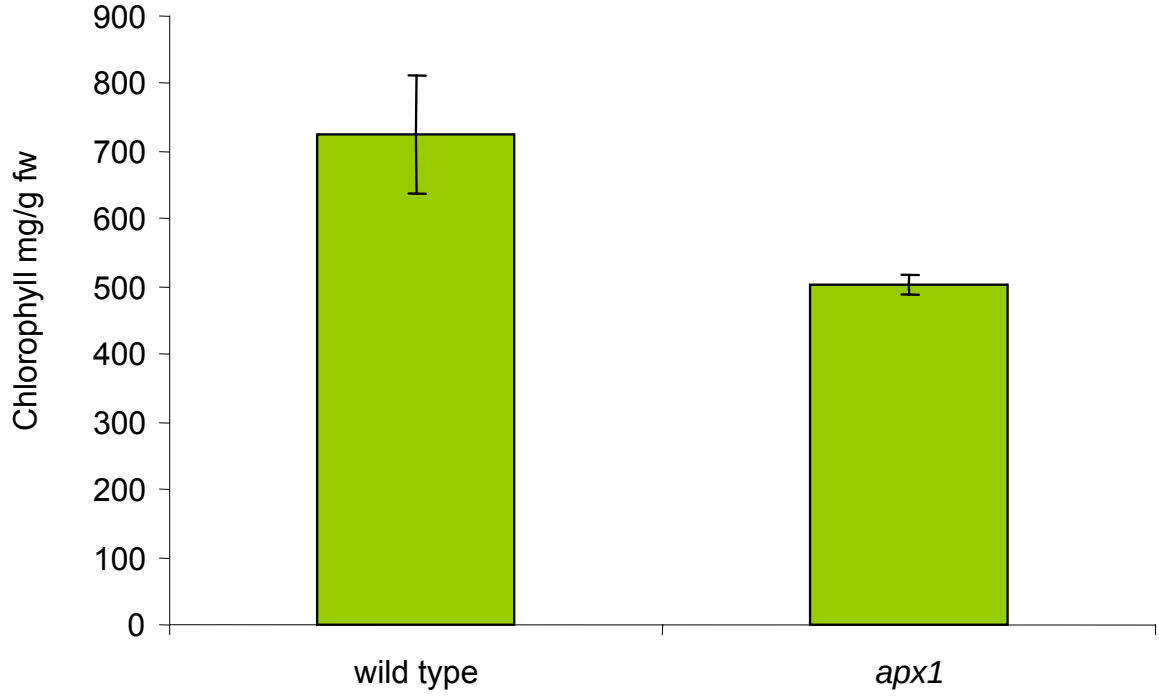

Figure S3. Chlorophyll concentration in wild type and *apx1* seedlings 1 week after a combined heat and drought stress.

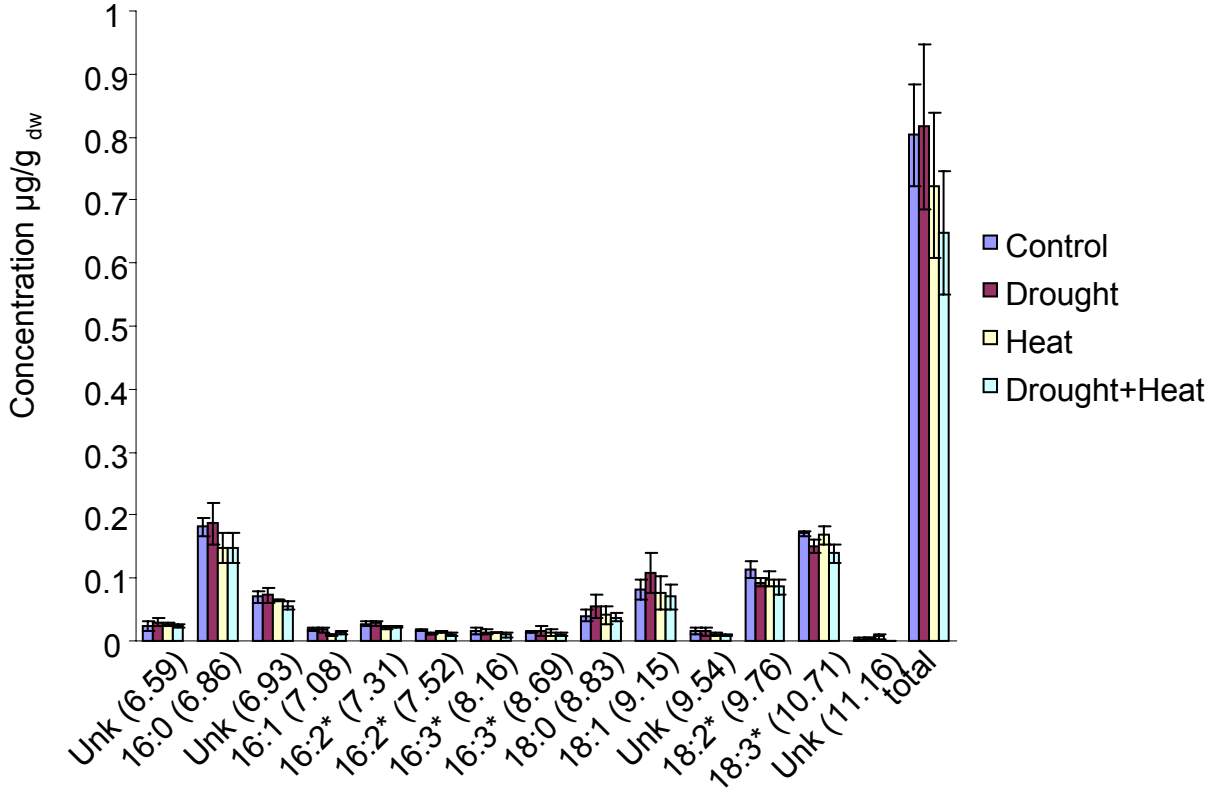

Figure S4. Accumulation of fatty acids in plants in response to drought, heat, or a combination of drought and heat. Numbers in parenthesis are the retention times for each compound, * indicates fatty acids that were identified only by a data base search, unk – unknown compounds that are likely fatty acids (based on mass spec).

For fatty acids determination, 50 mg tissue was ground in 2 ml of 50 mM Hepes (pH 7.9), 3 mM MgCl and added to a 1:2.5 mixture of chloroform/methanol solution containing 0.02% butylated hydroxytoluene, 250 ng of heptadecanoic acid (C17) were added to each sample as standard. Ratio of chloroform/methanol/buffer was adjusted until a strong emulsion developed. After centrifugation at 2000 rpm for 5 min the chloroform fraction was transferred to a new tube and evaporated with N2. Fatty acid methyl esters (FAME's) were prepared by re-suspending the dried fatty acids in 1.5 ml BF3 in methanol, aerating in N2, capping and heating to 55o C for 15 min. After samples were cooled 3 ml of petroleum ether were added and after vortex the top layer was transferred to a new tube containing a drying reagent (Na2SO4/NaHCO3 1:1 w/w) to remove methanol and neutralize the BF3. Samples were then dried to completeness under a stream of N2. The FAME's were reconstituted in 200 µl of carbon disulfide and placed into auto sampler vials with 250 µl inserts prior to mass spectral analysis. The GC-MS analysis was conducted on a Trace (ThermoFinnigan, San Jose; CA) GC interfaced with a Polaris Q ion trap mass spectrometer (ThermoFinnigan, San Jose; CA). The FAME's were separated using a 60 meter HP-INNOWAX column with an I.D. of 0.25 mm and 0.25 µm film thickness. The oven was set at an initial temperature of 200° C and immediately ramped up to 240° C at a rate of 5° C/min and then held at the final temperature for 30 minutes. The mass spectrometer was used in EI mode at 70eV with a scan range of 40 – 450 m/z with an ion source temperature of 200° C. Sample identification was done using authentic standards and the NIST2002 library.

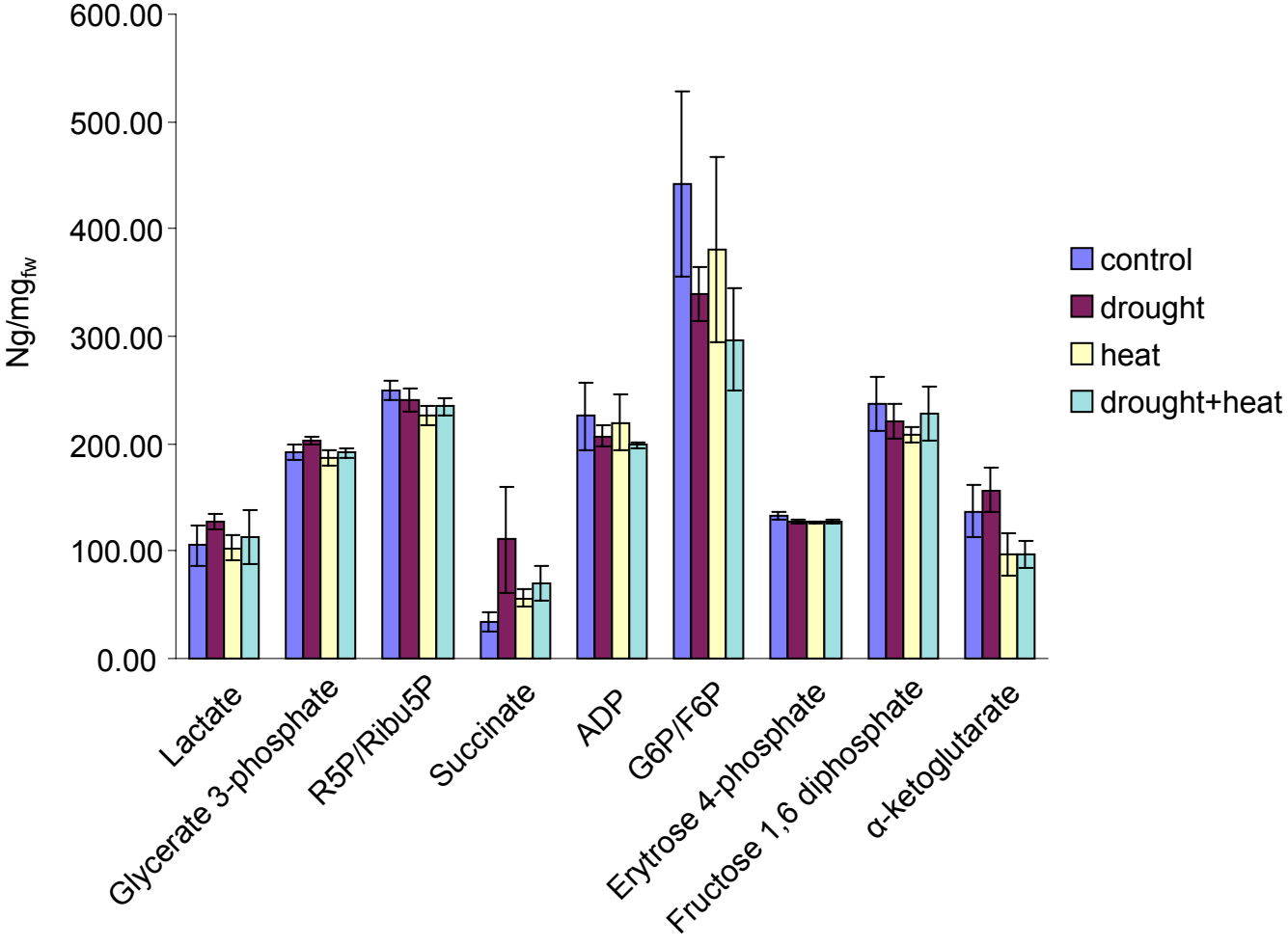

Figure S5. Accumulation of metabolites in plants in response to drought, heat, or a combination of drought and heat.