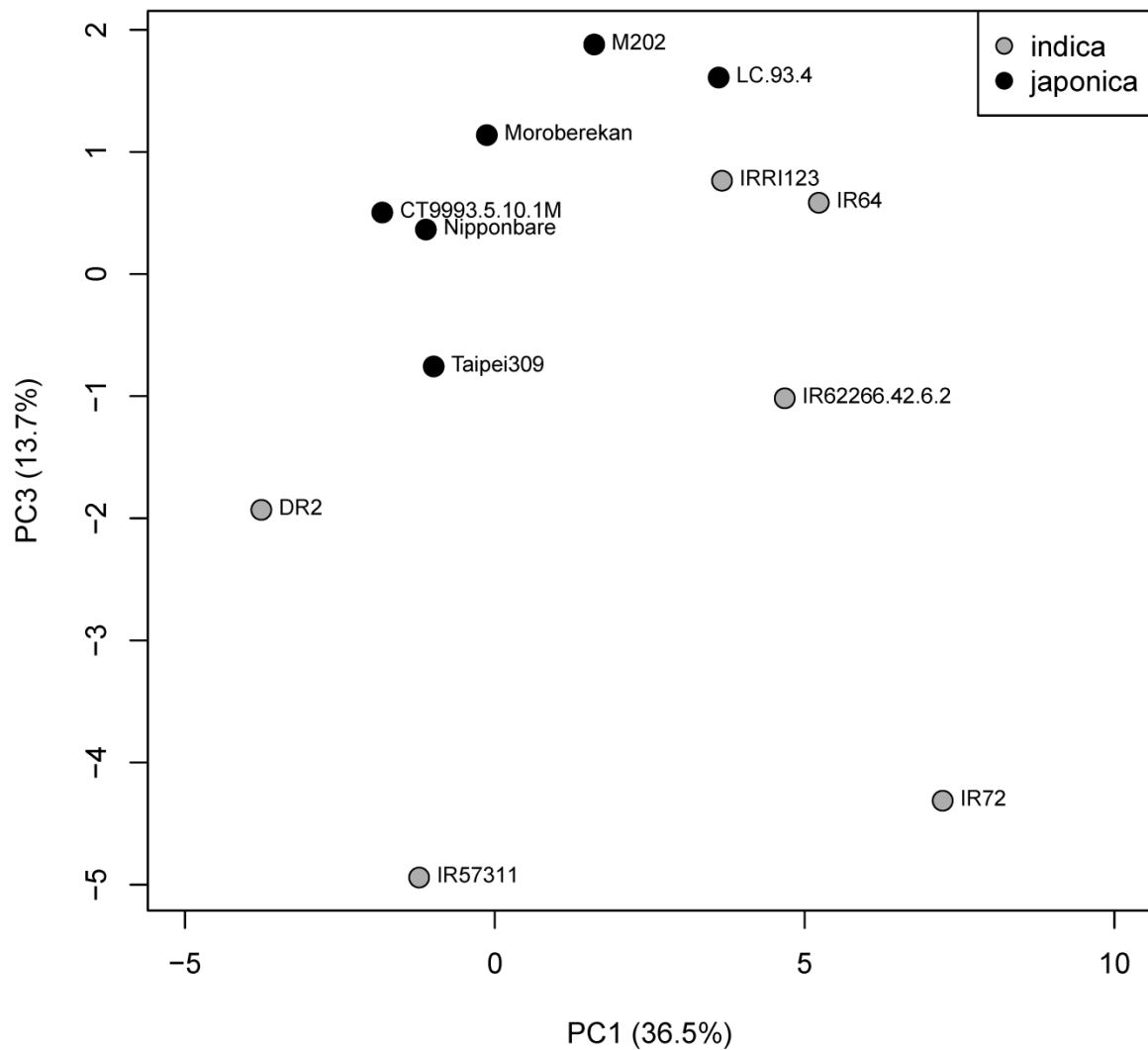


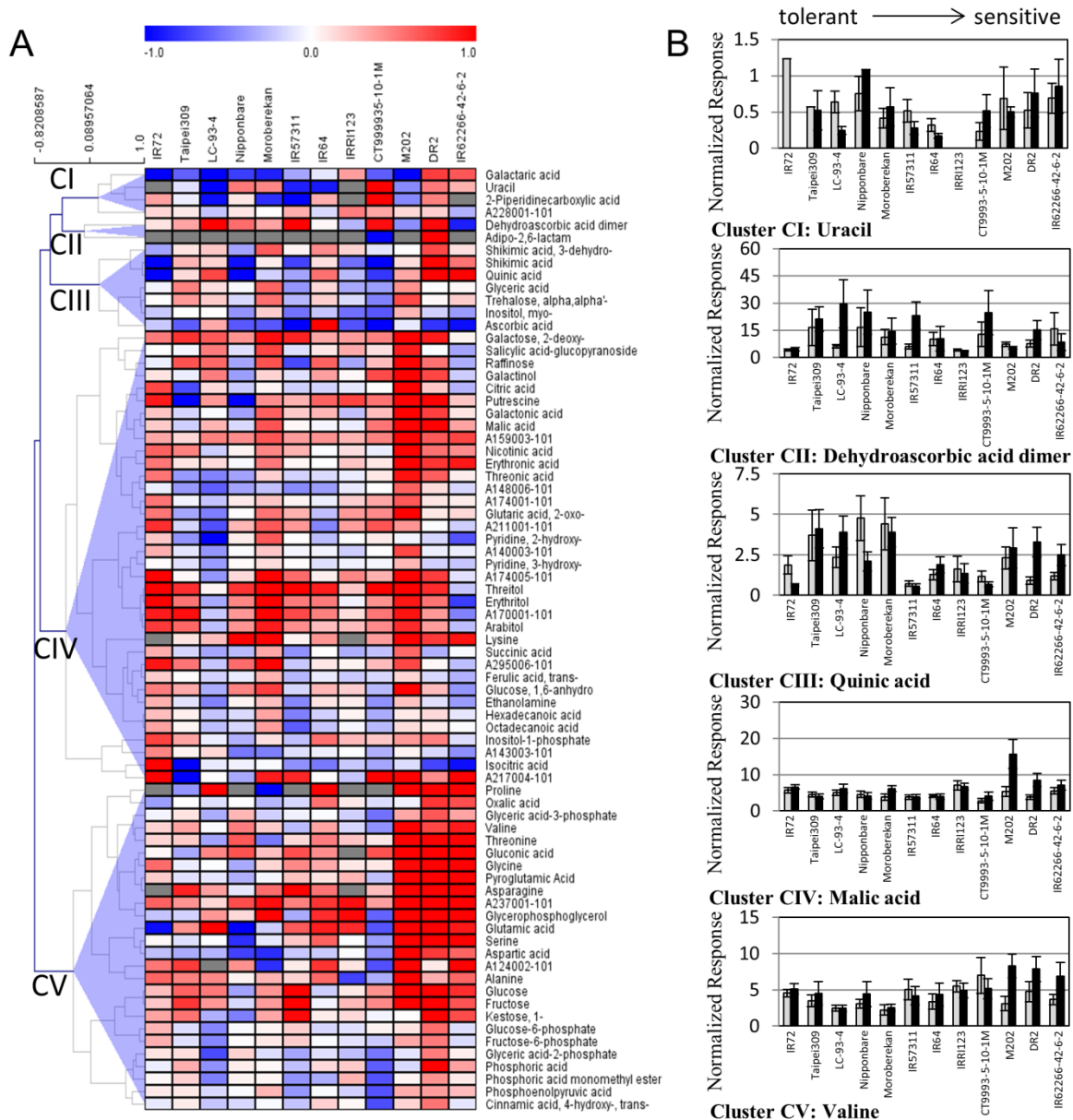
## High night temperature strongly impacts TCA cycle, amino acid and polyamine biosynthetic pathways in rice in a sensitivity dependent manner

Ulrike Glaubitz, Alexander Erban, Joachim Kopka, Dirk K. Hincha, Ellen Zuther



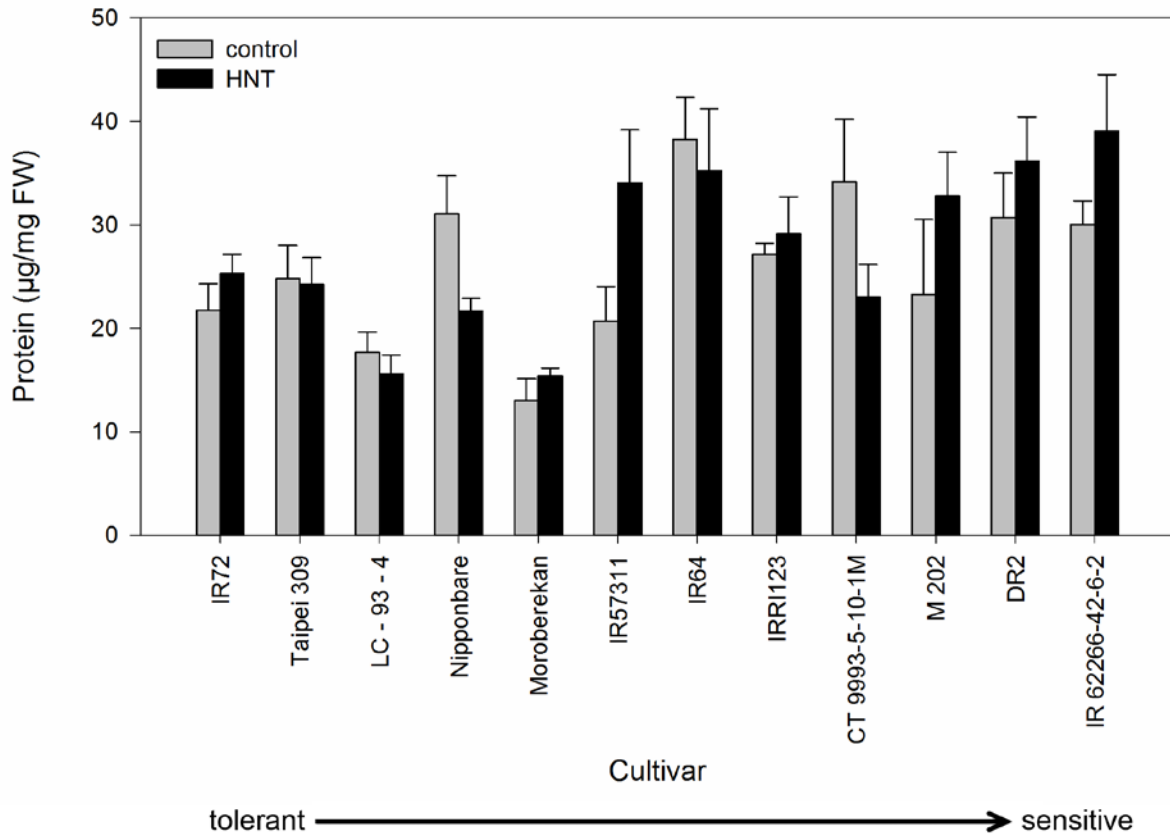
### Supplementary Figure S1

Principal Component Analysis (PCA) of metabolite profiles measured by GC-MS after 48 DAS under control conditions in 12 cultivars of rice.



### Supplementary Figure S2


(A) Hierarchical cluster analysis (HCA) with Pearson correlation used for  $\log_2$  fold changes of 75 metabolites measured by GC-MS after 23 days (48 DAS) of HNT in comparison to control conditions in 12 cultivars of rice. (B) Normalized responses of selected metabolites represent the response patterns of clusters CI to CV.



### Supplementary Figure S3

Protein content in leaves of 12 rice cultivars after 48 DAS under control and 23 days (48 DAS) under HNT

**Supplementary Table 1.** Cultivars of *Oryza sativa* L. used for HNT stress experiments. Seeds with two origins were propagated in the latter one.

<b>Cultivar</b>	<b>Species</b>	<b>Origin</b>	<b>Mean Rank</b>		
IR72	<i>Oryza sativa ssp. indica</i>	IRRI	4.1	tolerant	
Taipei 309	<i>Oryza sativa ssp. japonica</i>	IRRI, MPIMP	7.5		
LC-93-4	<i>Oryza sativa ssp. japonica</i>	IBT Vietnam, MPIMP	10.3		
Nipponbare	<i>Oryza sativa ssp. japonica</i>	IRRI, MPIMP	10.4		
Moroberekan	<i>Oryza sativa ssp. japonica</i>	IRRI	14.3		
IR57311	<i>Oryza sativa ssp. indica</i>	IRRI, MPIMP	19.1		
IR64	<i>Oryza sativa ssp. indica</i>	IRRI, MPIMP	20.8		
IRRI123	<i>Oryza sativa ssp. indica</i>	IRRI	26.5		
CT9993-5-10-1	<i>Oryza sativa ssp. japonica</i>	IRRI, MPIMP	26.5		
M202	<i>Oryza sativa ssp. japonica</i>	IRRI	44.1		
DR2	<i>Oryza sativa ssp. indica</i>	IBT Vietnam, MPIMP	45.2		
IR62266-42-6-2	<i>Oryza sativa ssp. indica</i>	IRRI, MPIMP	45.5		sensitive

**Supplementary Table 3.** Top ten most positive and negative loadings of PC1 and PC3 separating *indica* and *japonica* cultivars in a PCA of metabolite profiles measured by GC-MS after 48 DAS under control conditions.

PC1		PC3	
Galactaric_acid	0.717	Adipo_26_lactam	0.380
Adipo_26_lactam	0.218	Fructose	0.338
Raffinose	0.124	A217004_101	0.335
Gluconic_acid	0.089	Glucose	0.319
Cinnamic_acid_4_hydroxy_trans	0.086	Kestose_1_	0.294
Uracil	0.068	Proline	0.197
Glyceric_acid	0.059	Quinic_acid	0.188
Galactinol	0.049	Galactaric_acid	0.165
A228001_101	0.047	Shikimic_acid	0.163
Shikimic_acid_3_dehydro	0.045	A140003_101	0.152
Salicylic_acid_glucopyranoside	0.042	Galactose_2_deoxy	0.088
Erythritol	0.038	Glycine	0.079
Glutamic_acid	0.036	Inositol_myo_	0.076
Glutaric_acid_2_oxo	0.029	A237001_101	0.072
Asparagine	0.028	Cinnamic_acid_4_hydroxy_trans	0.069
Malic_acid	0.028	A174005_101	0.055
Dehydroascorbic_acid_dimer	0.021	Glutaric_acid_2_oxo	0.053
Glyceric_acid_3_phosphate	0.021	Ferulic_acid_trans	0.050
Arabitol	0.019	A211001_101	0.049
Glyceric_acid_2_phosphate	0.018	Inositol_1_phosphate	0.045
Inositol_1_phosphate	-0.014	Shikimic_acid_3_dehydro	-0.046
Fructose_6_phosphate	-0.016	A124002_101	-0.050
Alanine	-0.017	Pyroglutamic acid;Glutamine;Glutamic acid	-0.058
Octadecanoic_acid	-0.017	Citric_acid	-0.060
Aspartic_acid	-0.021	Threonine	-0.061
Pyridine_3_hydroxy	-0.022	Phosphoenolpyruvic_acid	-0.065
Nicotinic_acid	-0.023	Glyceric_acid_2_phosphate	-0.071
2_Piperidinecarboxylic_acid	-0.023	Glyceric_acid_3_phosphate	-0.072
Ethanolamine	-0.025	Glucose_16_anhydro	-0.073
A143003_101	-0.026	Valine	-0.082
Isocitric_acid	-0.033	Ascorbic_acid	-0.083
Threonine	-0.042	Oxalic_acid	-0.087
Lysine	-0.045	Uracil	-0.095
Valine	-0.046	Isocitric_acid	-0.110
Inositol_myo_	-0.047	Aspartic_acid	-0.114
Glycerophosphoglycerol	-0.055	Glutamic_acid	-0.129
Ascorbic_acid	-0.106	Glycerophosphoglycerol	-0.131
A140003_101	-0.134	Salicylic_acid_glucopyranoside	-0.166
A217004_101	-0.238	Raffinose	-0.192
Proline	-0.529	Lysine	-0.197

**Supplementary Table 4.** Spearman correlations calculated for correlations of 75 metabolites with HNT sensitivity (expressed as rank of damage by chlorosis, Glaubitz et al. 2014) under control and HNT conditions as well as the log<sub>2</sub> fold change. P-values < 0.05 are given in italic, p-values < 0.01 are given in bold. Metabolites and analytes are sorted alphabetically.

Metabolite	control		HNT		log <sub>2</sub> FC_MW	
	correlation coefficient r	p-value	correlation coefficient r	p-value	correlation coefficient r	p-value
2-Piperidinecarboxylic acid	-0.153	0.481	0.251	0.128	0.261	0.446
A124002-101	-0.219	0.251	0.097	0.577	0.164	0.614
A140003-101	0.0791	0.42	0.174	0.0821	-0.00699	0.974
A143003-101	0.133	0.175	0.0742	0.46	-0.434	0.15
A148006-101	0.0439	0.655	0.23	<i>0.0206</i>	0.559	0.0547
A159003-101	-0.0667	0.496	0.262	<b>0.00821</b>	0.594	<i>0.0387</i>
A170001-101	0.228	<i>0.0187</i>	-0.00946	0.925	-0.399	0.189
A174001-101	0.307	<b>0.00144</b>	0.252	<i>0.0111</i>	0.217	0.484
A174005-101	0.321	<b>0.00093</b>	0.257	<b>0.00968</b>	0.014	0.956
A211001-101	0.131	0.179	0.0673	0.503	-0.0839	0.783
A217004-101	0.133	0.173	0.328	<b>0.000843</b>	0.315	0.306
A228001-101	0.154	0.115	0.257	<b>0.00961</b>	0.126	0.683
A237001-101	-0.426	<b>6.31E-06</b>	0.128	0.201	0.65	<i>0.0203</i>
A295006-101	0.311	<i>0.0142</i>	0.0225	0.863	-0.413	0.173
Adipo-2,6-lactam	-1	0.333	-0.127	0.709	1	1
Alanine	0.168	0.0849	0.178	0.0754	-0.021	0.939
Arabitol	0.254	<b>0.00882</b>	0.18	0.0711	-0.252	0.415
Ascorbic acid	-0.0164	0.884	-0.128	0.228	-0.462	0.123
Asparagine	-0.306	0.069	0.378	<b>0.00548</b>	0.612	0.0537
Aspartic acid	0.212	<i>0.029</i>	0.525	<b>2.29E-08</b>	0.65	<i>0.0203</i>
Cinnamic acid, 4-hydroxy-, trans-	-0.133	0.175	-0.0367	0.715	0.217	0.484
Citric acid	0.0271	0.782	0.0215	0.83	0.0629	0.834
Dehydroascorbic acid dimer	0.155	0.165	-0.0608	0.569	-0.322	0.295
Erythritol	0.204	<i>0.0365</i>	0.0989	0.325	-0.273	0.377
Erythronic acid	0.178	0.068	0.372	<b>0.00014</b>	0.42	0.165
Ethanolamine	0.0141	0.886	0.0534	0.595	-0.196	0.527
Ferulic acid, trans-	0.0631	0.52	-0.0496	0.622	-0.441	0.143
Fructose	-0.226	<i>0.0198</i>	-0.0171	0.865	0.413	0.173
Fructose-6-phosphate	-0.00937	0.924	0.107	0.287	0.0979	0.749
Galactaric acid	0.0958	0.371	0.305	<b>0.00642</b>	0.573	<i>0.0478</i>
Galactinol	-0.121	0.353	0.0891	0.479	0.294	0.34
Galactonic acid	-0.169	0.0853	0.0664	0.509	0.42	0.165
Galactose, 2-deoxy-	0.199	0.0567	0.119	0.233	-0.0979	0.749
Gluconic acid	-0.276	0.0638	0.205	0.137	0.864	<b>2E-07</b>
Glucose	-0.232	<i>0.0171</i>	0.00273	0.978	0.392	0.197

Metabolite	control		HNT		log2FC_MW	
	correlation coefficient r	p-value	correlation coefficient r	p-value	correlation coefficient r	p-value
Glucose, 1,6-anhydro	0.466	<b>6.22E-07</b>	0.368	<b>0.000166</b>	-0.322	0.295
Glucose-6-phosphate	-0.00945	0.923	0.141	0.159	0.196	0.527
Glutamic acid	0.11	0.264	0.31	<b>0.00169</b>	0.58	<i>0.0446</i>
Glutaric acid, 2-oxo-	0.0277	0.778	0.138	0.169	0.252	0.415
Glyceric acid	0.204	<i>0.0362</i>	0.103	0.305	-0.112	0.716
Glyceric acid-2-phosphate	-0.112	0.258	0.0741	0.47	0.014	0.956
Glyceric acid-3-phosphate	-0.0766	0.434	0.0577	0.566	0.392	0.197
Glycerophosphoglycerol	-0.0824	0.484	0.383	<b>0.000494</b>	0.594	<i>0.0387</i>
Glycine	0.0365	0.71	0.408	<b>2.61E-05</b>	0.608	<i>0.0333</i>
Hexadecanoic acid	0.0821	0.402	0.0877	0.382	-0.0769	0.8
Inositol, myo-	-0.319	<b>0.000906</b>	-0.445	<b>3.84E-06</b>	-0.196	0.527
Inositol-1-phosphate	0.0321	0.756	0.0337	0.74	-0.021	0.939
Isocitric acid	0.104	0.286	-0.158	0.115	-0.462	0.123
Kestose, 1-	-0.0961	0.327	0.026	0.796	0.224	0.47
Lysine	-0.047	0.784	0.0165	0.916	0.382	0.258
Malic acid	-0.0495	0.614	0.235	<i>0.018</i>	0.559	0.0547
Nicotinic acid	0.196	<i>0.0455</i>	0.284	<b>0.00406</b>	0.0699	0.817
Octadecanoic acid	0.106	0.28	0.112	0.264	-0.0559	0.852
Oxalic acid	-0.103	0.294	0.135	0.178	0.58	<i>0.0446</i>
Phosphoenolpyruvic acid	0.0883	0.37	0.247	<i>0.0137</i>	0.245	0.429
Phosphoric acid	-0.088	0.369	0.102	0.309	0.287	0.352
Phosphoric acid monomethyl est	0.172	0.0781	0.242	<i>0.0151</i>	0.273	0.377
Proline	0.347	0.236	0.454	<b>0.0056</b>	0.786	<i>0.0251</i>
Putrescine	0.0888	0.457	0.344	<b>0.00228</b>	0.35	0.253
Pyridine, 2-hydroxy-	0.0902	0.357	0.0481	0.633	-0.21	0.498
Pyridine, 3-hydroxy-	0.0757	0.44	0.196	<i>0.0498</i>	-0.182	0.557
Pyroglutamic Acid	0.236	<i>0.0155</i>	0.466	<b>1.17E-06</b>	0.636	<i>0.0242</i>
Quinic acid	-0.288	<b>0.00288</b>	-0.102	0.308	0.517	0.0795
Raffinose	-0.148	0.131	-0.107	0.286	-0.042	0.886
Salicylic acid-glucopyranoside	0.345	<b>0.000309</b>	0.374	<b>0.00013</b>	-0.014	0.956
Serine	-0.03	0.759	0.529	<b>1.59E-08</b>	0.58	<i>0.0446</i>
Shikimic acid	-0.281	<b>0.00364</b>	-0.166	0.0965	0.406	0.181
Shikimic acid, 3-dehydro-	0.179	0.0685	0.194	0.0526	0.301	0.329
Succinic acid	0.12	0.22	0.157	0.117	0.035	0.904
Threitol	0.185	0.0574	0.0719	0.474	-0.168	0.588
Threonic acid	0.145	0.138	0.33	<b>0.000778</b>	0.497	0.0944
Threonine	0.106	0.278	0.381	<b>0.000112</b>	0.566	0.0512
Trehalose, alpha,alpha'-	-0.0014	0.989	-0.143	0.152	-0.028	0.921
Uracil	-0.159	0.367	0.243	0.126	0.321	0.346
Valine	0.0524	0.593	0.273	<b>0.00585</b>	0.336	0.273

**Supplementary Table 5.** List of genes and putative genes encoding enzymes involved in polyamine biosynthesis in rice. For selection of genes see Do et al. (2013). Annotation of genes is due to the Rice Genome Annotation Project (<http://rice.plantbiology.msu.edu>).

<b>Name</b>	<b>Locus TIGR</b>	<b>Annotation TIGR</b>
<i>ADC1</i>	LOC_Os06g04070	Pyridoxal-dependent decarboxylase protein, putative, expressed
<i>ADC2</i>	LOC_Os04g01690	Pyridoxal-dependent decarboxylase protein, putative, expressed
<i>ADC3</i>	LOC_Os08g33620	Arginine decarboxylase, putative, expressed
<i>AIH</i>	LOC_Os04g39210	Agmatine deiminase, putative, expressed
<i>CPA1</i>	LOC_Os03g07910	Nitrilase, putative, expressed
<i>CPA2</i>	LOC_Os06g10420	Nitrilase, putative, expressed
<i>CPA3</i>	LOC_Os12g31830	Nitrilase, putative, expressed
<i>CPA4</i>	LOC_Os02g33080	N-carbamoylputrescine amidase, putative, expressed
<i>ODC1</i>	LOC_Os09g37120	Pyridoxal-dependent decarboxylase protein, putative, expressed
<i>ODC2</i>	LOC_Os04g04980	Pyridoxal-dependent decarboxylase protein, putative, expressed
<i>SAMDC1</i>	LOC_Os02g39795	S-adenosyl-l-methionine decarboxylase leader peptide, putative, expressed
<i>SAMDC2</i>	LOC_Os04g42095	S-adenosyl-l-methionine decarboxylase leader peptide, putative, expressed
<i>SAMDC4</i>	LOC_Os09g25625	S-adenosyl-l-methionine decarboxylase leader peptide, putative, expressed
<i>SPD/SPM1</i>	LOC_Os02g14190	Spermidine synthase, putative, expressed
<i>SPD/SPM2</i>	LOC_Os02g15550	Spermidine synthase, putative, expressed
<i>SPD/SPM3</i>	LOC_Os06g33710	Spermidine synthase, putative, expressed
<i>SPD/SPM4</i>	LOC_Os07g22600	Spermidine synthase, putative, expressed



**Supplementary Table 7.** Spearman correlation calculated for 17 genes encoding enzymes involved in polyamine biosynthesis with HNT sensitivity (expressed as rank of damage by chlorosis, Glaubitz et al., 2014) under control and HNT conditions as well as the log<sub>2</sub> fold change. P-values < 0.05 are given in italic, p-values < 0.01 are given in bold. Genes are sorted according to their order in polyamine biosynthesis.

Gene	control		HNT		log <sub>2</sub> FC	
	correlation coefficient r	p-value	correlation coefficient r	p-value	correlation coefficient r	p-value
<i>ADC1</i>	-0.0998	0.638	0.373	0.0717	0.429	0.26
<i>ADC2</i>	-0.464	0.0782	0.651	<b>0.00183</b>	0.738	<i>0.0287</i>
<i>ADC3</i>	-0.336	0.106	0.36	0.0829	0.738	<i>0.0287</i>
<i>AIH</i>	-0.47	<i>0.0205</i>	-0.468	<i>0.0213</i>	0.571	0.12
<i>CPA1</i>	-0.733	<b>0.0000002</b>	-0.323	0.121	0.929	<b>0.0000002</b>
<i>CPA2</i>	-0.368	0.076	-0.289	0.169	0.69	<i>0.0474</i>
<i>CPA3</i>	-0.368	0.076	-0.407	<i>0.048</i>	0.571	0.12
<i>CPA4</i>	-0.208	0.326	0.725	<b>9.33E-06</b>	0.69	<i>0.0474</i>
<i>ODC1</i>	-0.363	0.105	0.667	<b>0.000339</b>	0.786	<i>0.0149</i>
<i>ODC2</i>	-0.254	0.288	0.0142	0.951	0.6	0.242
<i>SAMDC1</i>	-0.0762	0.719	0.567	<b>0.00393</b>	0.667	0.0588
<i>SAMDC2</i>	-0.144	0.496	-0.835	<b>0.0000002</b>	-0.19	0.619
<i>SAMDC4</i>	-0.236	0.262	0.368	0.076	0.762	<i>0.0212</i>
<i>SPD/SPM1</i>	-0.426	<i>0.0381</i>	0.129	0.545	0.357	0.353
<i>SPD/SPM2</i>	-0.57	<b>0.00463</b>	0.376	0.0696	0.714	<i>0.0374</i>
<i>SPD/SPM3</i>	0.256	0.272	0.665	<b>0.000369</b>	0.167	0.662
<i>SPD/SPM4</i>	-0.488	0.0533	0.0725	0.765	0.548	0.139