Supplemental Data

Title: Drought induces distinct growth response, protection and recovery mechanisms in the maize leaf growth zone

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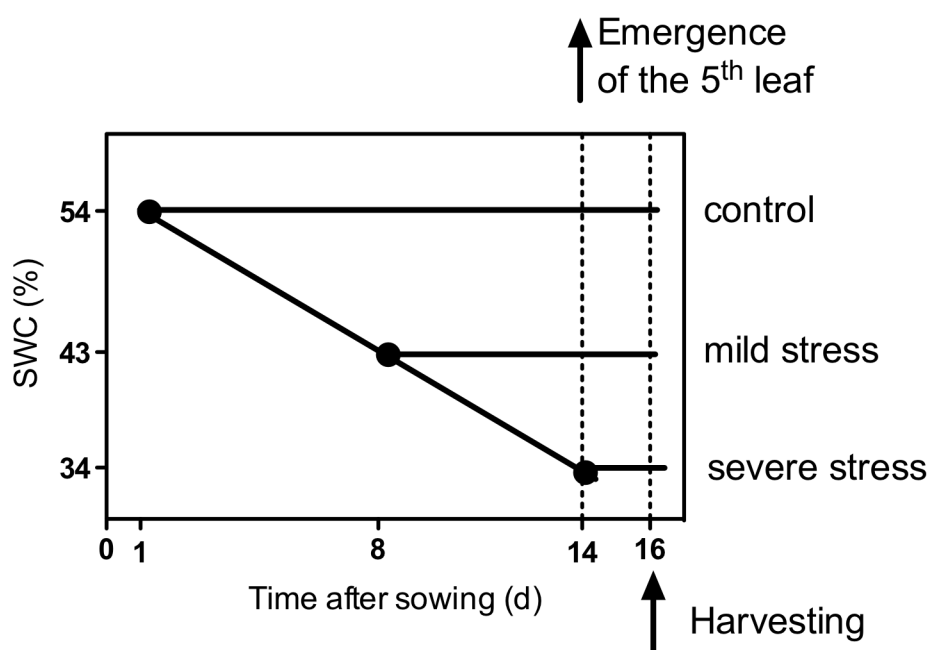
Figure S1. Soil Water Content (SWC) During the Growth Experiment. The symbols show the starting point of adding water in order to maintain the same SWC until the end of the experiment. The arrows show the time of emergence of the fifth leaf (when the soil conditions are stable in all treatments) and the time of harvesting and analysis (three days after emergence, during steady-state growth).
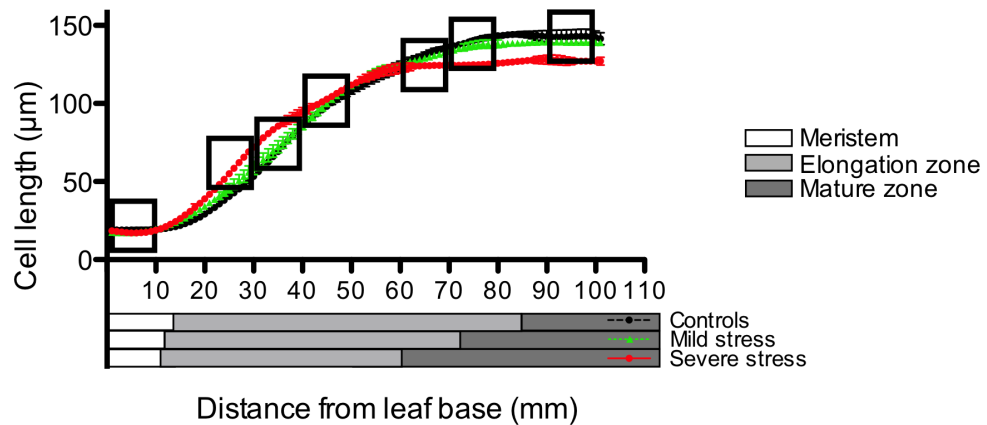
Figure S2. The Effect of Drought on the Cell Length Profile. The length of each developmental zone (meristem, elongation, mature) is marked for each condition (control, mild and severe drought stress). The boxes indicate where the sampling for the microarray analysis of equivalent developmental stages was done for each of the treatments. Data are averages +/- SE (n=5).
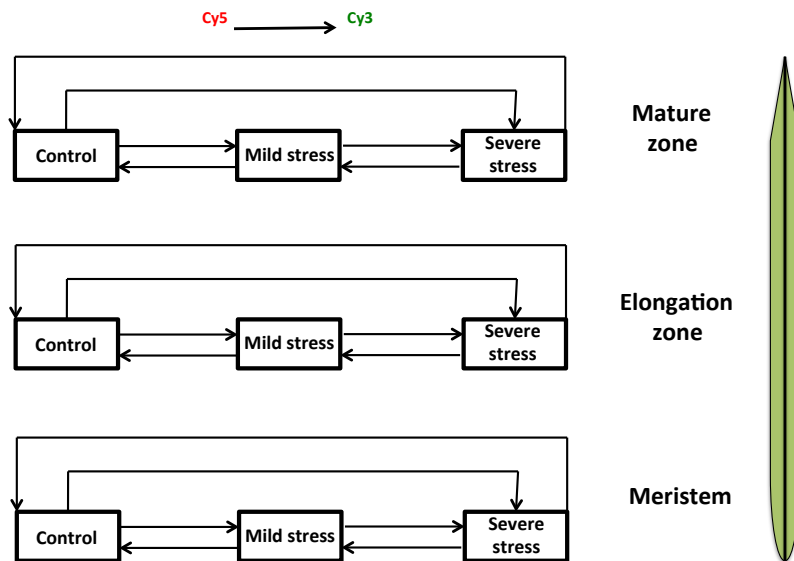

Figure S3. Hybridization Design. Three separate hybridization loops, each comprising 6 arrays, were used in order to directly contrast the three treatments (control, mild and severe drought stress) within each of the three zones (meristem, elongation zone and mature zone) of the growing maize leaf 5.
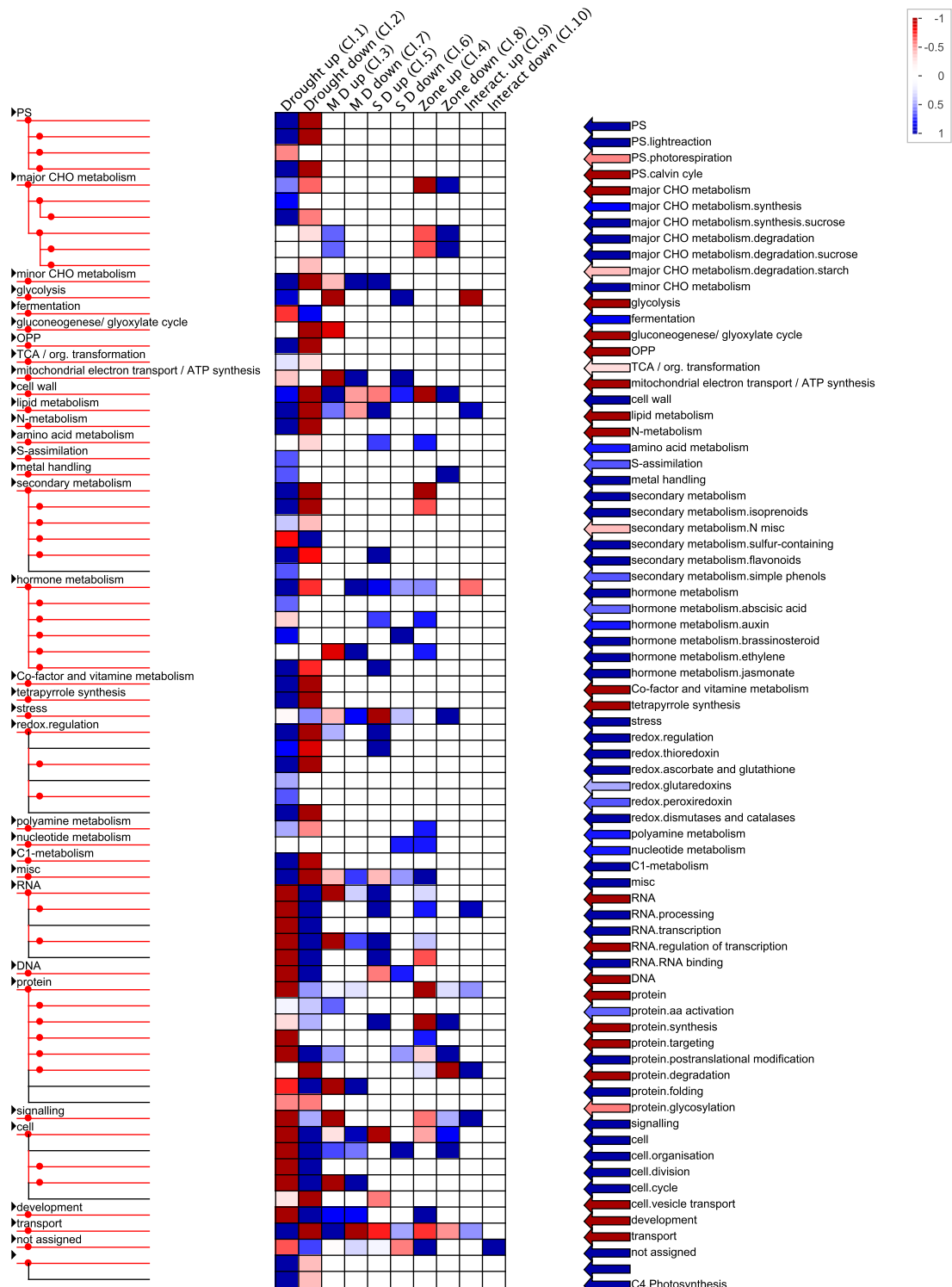
Figure S4. Gene Enrichment Analysis. GO categories, enriched among genes specifically affected by the drought stress conditions (clusters 1 and 2), specifically affected by mild drought stress (MD, clusters 3 and 7), and severe drought stress (SD, clusters 5 and 6), differentially expressed between the zones (clusters 4 and 8), and

affected by both stress and zone (clusters 9 and 10), ORA-Fisher test, PageMan. Blue color indicates overrepresentation and red: underrepresentation. The data represents all the significant genes from the two-way ANOVA analysis across all the zones from Fig. 1 A and C and the sample labels include cluster numbers from Fig. 1C (Cl.x).

Table S1. An Overview of Core Cell Cycle Genes in Maize. The table contains newly and previously described genes (1- annotated from the current dataset, by combining different databases as http://www.maizesequence.org, UNIPROT (http://www.uniprot.org/) and Plaza (http://bioinformatics.psb.ugent.be/plaza/), 2-Rymen et al. (2007), 3-Kakumanu et al. (2012)). A two-way ANOVA statistical test was applied to compare the gene expression in the three zones of the leaf (meristem, elongation and mature zone), and the three treatments (control, mild stress, severe stress) and the significant changes are marked in bold.

Zm GeneID	Gene Name	Synonym(s)	EST Accession No.	Protein Accession number	Agilent ID	Above BG level	Log2FC mild stress	Log2FC severe stress	Source of the gene description
GRMZM2G012220	*apc 1*		TC290187		A_92_P041111	no			1
GRMZM2G168886	*apc2*		TC286503		A_92_P012604	yes	**-0.504080092**	**-0.710388925**	1
GRMZM2G147603	*apc6;1*		TC283416	B4FWL0	A_92_P002299	no			1
GRMZM2G166684	*apc6;2*		CO454987		A_92_P013238	yes	**-1.507805192**	**-1.567607508**	1
GRMZM2G089296	*apc7*		TC306721		A_92_P021833	yes	**-1.059457833**	**-1.100621958**	1
GRMZM2G054247	*apc10*		TC311895	B6T5J2	A_92_P000813	yes	**-0.7096533**	**-0.649567683**	1
GRMZM2G162356	*apc11*		TC298221	B6TKR6	A_92_P017143	yes	**-1.1472785**	**-1.407176083**	1
GRMZM2G170591	*cdc23/Apc8*		TC313616	C4J4C0 B6SL38	A_92_P003265	yes	**-1.0721**	**-1.1293228**	1
GRMZM2G063192	*cdc20*		AI612495	B6U240	A_92_P008432	yes	**-3.052313992**	**-3.164055758**	1
GRMZM2G011520	*cdc25*		TC300846	B6TQK1	A_92_P022295	yes	0.193294458	**1.1379215**	2
GRMZM2G008327	*cdka1;1*	*Cdc2Zma*	TC292976	P23111	A_92_P026243	yes	-0.117478458	**-1.001422758**	2
			TC299222		A_92_P004318	yes	**0.429979533**	**0.465050175**	
GRMZM2G174596	*cdka2;1*		TC292977	D8L819	A_92_P008472	yes	**-1.467583108**	**-2.143914808**	2
GRMZM2G495626	*cdkb1;1*		TC287283	B4FWP9	A_92_P031231	yes	**-2.354959075**	**-2.656077792**	2
GRMZM2G070115	*cdkb2;1*		TC311057	C5Z786	A_92_P028552	no			2
GRMZM2G019267	*cdkc2*		TC304893	B4F9F3	A_92_P012950	yes	-0.174549233	-0.255549058	2
GRMZM2G149286	*cdkd1*		TC281529	B4FQK6	A_92_P025853	yes	**-0.171294083**	**-0.090427042**	2
GRMZM2G166771	*cdke1*		TC279290	B6TV49	A_92_P006921	no			2
GRMZM2G458799	*csk1*		TC292250		A_92_P013788	yes	**-2.621892458**	**-3.164654208**	2
GRMZM2G038485	*cks2*		TC312708	B6SLG7	A_92_P022467	yes	-0.051676692	-0.132153567	2
no seq	*cyca1;1*	*cyclIzm*	TC286394	Q41732	A_92_P031387	yes	**-3.271668908**	**-3.346761583**	2
GRMZM2G007071	*cyca1;2*	*cycZm2w*		Q43693	not on the array				2
GRMZM2G017081	*cyca2*		TC296008	C0HG87	A_92_P018228	yes	**-0.931325742**	**-0.778075267**	3
			TC313381		A_92_P009756	yes	**-1.903091842**	**-2.084325633**	
GRMZM2G060690	*cyca3;1*		CD447837	B6SKE9	not on the array				2
no seq	*cyca3;2*		TC292456		not on the array				2
GRMZM2G363437	*cyca3;3*		TC298919	B4FJ13	A_92_P013164	no			2
no seq	*cyca3;4*		TC298903		not on the array				2
GRMZM2G310115	*cycb1;1*	*cyclazm*	TC299155	Q41734	A_92_P007960	yes	**-1.865066317**	**-1.736511725**	2
GRMZM2G034647	*cycb1;2*	*cyclbzm*	TC288893	Q41733	A_92_P034339	yes	**-1.196039983**	**-1.079251292**	2
GRMZM2G005619	*cycb1;3*	*cycZme1*	TC295005	P93646	A_92_P027581	yes	**-1.496771358**	**-1.606615092**	2
GRMZM2G073671	*cycb2;1*	*cyclIIzm*	TC311432	Q41731	A_92_P019388	yes	**-2.198124142**	**-2.42463425**	2
GRMZM2G138886	*cycb2;2*		TC300622	C0P7P1	A_92_P009394	no			2
GRMZM2G476685	*cycd1;1*		TC284350	C0PDU6	A_92_P022470	no			2
			TC308600		A_92_P021047	yes	**-1.501842067**	**-2.759286575**	
GRMZM2G140633	*cycd1;2*	Cyclin delta-2	TC306119	B6TJ83	A_92_P004507	yes	**-1.773628525**	**-2.27767225**	3
GRMZM2G075117	*cycd2;1*	*CYCD2*	TC304897	Q8S524	A_92_P026759	yes	**0.110775708**	**-0.341425825**	2
			TC304890	Q8S524	A_92_P016104	yes	**0.1238227**	**-0.312027575**	
GRMZM2G133414 GRMZM2G133413	*cycd4;1*	*CYCD2;2, CYCD4*	TC306118	Q8S522 B8A1Q3	A_92_P012291	yes	**-1.4858**	**-2.7197073**	2
GRMZM2G178229	*cycd4;2*		TC290220	Q8S521	A_92_P018225	no			2
GRMZM2G006721	*cycd5;1*	*CYCD1*	TC301115	Q8S523	A_92_P038772	yes	**-0.82457225**	**-1.269019125**	2
GRMZM2G047637	*cycd5;2*		TC289521	C4J362	A_92_P041431	no			2
GRMZM2G050933	*cycd6*		TC291551	B6TAD6	A_92_P022300	yes	**0.213735167**	**-0.795834175**	1
GRMZM2G361662	*cych*		TC282470	B4G1I4	A_92_P034633	no			2
GRMZM2G361659	*del1*		TC295278	C0HFS9	A_92_P002096	no			2
GRMZM2G060000	*del2*		TC306241	C5Z7K6	A_92_P001624	yes	**-0.808996383**	**-0.8768665**	2
GRMZM2G462623	*dpa*		BE511883	C5XGP7	A_92_P031704	no			2
GRMZM2G139024	*dpb*		TC301910	B4FHH8	A_92_P020522	no			2
no seq	*dpc*		TC312155		not on the array				2
GRMZM2G086072	*dpd*		TC312156	B6TKJ8	A_92_P001200	yes	**-1.144267608**	**-1.784398208**	2
GRMZM2G050590	*e2f*		TC309263	C0P8W8	A_92_P010968	no			2
GRMZM2G116885	*krp1*			Q52PU9	not on the array				2
GRMZM2G101613	*krp2*	*cdki2*	TC297785	B6TIJ9 Q52PU8	A_92_P001257	yes	**2.46915**	**1.23848373**	2
GRMZM2G358931	*krp3*		TC306015	B4FGI1	A_92_P020763	yes	**-1.866751292**	**-1.333209808**	2
GRMZM2G037926	*krp4*		TC301833	C0P5I7	A_92_P036889	yes	-0.063066883	0.210832267	2
GRMZM2G162445	*mcm3*		TC293332	Q9SX02	A_92_P002202	yes	**-2.109759167**	**-2.2289333**	1
GRMZM2G100639	*mcm3;2*		TC293331	Q9SX04	A_92_P030312	yes	**-2.347820525**	**-2.521074542**	1

GRMZM2G066101	*mcm4;1*		TC311506	B7ZWV7	A_92_P011634	yes	**-1.997177717**	**-2.372373575**	1
GRMZM2G327032	*mcm4;2*		TC311507	C0PDH6	A_92_P011265	yes	**-2.076762692**	**-2.350265817**	1
GRMZM2G450055	*mcm5*		TC285664	C0HEP3	A_92_P039239	yes	**-2.23014045**	**-2.616655892**	1
GRMZM2G021069	*mcm6*		TC298385	Q5I285	A_92_P040623	yes	**-2.214225608**	**-1.866520958**	1
GRMZM2G065205	*mcm7*		TC285635	C0HFI8	A_92_P038901	yes	**-2.138947442**	**-2.30942825**	1
GRMZM2G133819	*mcm10*		TC289444	B6TE84	A_92_P010931	yes	**0.57351515**	**0.742147967**	1
GRMZM2G033828	*rbr1;1*	*RBR3*	TC282517	Q3LXA7	A_92_P007012	yes	**-2.282670625**	**-2.525713258**	2
GRMZM2G003043	*rbr2;1*	*RRB1*		O22344					2
		RBR1	TC281205	Q9LKX9	A_92_P017970	yes	0.017739533	0.213705775	
		RB1		Q7DLV4					
		ZmRB		Q41763					
GRMZM2G153150	*rbr2;2*	*RRB2*	TC281207	Q8H0J6	A_92_P029071	yes	-0.075069733	0.084041992	2
		RBR2a		O22346					
		RBR2b		O22345					
GRMZM5G878541	*wee1*		TC294770	Q9SPZ8	A_92_P023756	yes	**-0.66306805**	**-0.807602108**	2
1 Annotated by combining different sources as http://www.maizesequence.org, UNIPROT and http://bioinformatics.psb.ugent.be/plaza/;2 Rymen *et al*,2007;3 Kakumanu *et al*, 2012									

Table S2. An Overview of Expression Levels of Genes, Coding Different Isoforms of the Key Redox Enzymes in Response to Mild and Severe Drought Stress in the Different Positions of the Leaf Growth Zone. A two-way ANOVA statistical test was applied to compare the gene expression in the three zones of the leaf (meristem, elongation and mature zone), and the three treatments (control, mild stress, severe stress) and the significant changes are marked in bold.

| ZM Gene ID	Agilent ID	EST Accession Number	Above BG level	Name	Log2 FC mild stress	Log2 FC severe stress	log2FC elongation-meristem	log2FC mature-meristem
CAT								
GRMZM2G088212	A_92_P035919	TC292697	yes	*cat1*	**0.5710345**	**0.962720625**	-0.114605625	-0.181144875
GRMZM2G090568	A_92_P010403	TC312061	yes	*cat2*	**0.2504552**	**3.963655892**	-0.650855033	-0.292111025
GRMZM2G079348	A_92_P019193	TC309409	yes	*cat3*	**-0.921432633**	**-1.060040358**	-0.095424975	0.093026933
SOD								
GRMZM2G042080	A_92_P016286	TC295662	yes	*iron superoxide dismutase*	**1.236105333**	**1.4377495**	0.000627208	-0.36237275
	A_92_P035479	TC307686	yes		**1.102884533**	**1.340133683**	**-0.199645675**	**-0.810436708**
GRMZM5G864424	A_92_P009291	TC312005	yes	*iron superoxide dismutase 3*	**1.274814**	**1.677618125**	0.143398833	-0.139173333
GRMZM2G059991	A_92_P019990	CD956453	no	*Superoxide dismutase [Mn] 3.1, mitochondrial*				
GRMZM2G124455	A_92_P027370	TC301296	yes	*Superoxide dismutase [Mn] 3.4, mitochondrial*	**-0.177955458**	**-0.23672975**	**0.243224625**	**0.052382417**
GRMZM5G891739	A_92_P037327	TC286793	yes	*Superoxide dismutase [Cu-Zn]*	**0.945944517**	**2.319420658**	-0.196884758	-0.441819842
GRMZM2G106928	A_92_P007498	CO443266	yes	*Superoxide dismutase [Cu-Zn]*	**1.378868875**	**2.117176667**	-0.15551075	-0.345029333
GRMZM2G025992	A_92_P007360	CB886186	no	*Superoxide dismutase [Cu-Zn] 2*				
GRMZM2G169890	A_92_P013190	TC304922	no	*Superoxide dismutase [Cu-Zn] 4A*				
GRMZM2G058522	A_92_P040075	TC299523	no	*Superoxide dismutase [Cu-Zn] 4AP*				
NOX								
GRMZM2G426953	not on the array			*Zmrboh A*				
	A_92_P031602	TC311766	yes		**0.642347508**	**1.191182208**	-0.239258867	-0.219641942
GRMZM2G138152	A_92_P019915	TC282008	yes	*Zmrboh B*	**0.611732917**	**1.135101075**	-0.006003992	0.111273283
	not on the array							
GRMZM2G431506	not on the array							
GRMZM2G022547	A_92_P035351	CF036497	yes	*respiratory burst oxidase protein b*	**0.384864467**	**0.278254392**	0.000399783	0.0096326
	A_92_P017675	DR818840	yes		**0.419170917**	**0.049476467**	0.137440742	0.377926242
GRMZM2G034896	A_92_P027911	TC306238	yes	*respiratory burst oxidase-like protein B2*	0.154802917	0.367331117	0.107933642	0.316032492
GRMZM2G043435	A_92_P039989	TC281675	no	*Zmrboh C*				
	A_92_P024538	TC306864	no					
	A_92_P001607	CO448228	no					
GRMZM2G441541	A_92_P011018	CA452791	no	*Zmrboh D*				
	A_92_P007144	TC294962	no					
POX								
GRMZM2G104394	A_92_P008062	TC295344	yes	*pmpox1*	**-0.288150792**	**-1.100907267**	0.1572933	0.017432467
GRMZM2G104109	A_92_P031214	BM500461	yes	*peroxidase1*	**0.316913533**	**0.274578967**	-0.036847142	-0.097476508
GRMZM2G126261	A_92_P022169	TC306090	yes	*pmpox3-1*	**0.324803408**	**2.4549677**	0.363149925	-0.415227792
GRMZM2G133475	A_92_P019188	TC302528	yes	*pmpox3-2*	**1.691313725**	**3.569904925**	-0.7038954	-0.10656895
GRMZM2G080689	A_92_P009418	TC294882	yes	*peroxidase52*	**0.107482783**	**-0.6200205**	**0.552242242**	**0.649995417**
GRMZM2G382379	A_92_P010677	CO442337	yes	*peroxidase68*	0.204101792	0.028471725	0.178375375	0.704254317
LOX								
GRMZM2G156861	A_92_P015840	BE051732	yes	*lox2*	**1.746592683**	**2.533807342**	**0.21244645**	**0.7784179**
	A_92_P017031	TC299182	yes		**3.203277475**	**3.5925821**	-0.241316333	0.889223458
	A_92_P015218	TC299191	yes		**2.020845833**	**2.580663333**	**0.127247917**	**1.07472375**
GRMZM2G109056	A_92_P007859	TC299188	yes	*lox4*	-0.411169917	-0.344087708	0.474599917	0.400542083
GRMZM2G102760	A_92_P024692	TC299185	yes	*lox5*	-0.051442042	0.241035542	-0.288532183	0.042697458
GRMZM2G040095	A_92_P018509	TC290587	yes	*lox6*	**0.474666817**	**0.729746133**	**0.003948208**	**0.396724317**
	A_92_P007454	TC294707	yes		**0.826932775**	**1.936698392**	-0.095991492	0.183463533
GRMZM2G015419	A_92_P041145	TC298883	yes	*lox10*	**-0.08685205**	**2.109618167**	**-0.105175758**	**0.823767425**
GRMZM2G009479	A_92_P018298	TC298881	yes	*lox11*	**-2.486035833**	**-1.812820158**	-0.277310692	0.000970775
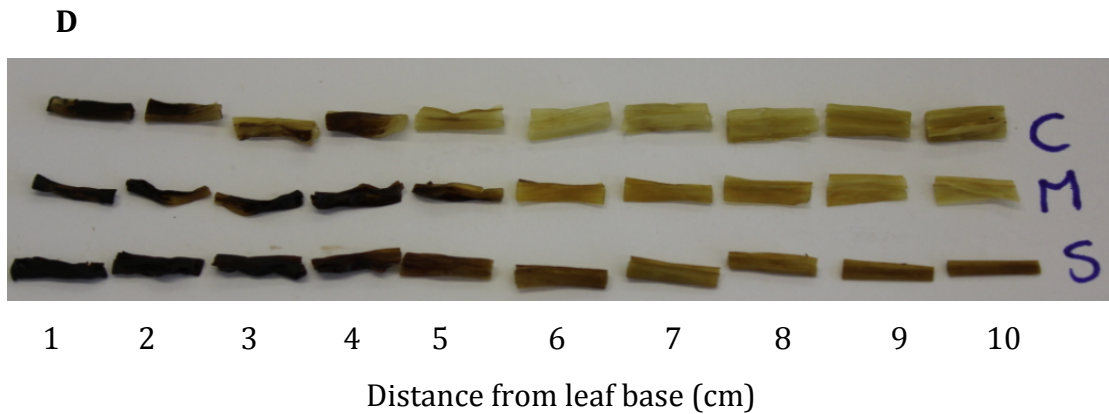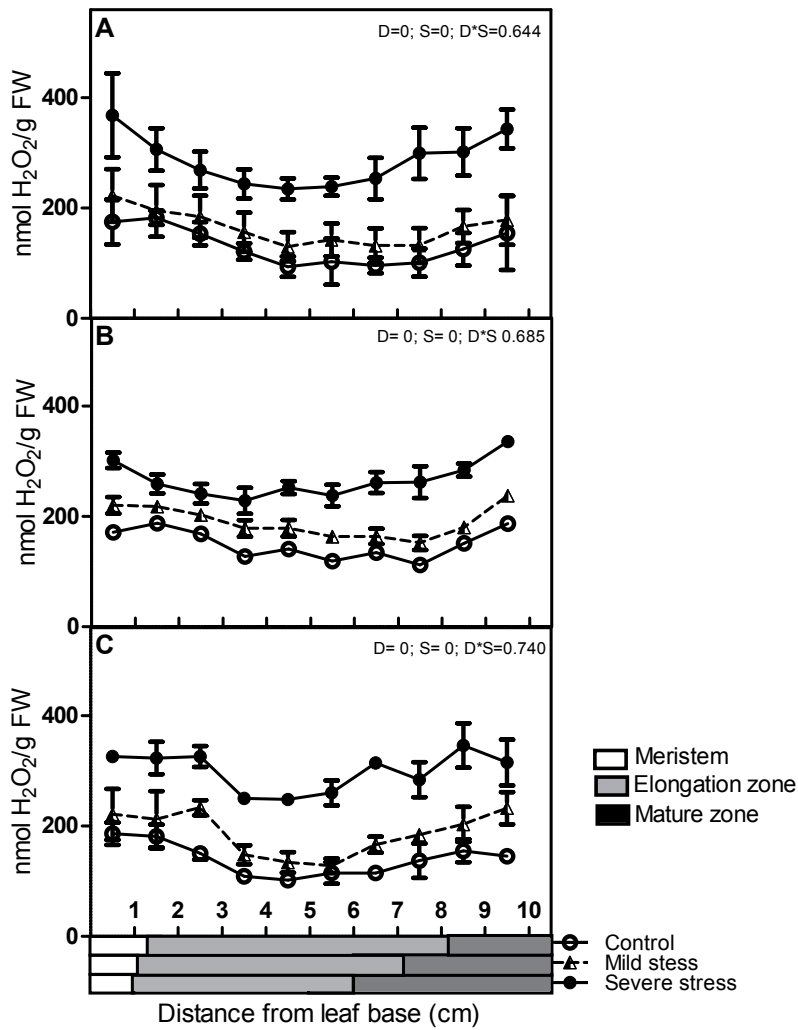
Figure S5. Comparison Between Different Methods of Determination of H$_2$O$_2$ Concentration Across the Growth Zone of Maize Leaves, Subjected to Mild and Severe Drought Stress.

(A) Extraction in phosphate buffer (pH=6.5), containing catalase inhibitor (1 mM hydroxylamine) followed by quantification with xylenol orange reagent. **(B)** Extraction in 5% TCA, followed by quantification with xylenol orange reagent. **(C)** Extraction in phosphate buffer (pH=6.5), containing catalase inhibitor (1 mM hydroxylamine), followed by a quantification with the Amplex red hydrogen peroxide/peroxidase assay (Molecular Probes). **D)** H_2O_2 localization trough 3,3'-diaminobenzidine **(**DAB) staining of the growth zone of maize leaves, from control (C), mildly (M) and severely (S) stressed plants. H_2O_2 content measurements by extracting H_2O_2 in 5% TCA. A two-way ANOVA was used as a statistical test and p values for the two factors, drought (D) and segment (S), as well as the interaction between them, are present on each graph panel. Data are averages +/- SE (n=5). The length of each developmental zone (meristem, elongation, mature) in each treatment (control, mild, and severe stress) is marked on the x axes of the graphs according to Figure S2.

References

Kakumanu A, Ambavaram MMR, Klumas C, Krishnan A, Batlang U, Myers E, Grene R, Pereira A (2012) Effects of Drought on Gene Expression in Maize Reproductive and Leaf Meristem Tissue Revealed by RNA-Seq. Plant Physiology **160:** 846-867

Rymen B, Fiorani F, Kartal F, Vandepoele K, Inze D, Beemster GTS (2007) Cold nights impair leaf growth and cell cycle progression in maize through transcriptional changes of cell cycle genes. Plant Physiology **143:** 1429-1438