Rhizobacteria-induced systemic tolerance against drought stress in *Sorghum bicolor* (L.) Moench

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Supplementary material

Supplementary examples of the chemometric modelling employed in this chapter are provided here. However, considering the enormous scale of the raw data in addition to the extent of the treatment interactions [Rhizobacteria (6) × Stress (2) × Tissue (2) × electrospray ionization (ESI)-charge (2)], not all of the chemometric models were included, but can be made available upon request.

Abbreviations

ACC, 1-aminocyclopropane-1-carboxylate; BPI, base peak intensity; ESI, electrospray ionization; HD, high definition; LSD, least significant difference; MALDI, matrix-assisted laser desorption/ionization; MF, Molecular formula; MVDA, multivariate data analyses; MS, mass spectrometry; OPLS-DA, orthogonal partial least square-discriminant analysis; QC, quality control; Rt, retention time; RWC, relative water content; SAF, stress alleviation factor; TOF, time of flight; UCNSNF, Untreated control receiving no stress and no fertilizer; UCNS, untreated no-stress control; UCS, untreated stress control; UHPLC, ultra-high performance liquid chromatography, VIP, variable importance in projection.

Materials and methods for ultra-high performance liquid chromatography-high definition mass spectrometry

Sample collection

Fresh samples for ultra-high performance liquid chromatography-high definition mass spectrometry (UHPLC-HDMS) analyses were taken 24 h post drought, which coincided with 5 d post treatment with each of the selected rhizobacterial isolates. The samples were collected from roots and shoots of drought stressed and non-stressed *S. bicolor* plants of which each group was primed with each of the five selected rhizobacterial isolates or left naïve (untreated control). Samples were taken from each seedling, first from shoots and then roots. Root

samples were carefully removed from the growing medium (pure silica sand) by adding sterilized dH_2O to each pot (up to field capacity) to loosen the roots. The roots were then carefully washed with sterilized dH_2O and blotted dry with tissue paper. Each sample was immediately weighed, placed in a 50 mL centrifuge tube, instantly flash-frozen with liquid nitrogen and kept at -72 °C until time of metabolite extraction. Immediately before extraction, each frozen sample was carefully crushed to a powder by making use of a clean spatula. During the crushing process, each sample was kept frozen by adding liquid nitrogen as needed. One gram of the crushed sample was then carefully transferred to a 50 mL centrifuge tube. The extraction process followed immediately thereafter making sure that the sample remained frozen up to this point. It is important to note here that in addition to metabolites originating from *S. bicolor*, root samples also contained metabolites originating from the different rhizobacterial treatments, whereas leaf samples only contained metabolites originating from *S. bicolor*.

Metabolite extraction

Intracellular metabolites were extracted with 80% methanol [1:10 (w/v)] from roots and shoots of drought stressed and non-stressed *S. bicolor* plants of which each group was primed with each of the five selected rhizobacterial isolates or left naïve (untreated control). The 80% methanol mixture was homogenized using an Ultra Turrax homogenizer. The samples were subsequently centrifuged for 20 min at 5100 rpm at 4°C. Supernatants were removed and evaporated under vacuum by using a rotary evaporator at 55 °C to a final volume of approximately 1 mL and transferred to Eppendorf tubes where it was dried in a vacuum centrifuge at 40 °C for 6 h to complete dryness. The dried samples were subsequently resuspended to a final volume of 500 µL (50:50, MeOH:MilQ water) and filtered through 0.22 µm nylon syringe filters (Anatech, Randburg, South Africa) into HPLC glass vials fitted with 500 µL inserts and stored at -20 °C. For quality control (QC) purposes, pooled samples were prepared by pipetting and mixing aliquots of equal volume from all samples.

Ultra-high performance liquid chromatography-high definition mass spectrometry analysis

The methanolic extracts were analysed on a Waters Acquity UHPLC coupled in tandem to a Waters SYNAPT G1 Q-TOF mass spectrometer (Waters Corporation Milford, USA). The batch consisted of a total of 72 samples comprising 2 tissues (roots and shoots), 12 treatments [rhizobacterial treatment (6) *vs.* stress (2)], 3 biological replicates and each sample was injected 3 times (a total of 216 injections) to account for any technical variability. It is important to note here that in addition to metabolites originating from *S. bicolor*, root samples also contained metabolites originating from the different rhizobacterial treatments, whereas leaf samples only contained metabolites originating from *S. bicolor*. Chromatographic separation was attained using a Waters HSS T3 C18 column (150 mm × 2.1 mm × 1.8 µm) thermostatted at 60 °C. The T3 column is C18 column (reverse phase) with imbedded polar structures, thus enabling the separation of both polar- and non-polar compounds. A binary solvent system consisting of 0.1% aqueous formic acid (Sigma-Aldrich, Munich, Germany) (solvent A) and 0.1% formic acid in acetonitrile (Romil Pure Chemistry, Cambridge, UK) (solvent B) was used, with a gradient elution at a flow rate of 0.4 mL min^{-1}. The initial conditions of 98% A and 2% B were held for 13 min followed by 30% A and 70% B at 14 min. At 15 min the conditions were changed to 5% A and 95% B, these conditions were held for 2 min and then changed to the initial conditions. The analytical column was allowed to equilibrate for 2 min before the next

injection. The total chromatographic run time was 20 min and the injection volume was 2 µL. For the MS analyses, data were acquired in both positive and negative ESI modes; and the MS conditions were set as follows: capillary voltage of 2.5 kV, sampling cone at 30 V, extraction cone at 4 V, cone gas flow 50 L h^{-1}, desolvation gas flow 550 L h^{-1}, source temperature at 120 °C, desolvation temperature at 450 °C, scan time of 0.1 s and mass range of 100–1000 Da. Leucine encephalin (50 pg mL^{-1}) was used as a calibrant to acquire mass accuracies between 1 and 3 mDa and data were acquired at different collision energies (MSE, 10–50 eV) to aid with structural elucidation and annotation of the analytes. The sample acquisition was done in a randomised order. For quality assurance and control, solvent blanks (to monitor background noise from the solvent) and the QC samples were included in the batch. For QC samples, six QC injections were done at the beginning of the batch, and six QC injections were placed after every 30 sample injections in the QC sample (6 injections).

Data analysis

The datasets were processed using MarkerlynxXS™ software (Waters Corporation, Milford, USA). Alignment, peak finding, peak integration and retention time (Rt) correction were done on a Rt range of 1.5-15 min, *m/z* range of 100-1000 Da, mass tolerance of 0.05 Da and Rt window of 0.2 min. Data were normalized to total intensity using MarkerlynxXS. The datasets were exported to the SIMCA (soft independent modelling of class analogy) software version 14 (Umetrics, Umea, Sweden) for data pre-treatment and multivariate data analyses (MVDA) including principal component analysis (PCA), hierarchical clustering analysis (HCA) and orthogonal partial least square-discriminant analysis (OPLS-DA). Before computing these MVDA models, data were mean centred and Pareto-scaled. The computed and used models were validated as described in the results section.

Metabolite annotation

The annotation of metabolites were performed using Taverna workbench (www.taverna.org.uk) for PUTMEDID_LCMS Metabolite ID Workflows (Brown et al., 2011, 2009). Taverna workbench allows for the integrated, automated and high-throughput annotation and putative identification of metabolites from liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) metabolomic data. The workflows consist of correlation analysis, metabolic feature annotation and metabolite annotation. The MarkerLynx-based data matrix was first formatted to match the Taverna workbench requirements. Three main workflows formed the Taverna metabolite identification procedure: (i) Pearson-based correlation analysis (List_CorrData), (ii) metabolic feature annotation (annotate_Massmatch) – allowing for grouping together ion peaks with similar features such as Rt, and annotating features with the type of *m/z* ion (molecular ion, isotope, adduct, others) believed to originate from the same compound. The elemental composition / molecular formula (MF) of each *m/z* ion was then automatically calculated; and (iii) metabolite annotation (matchMF-MF) of the calculated MF (from the output file from workflow 2) was automatically compared and matched to the MF from a pre-defined reference file of metabolites (inhouse library). Metabolite annotation was confirmed by the following steps: (i) the MF of a selected metabolite candidate was manually searched on bioinformatics tools and database, such as SorghumbicolorCyc (https://www.plantcyc.org/databases/sorghumbicolorcyc/5.0), KEGG (Kyoto Encyclopedia of Genes and Genomes, www.genome.jp/kegg/) and Chemspider (www.chemspider.com), and (ii) structural confirmation through careful inspection of fragmentation patterns by examining the MS^1 and MS^E spectra of the selected metabolite candidate; (iii) comparative assessment with/against annotation details of metabolites in *S.*

bicolor, reported in literature, particularly in Dykes & Rooney (2006) and Kang et al. (2016). Metabolites were annotated to level 2 as classified by the Metabolomics Standard Initiative (MSI) (Sumner et al., 2007).

Metabolic pathway analysis

The MetaboAnalyst bioinformatics tool suite (version 3.0; http://www.metaboanalyst.ca/) was used to perform metabolomics pathway analysis (MetPA). The input into MetPA tool were the annotated metabolites from the OPLS-DA models. MetPA is a dedicated web-based pathway analysis and visualisation tool that comprises several pathway enrichment analysis methods and the analysis pathway topological characteristics to enable the elucidation of most relevant and altered pathways involved in the conditions under consideration (Xia et al., 2015).

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Table S1

Screening of the rhizobacterial isolates for their potential to induce drought stress tolerance in *Sorghum bicolor*.

Isolate code	Parameters[1]						Percentage stress alleviation (%)[2]			SAF-IST[3]	Species identification		ACC deaminase	
	Plant height (cm)		Roots (g)		Shoots (g)		Height	Roots	Shoots	(%)	MALDI-TOF MS (Score Value)[4]	16S RRNA	*In vitro* assay[5]	*acdS*[6]
A02	0.315	c-p	0.995	b-e	0.163	g-q	7.95	-12.14	-5.06	-3.08	*Bacillus pumilus* (2.041)	-	-	-
A03	0.315	d-p	0.990	b-e	0.164	g-q	7.77	-12.53	-4.50	-3.08	*Bacillus cereus* (2.296)	-	-	-
A05	0.312	e-q	0.992	b-e	0.184	e-p	6.99	-12.40	7.16	0.58	*Bacillus cereus* (2.171)	-	-	-
A07	0.305	k-r	1.099	a-e	0.175	f-q	4.32	-2.88	1.50	0.98	*Bacillus megaterium* (1.995)	-	-	-
A08	0.322	a-m	1.202	a-e	0.191	c-p	10.10	6.15	10.78	9.01	*Bacillus megaterium* (2.066)	-	-	-
A10	0.326	a-l	0.830	c-e	0.154	o-q	11.51	-26.68	-10.41	-8.53	*Bacillus pumilus* (1.933)	-	-	-
A11W	0.318	b-o	1.063	a-e	0.184	e-p	8.73	-6.09	7.17	3.27	*Bacillus cereus* (2.176)	-	-	-
A11Y	0.317	b-p	0.930	b-e	0.197	c-n	8.56	-17.89	14.42	1.70	*Bacillus megaterium* (1.725)	-	-	-
A12	0.306	j-q	0.903	c-e	0.162	h-q	4.62	-20.22	-5.92	-7.17	*Bacillus megaterium* (2.226)	-	-	-
A13	0.323	a-l	0.996	b-e	0.174	f-q	10.65	-11.99	0.86	-0.16	*Bacillus pumilus* (2.06)	-	-	-
A14	0.325	a-l	0.998	b-e	0.171	f-q	11.27	-11.85	-0.54	-0.37	-	-	-	-
A15	0.328	a-l	1.322	a-d	0.230	a-d	12.26	16.82	33.55	20.88	*Bacillus cereus* (2.112)	-	-	-
A17	0.330	a-k	0.914	b-e	0.185	e-p	13.08	-19.28	7.23	0.35	*Bacillus cereus* (2.286)	-	-	-
A18B	0.322	a-m	1.188	a-e	0.233	a-c	10.31	4.91	35.36	16.86	*Arthrobacter parietis* (2.05)	-	-	-
A18W	0.333	a-i	1.111	a-e	0.179	f-q	14.01	-1.85	3.89	5.35	*Bacillus cereus* (2.184)	-	-	-
A18Y	0.321	a-n	1.093	a-e	0.176	f-q	9.97	-3.43	2.15	2.90	*Bacillus megaterium* (2.098)	-	-	-
A19	0.323	a-l	1.239	a-e	0.196	c-o	10.62	9.49	13.80	11.30	*Bacillus cereus* (2.067)	-	-	-
A20	0.321	a-n	1.082	a-e	0.183	e-p	9.90	-4.42	6.31	3.93	*Bacillus megaterium* (2.1)	-	-	-
A21	0.313	d-p	1.128	a-e	0.196	c-n	7.26	-0.33	14.13	7.02	*Bacillus thuringiensis* (2.126)	-	-	-
A22	0.316	b-p	0.864	c-e	0.171	f-q	8.12	-23.70	-0.36	-5.32	*Bacillus pumilus* (1.999)	-	-	-
A23	0.296	m-r	0.951	b-e	0.155	n-q	1.47	-16.00	-10.12	-8.22	*Bacillus thuringiensis* (2.05)	-	-	-
A25W	0.303	l-r	1.025	a-e	0.173	f-q	3.87	-9.44	0.29	-1.76	*Bacillus pumilus* (1.985)	-	-	-
A26	0.311	f-q	1.240	a-e	0.169	f-q	6.64	9.52	-1.67	4.83	*Bacillus pumilus* (2.054)	-	-	-
A27	0.311	f-q	0.858	c-e	0.156	m-q	6.34	-24.18	-9.56	-9.13	*Bacillus cereus* (2.152)	-	-	-
A28[7]	**0.342**	**a-c**	**1.433**	**ab**	**0.222**	**a-f**	**17.12**	**26.59**	**28.96**	**24.22**	***Bacillus pumilus* (2.036)**	***Bacillus* sp.**	**Negative**	**Negative**
A31	0.320	a-n	1.128	a-e	0.195	c-o	9.42	-0.32	13.48	7.53	*Bacillus megaterium* (2.135)	-	-	-

Table S1 *Cont.*

Isolate code	Parameters[1]						Percentage stress alleviation (%)[2]			SAF-IST[3]	Species identification		ACC deaminase	
	Plant height (cm)		Roots (g)		Shoots (g)		Height	Roots	Shoots	(%)	MALDI-TOF MS (Score Value)[4]	16S RRNA	*In vitro* assay[5]	*acdS*[6]
A35	0.332	a-j	1.167	a-e	0.178	f-q	13.53	3.12	3.17	6.61	*Bacillus megaterium* (1.957)	-	-	-
A39	0.310	f-q	1.003	b-e	0.177	f-q	6.23	-11.39	3.16	-0.67	*Bacillus megaterium* (1.988)	-	-	-
A44	0.323	a-l	1.181	a-e	0.198	c-m	10.55	4.31	14.95	9.94	*Bacillus pumilus* (2.062)	-	-	-
A47A	0.308	h-q	0.873	c-e	0.187	e-p	5.45	-22.84	8.76	-2.88	*Bacillus pumilus* (2.001)	-	-	-
N09	0.337	a-f	1.144	a-e	0.199	c-l	15.51	1.05	15.62	10.73	*Arthrobacter aurescens* (2.131)	-	-	-
N1	0.320	a-n	1.036	a-e	0.171	f-q	9.45	-8.51	-0.65	0.10	*Pseudomonas koreensis* (2.011)	-	-	-
N10W	0.327	a-l	1.228	a-e	0.202	c-i	11.88	8.50	17.13	12.50	*Bacillus cereus* (2.398)	-	-	-
N11	0.335	a-g	1.156	a-e	0.187	e-p	14.69	2.10	8.71	8.50	-	-	-	-
N16W	0.319	b-n	0.947	b-e	0.149	pq	9.14	-16.33	-13.69	-6.96	*Bacillus cereus* (2.361)	-	-	-
N17	0.312	f-q	0.987	b-e	0.165	f-q	6.82	-12.80	-3.83	-3.27	*Bacillus pumilus* (2.013)	-	-	-
N18[7]	**0.330**	**a-k**	**1.071**	**a-e**	**0.223**	**a-e**	**13.01**	**-5.39**	**29.45**	**12.36**	***Bacillus pumilus* (1.817)**	***Bacillus* sp.**	**Negative**	**Negative**
N19	0.340	a-d	1.091	a-e	0.195	c-o	16.54	-3.65	13.35	8.75	*Pseudomonas koreensis* (2.14)	-	-	-
N2	0.340	a-d	1.119	a-e	0.191	c-o	16.27	-1.12	11.05	8.73	*Bacillus cereus* (2.232)	-	-	-
N20	0.339	a-e	1.122	a-e	0.192	c-o	15.96	-0.90	11.33	8.80	*Pseudomonas koreensis* (2.12)	-	-	-
N22A	0.323	a-l	1.035	a-e	0.194	c-o	10.51	-8.58	12.57	4.84	*Bacillus pumilus* (2.051)	-	-	-
N23	0.295	n-r	1.014	a-e	0.159	j-q	1.10	-10.40	-7.86	-5.72	*Bacillus pumilus* (2.153)	-	-	-
N26	0.279	r	0.927	b-e	0.162	h-q	-4.42	-18.12	-5.91	-9.48	*Bacillus muralis* (1.874)	-	-	-
N28	0.303	l-r	0.866	c-e	0.157	l-q	3.90	-23.52	-8.73	-9.45	*Bacillus subtilis* (1.81)	-	-	-
N29	0.327	a-l	0.964	b-e	0.161	i-q	11.88	-14.88	-6.69	-3.23	*Pseudomonas koreensis* (2.023)	-	-	-
N30	0.336	a-g	1.155	a-e	0.194	c-o	15.07	1.99	12.59	9.88	*Pseudomonas chlororaphis* (2.119)	-	-	-
N31	0.303	l-r	0.882	c-e	0.184	e-p	3.84	-22.08	6.71	-3.84	*Pseudomonas koreensis* (2.028)	-	-	-
N36	0.329	a-l	1.010	b-e	0.177	f-q	12.57	-10.77	2.72	1.51	*Bacillus pumilus* (2.042)	-	-	-
N4	0.316	c-p	0.974	b-e	0.179	f-q	8.05	-13.95	3.97	-0.64	*Pseudomonas chlororaphis* (2.125)	-	-	-
N40	0.307	i-q	0.796	e	0.173	f-q	5.07	-29.67	0.31	-8.10	*Bacillus weihenstephanensis* (2.085)	-	-	-

Table S1 *Cont.*

Isolate code	Parameters[1]						Percentage stress alleviation (%)[2]			SAF-IST[3]	Species identification		ACC deaminase	
	Plant height (cm)		Roots (g)		Shoots (g)		Height	Roots	Shoots	(%)	MALDI-TOF MS (Score Value)[4]	16S RRNA	*In vitro* assay[5]	*acdS*[6]
N43	0.323	a-l	1.092	a-e	0.181	e-q	10.68	-3.52	5.07	4.08	*Pseudomonas koreensis* (2.12)	-	-	-
N45	0.328	a-l	1.037	a-e	0.163	g-q	12.47	-8.43	-5.04	-0.33	*Pseudomonas chlororaphis* (2.299)	-	-	-
N47	0.328	a-l	1.221	a-e	0.203	b-h	12.29	7.88	18.18	12.78	*Bacillus pumilus* (1.832)	-	-	-
N49	0.291	p-r	1.017	a-e	0.182	e-p	-0.41	-10.16	5.79	-1.59	*Bacillus pumilus* (2.076)	-	-	-
N52[7]	**0.332**	**a-j**	**1.138**	**a-e**	**0.205**	**b-g**	**13.70**	**0.53**	**18.95**	**11.06**	***Bacillus pumilus* (1.879)**	***Bacillus* sp.**	**Negative**	**Negative**
N53	0.313	e-p	0.819	de	0.171	f-q	7.23	-27.64	-0.47	-6.96	*Bacillus pumilus* (2.124)	-	-	-
N54	0.310	g-q	0.981	b-e	0.164	g-q	6.16	-13.37	-4.95	-4.05	*Bacillus subtilis* (1.95)	-	-	-
N55B	0.320	a-n	1.016	a-e	0.162	h-q	9.63	-10.25	-5.87	-2.16	*Bacillus cereus* (2.124)	-	-	-
N59	0.313	d-p	0.902	c-e	0.158	k-q	7.26	-20.36	-8.09	-7.06	*Pseudomonas chlororaphis* (1.708)	-	-	-
N60	0.312	f-q	1.083	a-e	0.187	e-p	6.68	-4.33	8.87	3.74	*Bacillus cereus* (2.35)	-	-	-
N61	0.338	a-f	1.202	a-e	0.207	b-f	15.72	6.17	20.18	14.02	*Bacillus cereus* (2.223)	-	-	-
N62	0.286	qr	0.996	b-e	0.168	f-q	-1.99	-12.03	-2.35	-5.46	*Pseudomonas koreensis* (2.13)	-	-	-
N65	0.341	a-d	0.934	b-e	0.139	q	16.78	-17.51	-19.22	-6.65	*Pseudomonas monteilii* (2.06)	-	-	-
N66[7]	**0.331**	**a-k**	**1.194**	**a-e**	**0.246**	**ab**	**13.36**	**5.48**	**42.75**	**20.53**	***Pseudomonas putida* (2.038)**	***Pseudomonas* sp.**	**Positive**	**Positive**
N67	0.315	c-p	1.347	a-c	0.200	c-k	7.95	19.02	16.12	14.36	*Pseudomonas rhodesiae* (2.034)	-	-	-
N69	0.326	a-l	1.025	a-e	0.175	f-q	11.64	-9.47	1.50	1.23	*Pseudomonas rhodesiae* (2.085)	-	-	-
N71	0.337	a-f	1.191	a-e	0.207	b-f	15.51	5.25	20.22	13.66	*Bacillus pumilus* (1.961)	-	-	-
N74	0.318	b-o	1.219	a-e	0.198	c-m	8.87	7.69	14.95	10.50	*Pseudomonas vancouverensis* (1.96)	-	-	-
N76	0.341	a-d	1.024	a-e	0.190	d-p	16.68	-9.52	10.55	5.90	*Pseudomonas chlororaphis* (1.841)	-	-	-
N77	0.335	a-g	1.247	a-e	0.201	c-j	14.83	10.15	16.73	13.90	*Bacillus cereus* (2.352)	-	-	-
N77Y	0.321	a-n	1.042	a-e	0.173	f-q	9.93	-7.94	0.32	0.77	*Pseudomonas chlororaphis* (1.947)	-	-	-
N79	0.338	a-f	0.991	b-e	0.192	c-o	15.68	-12.44	11.85	5.03	*Bacillus pumilus* (2.138)	-	-	-
N80	0.334	a-h	1.012	a-e	0.185	e-p	14.38	-10.62	7.26	3.68	*Bacillus pumilus* (2.057)	-	-	-
NAS-6G6[7]	**0.321**	**a-n**	**1.180**	**a-e**	**0.198**	**c-l**	**9.93**	**4.24**	**15.26**	**9.81**	**-**	***Paenibacillus alvei***	**Negative**	**Negative**

Table S1 *Cont.*

Isolate code	Parameters[1]						Percentage stress alleviation (%)[2]			SAF-IST[3]	Species identification		ACC deaminase	
	Plant height (cm)		Roots (g)		Shoots (g)		Height	Roots	Shoots	(%)	MALDI-TOF MS (Score Value)[4]	16S RRNA	*In vitro* assay[5]	*acdS*[6]
RhizoVital®	0.334	a-h	1.165	a-e	0.182	e-p	14.25	2.90	5.75	7.63	*Bacillus subtilis* (1.975)	-	-	-
S5	0.345	ab	1.281	a-e	0.199	c-l	18.22	13.17	15.42	15.60	*Bacillus cereus* (2.021)	-	-	-
T29	0.329	a-l	1.082	a-e	0.190	d-p	12.57	-4.46	10.31	6.14	-	-	-	-
UCNS[8]	0.361	a	1.533	a	0.262	a	23.77	35.42	52.56	37.25	-	-	-	-
UCNSNF[9]	0.295	n-r	1.093	a-e	0.156	m-q	1.06	-3.45	-9.62	-4.00	-	-	-	-
UCS[10]	0.292	o-r	1.132	a-e	0.172	f-q	0.00	0.00	0.00	0.00	-	-	-	-

[1] Means followed by the same letter within a column does not differ significantly according to Tukey's least significant difference (LSD) test at a significance level of $p < 0.05$.
[2] The percentage stress alleviation was calculated as the percentage increase in each of the growth parameters (plant height, root- and shoot mass) by treating *S. bicolor* plants with each of the 77 rhizobacterial isolates compared to that of the untreated control (UCS) under drought stress conditions.
[3] The stress alleviation factor (SAF) was calculated with the formula as outlined in section 4.2.2.6. and incorporated the percentage increase in (1) plant height, (2) root- and (3) leaf biomass.
[4] Meaning of matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) score values: 2.300-3.000= highly probable species identification; 2.000-2.299= secure genus identification, probable species identification; 1.700-1.999= probable genus identification; 0.000-1.699= not reliable identification.
[5] An *in vitro* assay was done to determine 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity as outlined in section 4.2.4.1.
[6] The presence of the *acdS* gene encoding ACC deaminase activity was confirmed as outlined in section 4.2.4.2.
[7] The rhizobacterial isolates A28, N18, N52 and N66 (selected as selected isolates for eliciting induced systemic tolerance against drought stress in *S. bicolor* plants) and the semi-commercial strain NAS-6G6 (previously identified as *Paenibacillus alvei* by means of 16S rRNA gene sequencing) are indicated in **bold**.
[8] Untreated control receiving no stress (UCNS).
[9] Untreated control receiving no stress and no fertilizer (UCNSNF).
[10] Untreated control receiving drought stress (UCS).

Table S2 Plant growth promotion[1] and induction of drought stress tolerance in *S. bicolor* after treatment with the selected rhizobacterial isolates for induced systemic tolerance.

Isolate	Stress	Leaf weight (g)				Root weight (g)				Plant height		Root length		Root to shoot ratio[1]		RWC[2]	
		Fresh		Dry		Fresh		Dry		(cm)		(cm)					
A28	None	0.25	c	0.04	c	0.17	bc	0.03	bc	23.56	abcd	10.94	a	0.47	a	-	
N18	None	0.39	ab	0.56	ab	0.24	ab	0.04	abc	26.44	a	10.67	a	0.41	a	-	
N52	None	0.32	bc	0.05	abc	0.19	b	0.03	cd	25.44	ab	9.52	ab	0.38	a	-	
N66	None	0.39	ab	0.06	ab	0.25	ab	0.04	abc	25.54	ab	7.46	abc	0.29	a	-	
NAS-6G6	None	0.41	ab	0.06	a	0.23	ab	0.04	bc	26.98	a	10.31	ab	0.38	a	-	
Untreated	None	0.37	ab	0.05	ab	0.21	ab	0.03	bc	26.69	a	9.85	ab	0.37	a	100.60	a
A28	Drought	0.10	d	**0.04**	**c**	0.09	cd	0.01	de	20.96	cd	7.38	abc	0.35	a	88.60	a
N18	Drought	**0.35**	**ab**	**0.06**	**ab**	**0.26**	**ab**	**0.05**	**a**	**24.04**	**abc**	7.33	abc	0.30	a	96.40	a
N52	Drought	**0.34**	**abc**	**0.05**	**abc**	**0.19**	**b**	**0.04**	**bc**	**23.15**	**abcd**	7.80	abc	0.33	a	91.80	a
N66	Drought	**0.43**	**a**	**0.05**	**ab**	**0.29**	**a**	**0.04**	**ab**	**24.73**	**abc**	**8.29**	**ab**	0.34	a	87.00	a
NAS-6G6	Drought	**0.31**	**bc**	**0.04**	**bc**	**0.24**	**ab**	**0.04**	**ab**	**22.29**	**bcd**	6.17	bc	0.28	a	88.80	a
Untreated	Drought	0.05	d	0.01	d	0.04	d	0.01	e	20.54	d	3.67	c	0.18	a	87.10	a

[1] The plant growth promotion parameters recorded included leaf and root biomass, plant height, root length and root to shoot ratio. Means within columns followed by the same letter does not differ significantly according to Tukey's LSD test at a significance level of $p < 0.05$. A statistically significant ($p < 0.05$) promotion of *S. bicolor* growth under drought stress conditions, in response to treatment with each of the five rhizobacterial isolates compared to that of the untreated (naïve) stress control, is shown here in **bold**.[2] Relative water content (RWC) was calculated as described by Seelig *et al.*, (2008, section 4.2.5.1).

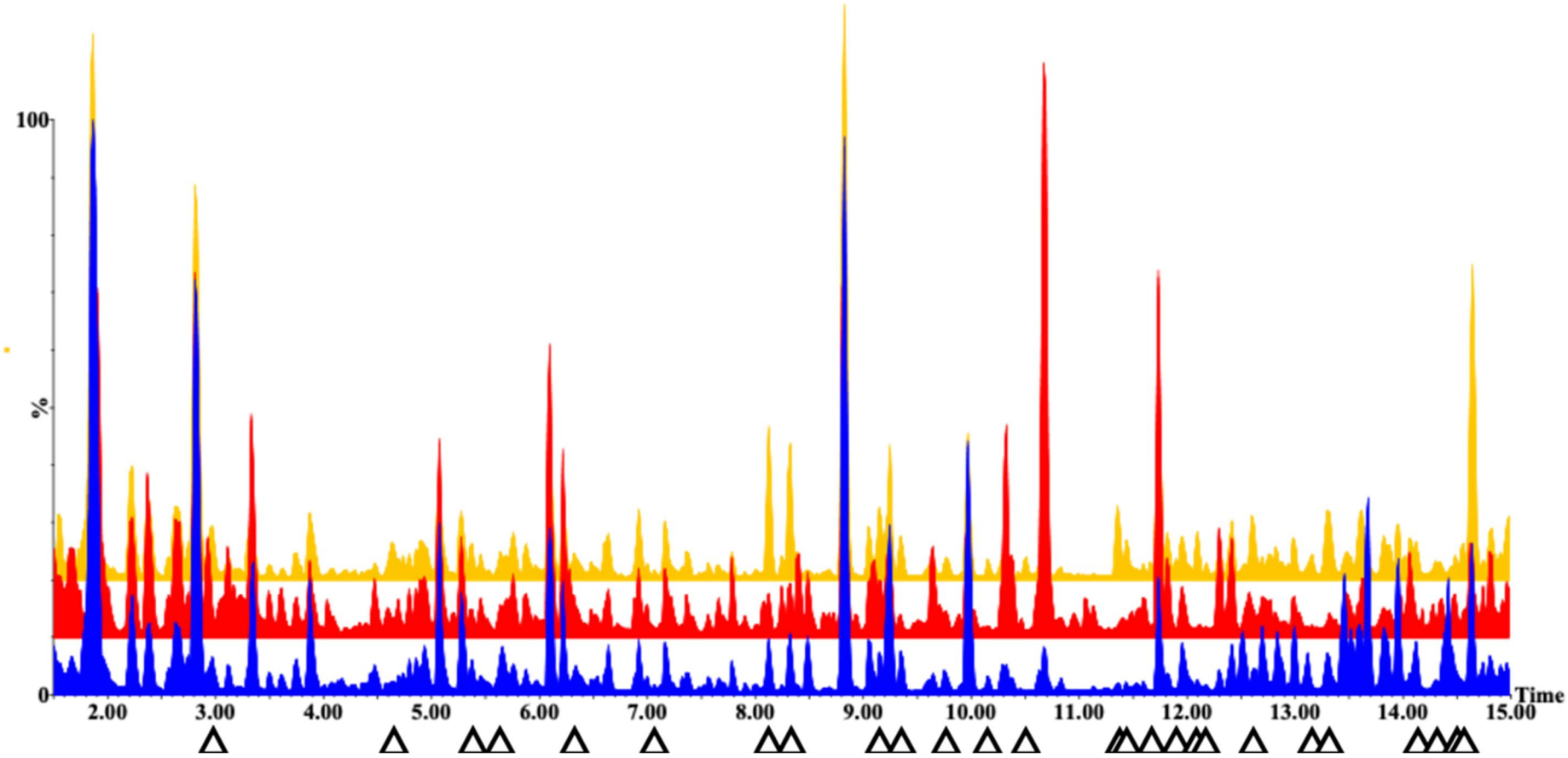

Fig. S1 UHPLC-HDMS BPI chromatograms of ESI-positive data obtained from *S. bicolor* roots, indicating the metabolomic profiles of treatments (from bottom to top): (1) untreated (naïve) control receiving no drought stress (blue); (2) untreated (naïve) stress control receiving drought stress alone (red); (3) treatment with rhizobacterial isolate N66 and drought stress (yellow). The triangles below the x-axis indicate the peaks of features that were upregulated in N66-primed *S. bicolor* shoots (yellow) *vs.* those left naïve (red) under drought stress conditions.

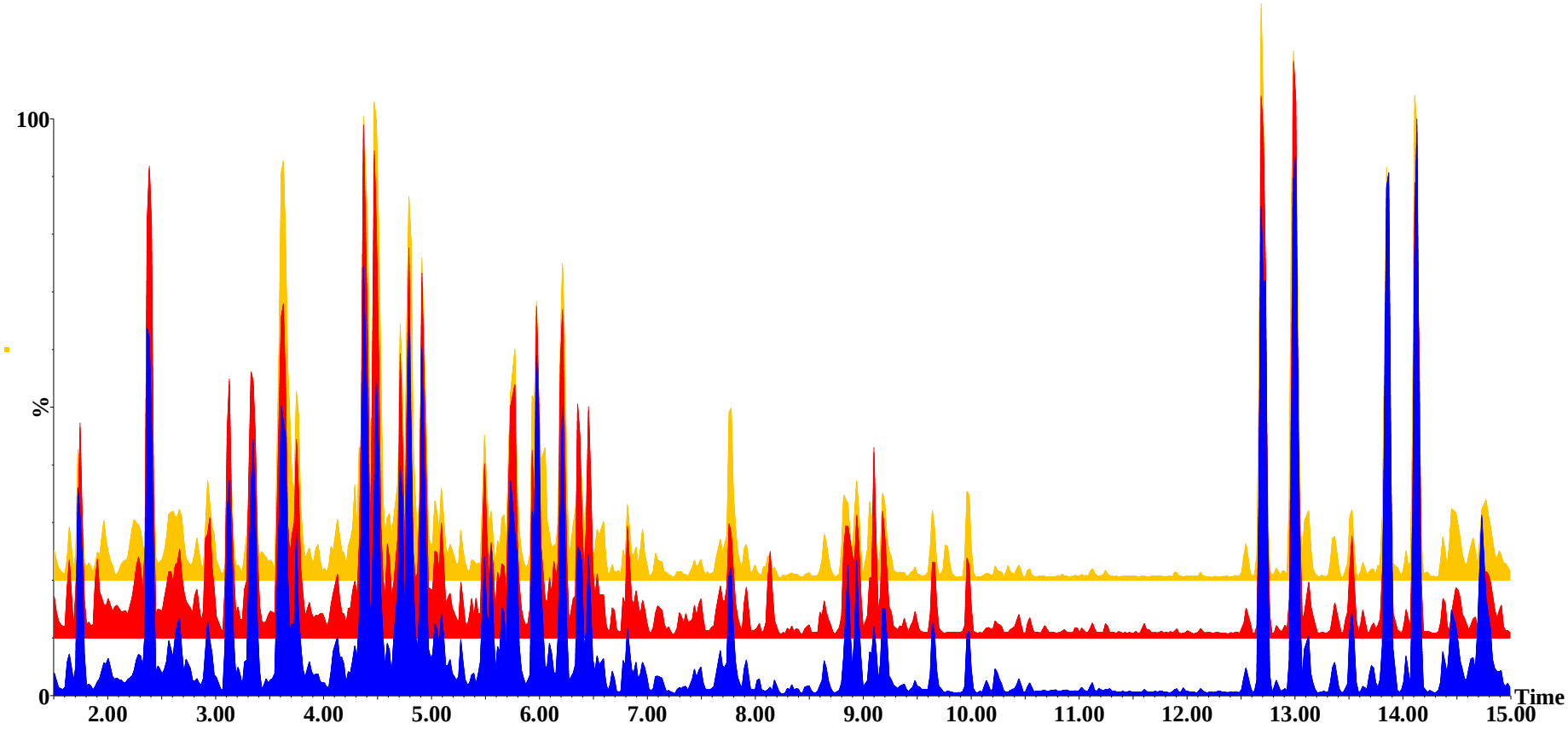

Fig. S2 UHPLC-HDMS BPI chromatograms of ESI-negative data obtained from *S. bicolor* **shoots**, indicating the metabolomic profiles of treatments (from bottom to top): (1) untreated (naïve) control receiving no drought stress (**blue**); (2) untreated (naïve) stress control receiving drought stress alone (**red**); (3) treatment with rhizobacterial isolate **N66** and drought stress (**yellow**).

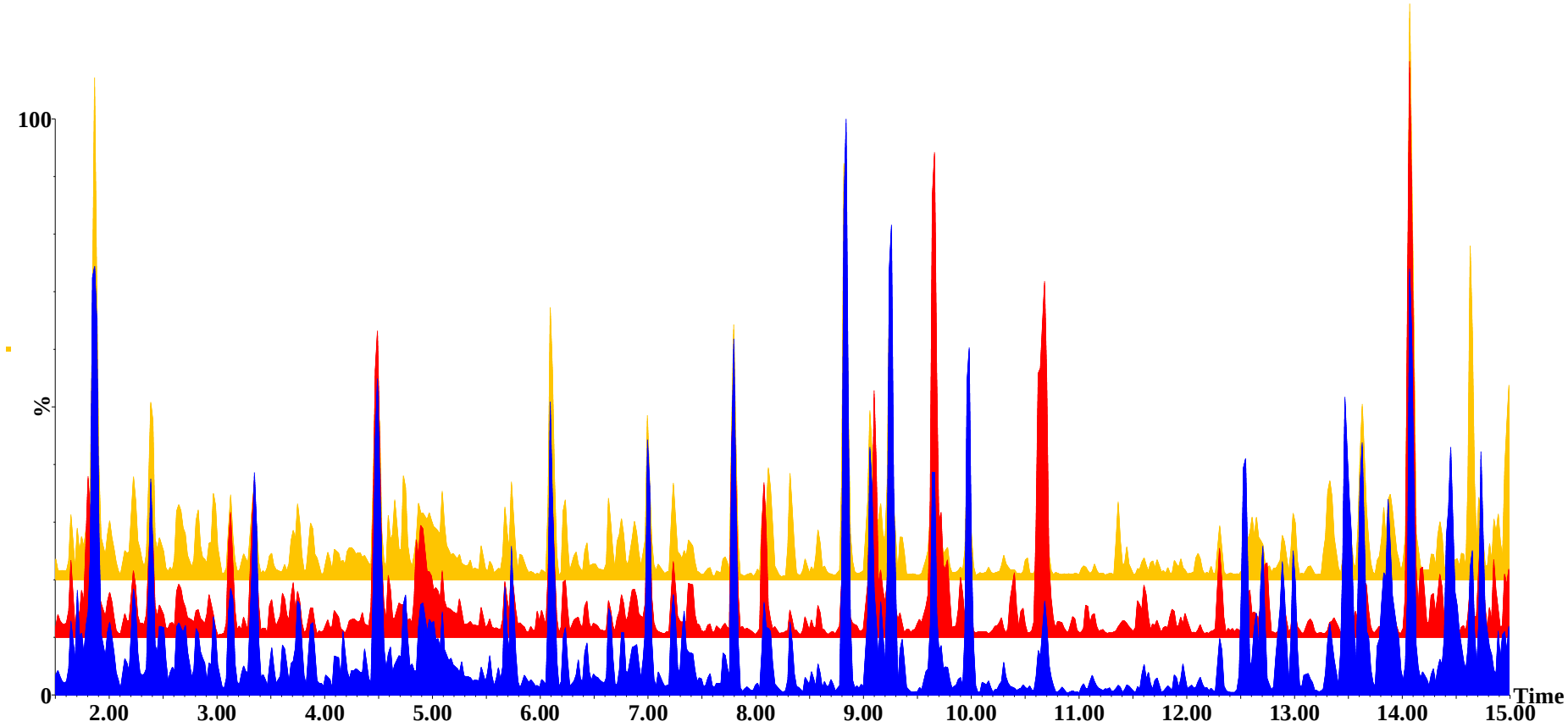

Fig. S3 UHPLC-HDMS BPI chromatograms of ESI-negative data obtained from *S. bicolor* **roots**, indicating the metabolomic profiles of treatments (from bottom to top): (1) untreated (naïve) control receiving no drought stress (**blue**); (2) untreated (naïve) stress control receiving drought stress alone (**red**); (3) treatment with rhizobacterial isolate **N66** and drought stress (**yellow**).

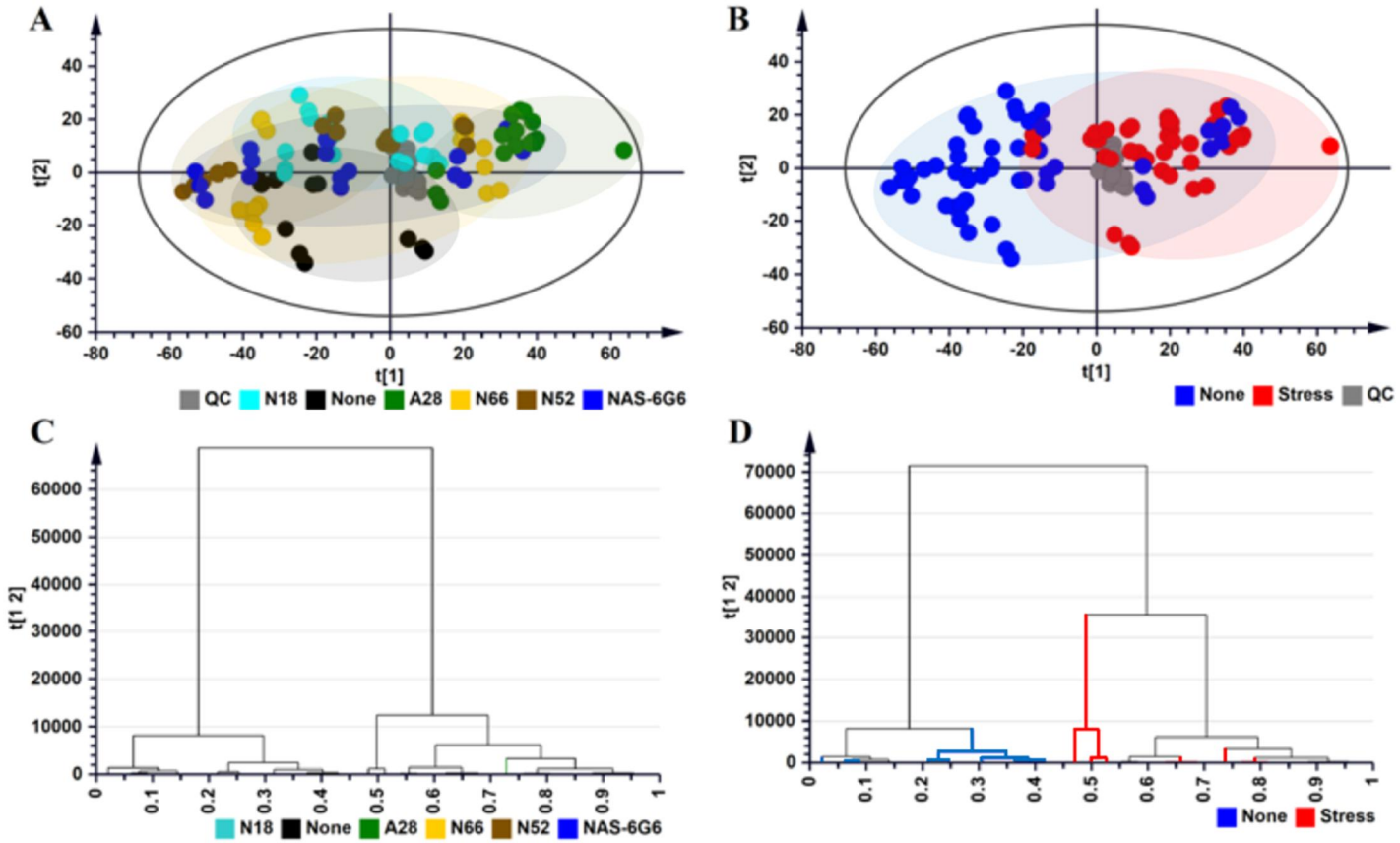

Fig. S4 PCA score / scatter plot of *S. bicolor* root samples computed from ESI-positive data representing the first two PCs of a 15-component PCA model. The model explains 66.6% variation in the Pareto-scaled data (R^2X = 0.666) and 47.8% predicted variation according to cross-validation (Q^2 =0.478). (A and B) represents the same PCA scores plot with (A) showing the rhizobacteria treatment-related clustering and (B) showing the drought stress-related clustering. (C and D) HCA dendrogram corresponding to (A and B). Legend: (A and C) QC: Quality control samples (grey); N18: treatment with rhizobacterial isolate N18 (light blue); None: untreated control (black); A28: treatment with rhizobacterial isolate A28 (green); N66: treatment with rhizobacterial isolate N66 (yellow); N52: treatment with rhizobacterial isolate N52 (brown); NAS-6G6: treatment with the semi-commercial strain NAS-6G6 (dark blue) and (B and D) None: no-drought stress (blue); Stress: drought stress (red).

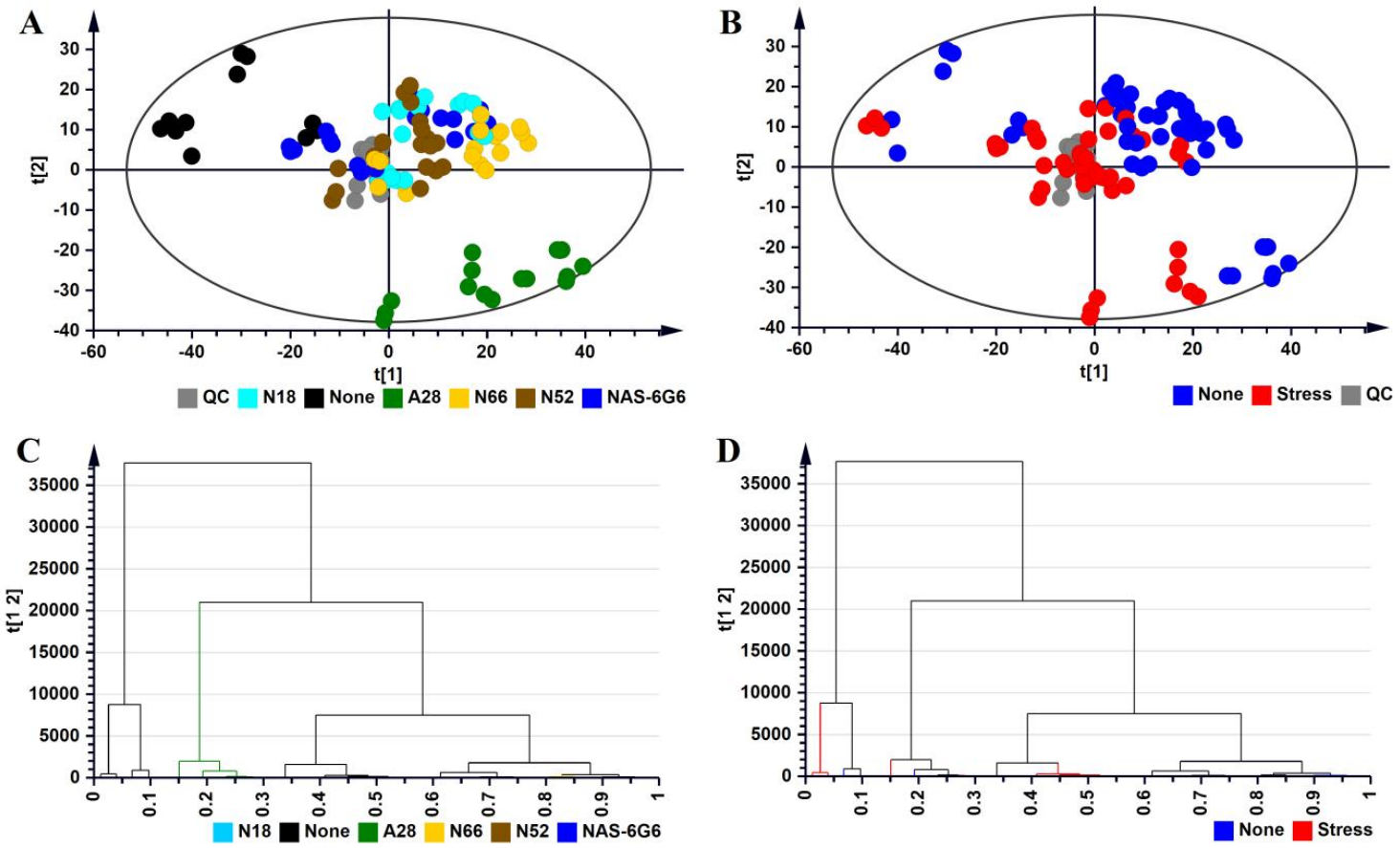

Fig. S5 PCA score scatter plot of *S. bicolor* shoot samples computed from ESI-negative data representing the first two PCs of a 16-component PCA model. The model explains 62.5% variation in the Pareto-scaled data ($R^2X = 0.625$) and 40.9% predicted variation according to cross-validation ($Q^2 = 0.409$). (**A** and **B**) represents the same PCA scores plot with (**A**) showing the rhizobacteria treatment-related clustering and (**B**) showing the drought stress-related clustering. (**C** and **D**) HCA dendrogram corresponding to (A and B). Legend: (**A** and **C**) QC: Quality control samples (grey); N18: treatment with rhizobacterial isolate N18 (light blue); None: untreated control (black); A28: treatment with rhizobacterial isolate A28 (green); N66: treatment with rhizobacterial isolate N66 (yellow); N52: treatment with rhizobacterial isolate N52 (brown); NAS-6G6: treatment with semi-commercial strain NAS-6G6 (dark blue) and (**B** and **D**) None: no-drought stress (blue); Stress: drought stress (red).

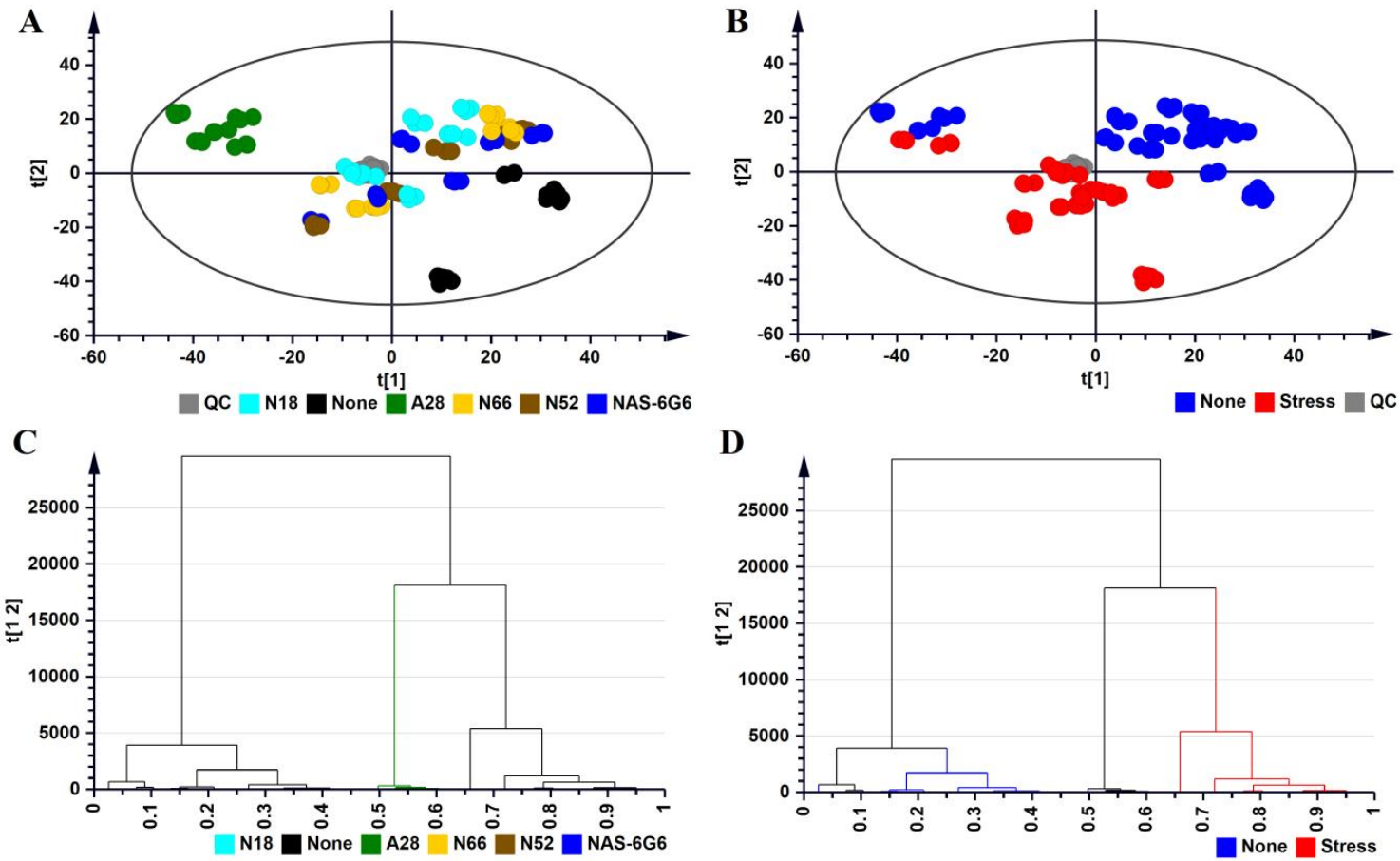

Fig. S6 PCA score scatter plot of *S. bicolor* root samples computed from ESI-negative data representing the first two PCs of a 15-component PCA model. The model explains 65.5% variation in the Pareto-scaled data (R^2X = 0.655) and 46.7% predicted variation according to cross-validation (Q^2 =0.467). (**A** and **B**) represents the same PCA scores plot with (**A**) showing the rhizobacteria treatment-related clustering and (**B**) showing the drought stress-related clustering. (**C** and **D**) HCA dendrogram corresponding to (A and B). Legend: (**A** and **C**) QC: Quality control samples (grey); N18: treatment with rhizobacterial isolate N18 (light blue); None: untreated control (black); A28: treatment with rhizobacterial isolate A28 (green); N66: treatment with rhizobacterial isolate N66 (yellow); N52: treatment with rhizobacterial isolate N52 (brown); NAS-6G6: treatment with semi-commercial strain NAS-6G6 (dark blue) and (**B** and **D**) None: no-drought stress (blue); Stress: drought stress (red).

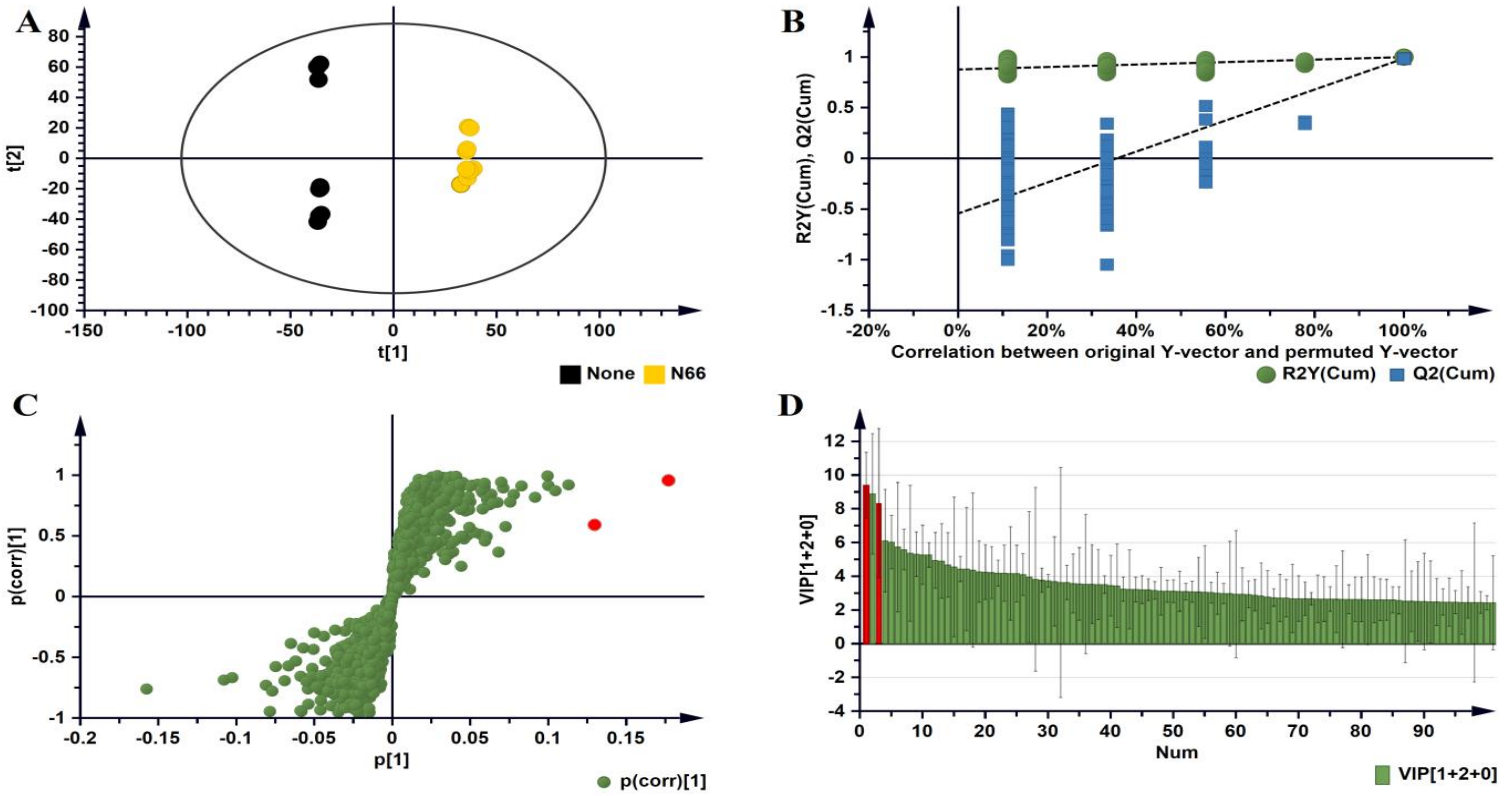

Fig. S7 OPLS-DA modelling of *S. bicolor* root samples and variable/feature selection ESI-positive data. (A) A typical scores scatter plot for the OPLS-DA model separating drought stressed plants that were (1) left untreated (None) *vs* (2) N66-treated (N66) (1 + 2 + 0 components, $R^2X = 0.569$, $Q^2 = 0.985$, CV-ANOVA $p < 0.05$). In the scores plot, it is evident that the two groups are clearly separated: None *vs* N66. (B) A typical response permutation test plot (n = 100) for the OPLS-DA model in (A); the R^2 and Q^2 values of the permutated models correspond to *y*-axis intercepts: $R^2 = (0.0, 0.89)$ and $Q^2 = (0.0, -0.52)$; (C) An OPLS-DA loadings S-plot for the same model in (A); variables situated in the extreme end of the S-plot are statistically relevant and represent prime candidates as discriminating variables/features. (D) A variable importance in projection (VIP) plot for the same model; pointing mathematically to the importance of each variable (feature) in contributing to group separation in the OPLS-DA model. (C and D) Examples of the variables that were significantly upregulated in primed plants are highlighted in red.

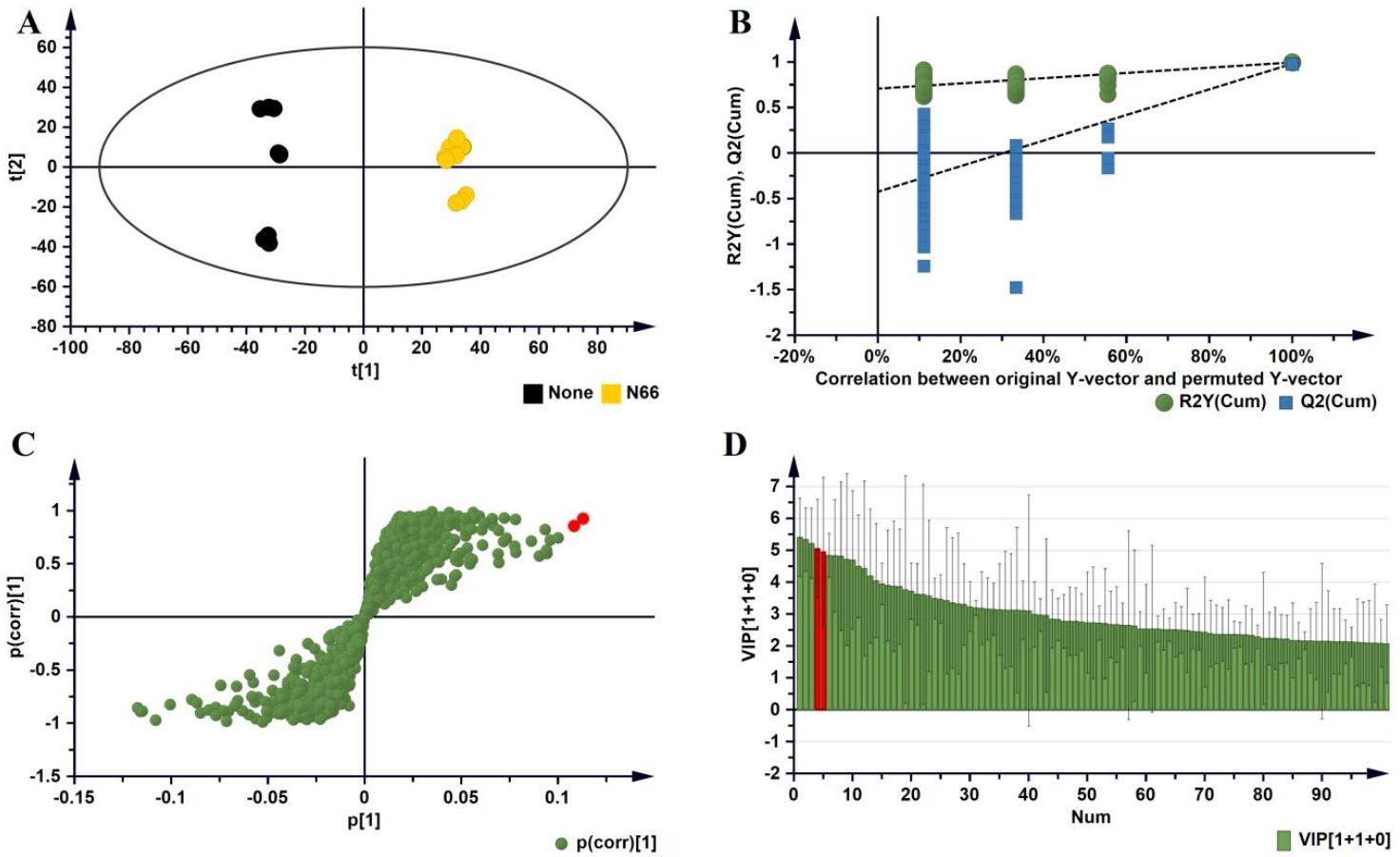

Fig. S8 OPLS-DA modelling of *S. bicolor* shoots samples and variable/feature selection ESI-negative data. (**A**) A typical scores scatter plot for the OPLS-DA model separating drought stressed plants that were (1) left untreated (None) *vs* (2) N66-treated (N66) (1 + 1 + 0 components, $R^2X = 0.490$, $Q^2 = 0.980$, CV-ANOVA $p < 0.05$). In the scores plot, it is evident that the two groups are clearly separated: None *vs* N66. (**B**) A typical response permutation test plot (n = 100) for the OPLS-DA model in (A); the R^2 and Q^2 values of the permutated models correspond to *y*-axis intercepts: $R^2 = (0.0, 0.75)$ and $Q^2 = (0.0, -0.5)$; **(C)** An OPLS-DA loadings S-plot for the same model in (A); variables situated in the extreme end of the S-plot are statistically relevant and represent prime candidates as discriminating variables/features. (**D**) A VIP plot for the same model; pointing mathematically to the importance of each variable (feature) in contributing to group separation in the OPLS-DA model. (**C** and **D**) Examples of the variables that were significantly upregulated in primed plants are highlighted in red

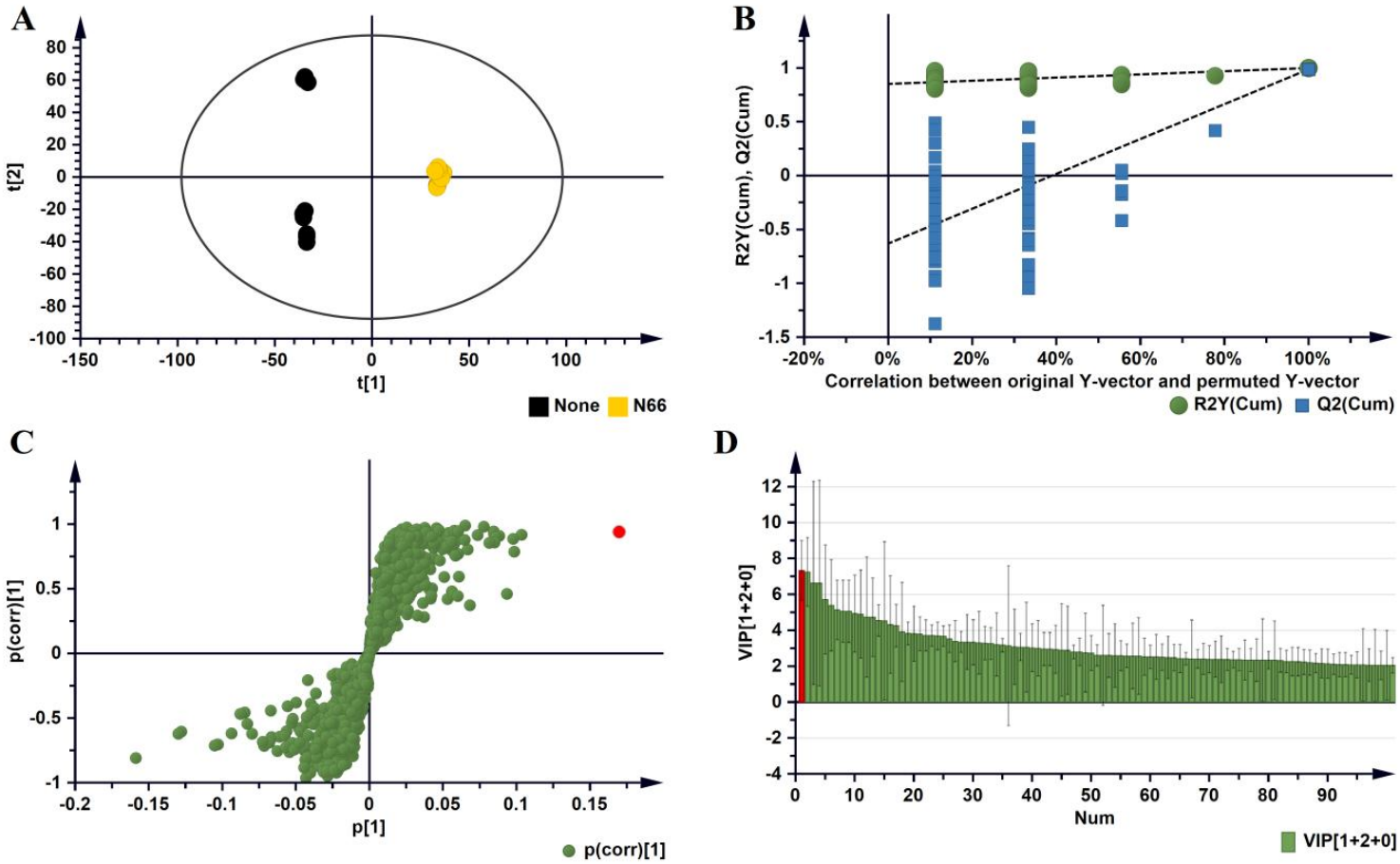

Fig. S9 OPLS-DA modelling of *S. bicolor* roots samples and variable/feature selection ESI-negative data. (**A**) A typical scores scatter plot for the OPLS-DA model separating drought stressed plants that were (1) left untreated (None) *vs* (2) N66-treated (N66) (1 + 2 + 0 components, $R^2X = 0.605$, $Q^2 = 0.987$, CV-ANOVA $p < 0.05$). In the scores plot, it is evident that the two groups are clearly separated: None *vs* N66. (**B**) A typical response permutation test plot (n = 100) for the OPLS-DA model in (A); the R^2 and Q^2 values of the permutated models correspond to *y*-axis intercepts: $R^2 = (0.0, 0.75)$ and $Q^2 = (0.0, -0.73)$; (**C**) An OPLS-DA loadings S-plot for the same model in (A); variables situated in the extreme end of the S-plot are statistically relevant and represent prime candidates as discriminating variables/features. (**D**) A VIP plot for the same model; pointing mathematically to the importance of each variable (feature) in contributing to group separation in the OPLS-DA model. (**C** and **D**) Examples of the variables that were significantly upregulated in primed plants are highlighted in red.