Electronic Supplimentary Information

Attenuated total reflection Fourier-transform infrared spectroscopy for the prediction of hormone concentrations in plants

Claire A Holden[1], Martin McAinsh[1], Jane E Taylor[1], Paul Beckett[2], Alfonso Albacete[3,4], Cristina Martínez-Andújar[4], Camilo L. M. Morais[5,6], Francis L Martin[7,8]*

[1] Lancaster Environment Centre, Lancaster University, UK

[2] Phlorum Ltd, UK

[3] Institute for Agro-Environmental Research and Development of Murcia (IMIDA), Department of Plant Production and Agrotechnology, C/ Mayor s/n, E-30150 La Alberca, Murcia, Spain
[4] CEBAS-CSIC. Department of Plant Nutrition. Campus Universitario de Espinardo, E-30100 Murcia, Spain
[5] Center for Education, Science and Technology of the Inhamuns Region, State University of Ceará, Tauá 63660-000, Brazil

[6] Graduate Program in Chemistry, Institute of Chemistry, Federal University of Rio Grande do Norte, Natal 59072-970, Brazil
[7] Department of Cellular Pathology, Blackpool Teaching Hospitals NHS Foundation Trust, Whinney Heys Road, Blackpool FY3 8NR, UK

[8] Biocel UK Ltd., Hull HU10 6TS, UK

*Corresponding author: Francis L Martin; Email: francis.martin2@nhs.net

Table S1: Lighting conditions within each Snijder cabinet

Light Quality	**'Light' Groups: LC, LD, LN and LLN**	**'Shade' Groups: SC, SD, SN and SLN**
PFD-R(700-780 nm)	72.51	49.28
PFD-FR(600-700 nm)	12.89	116.5
photosynthetic photon flux density PPFD(400-700 nm)	189.8	124.7
PFD-UV(380-400 nm)	0.5677	0.4402
PFD-B(400-500 nm)	33.93	21.58
PFD-G(500-600 nm)	83.40	53.87
peak wavelength λp / nm	545	741
peak wavelength value λpV / $mWm^{-2}nm^{-1}$	827.7	576.0
Irradiance	43.2	45.8
Illuminance/ lux.	15128	9617

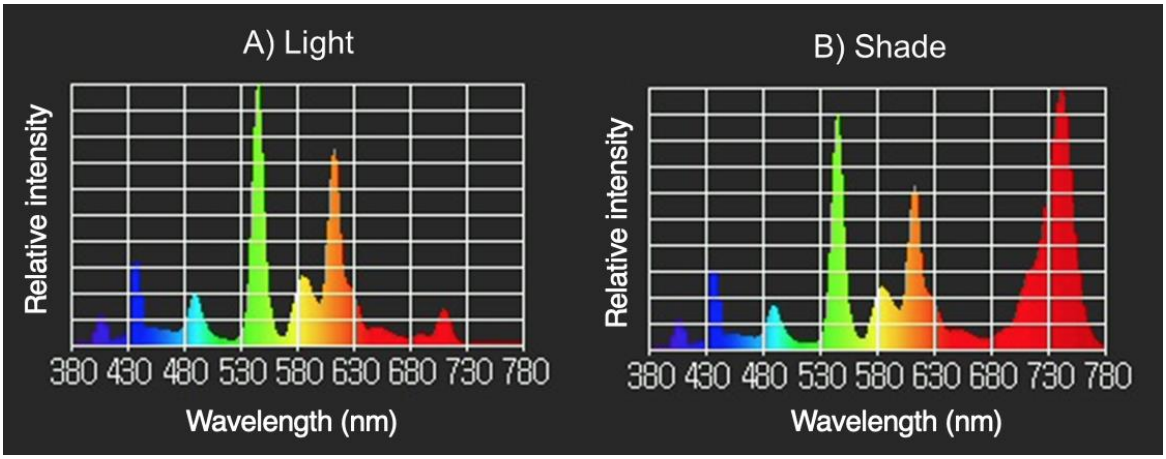

Figure S1: Spectra from a) 'Light' b) 'Shade' cabinets, providing red: far-red ratios of 5.6 and 0.4 respectively.

Table S2: Reagents used for Hoagland's solution. Full strength Hoagland's solution was made using 100 mL of solution A, 100 mL of solution B and 10 mL of solution C in 10 L of deionised water.

Solution	**Reagent**	**Concentration/ gL^{-1}**
A (100 mL)	NH_4NO_3	8.000
	$Ca(NO_3)_2.4H_2O$	82.600
	KNO_3	35.700
B (100 mL)	KNO_3	5.000
	KH_2PO_4	27.400
	$MgSO_4.7H_2O$ *added first	24.600
	$MnSO_4.5H_2O$	0.053
	H_3BO_3	0.140
	$CuSO_4.5H_2O$	0.015
	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.008
	$ZnSO_4.7H_2O$	0.060
C (10 mL)	Fe-EDTA	36.71

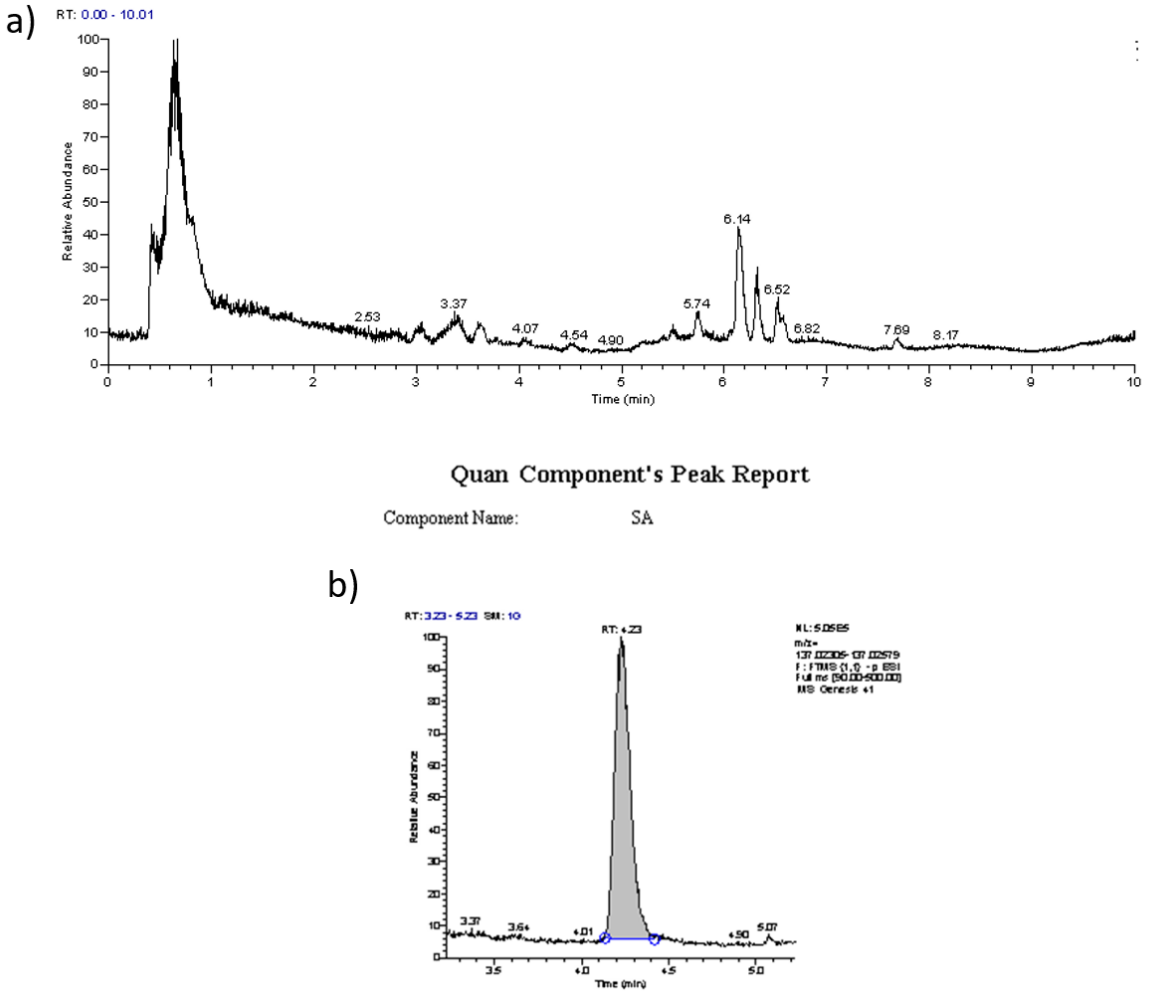

Figure S2: Total ion current and mass chromatogram (m/z 137.02442) for salicylic acid.

Table S3: Hormone descriptions and molecular ion masses

Hormone class	**Abbreviation**	**Hormone**	**Molecular formula**	**$[M-H]^-$**
Ethylene precursor	ACC	1-Aminocyclopropane-1-carboxylic acid	$C_4H_7NO_2$	100.04040
Cytokinins	t-Z	*trans*-Zeatin	$C_{10}H_{13}N_5O$	218.10473
	t-ZR	*trans*-Zeatin riboside	$C_{15}H_{21}N_5O_5$	350.14699
	iP	Isopentenyladenine	$C_{10}H_{13}N_5$	202.10982
Gibberellins	GA1	Gibberellin A1	$C_{19}H_{24}O_6$	347.15001
	GA3	Gibberellin A3	$C_{19}H_{22}O_6$	345.13436
	GA4	Gibberellin A4	$C_{19}H_{24}O_5$	331.15510
Auxins	IAA	Indole-3-acetic acid	$C_{10}H_9NO_2$	174.05605
Abscisic acid	ABA	Abscisic acid	$C_{15}H_{20}O_4$	263.12888
Salicylates	SA	Salicylic acid	$C_7H_6O_3$	137.02442
Jasmonates	JA	Jasmonic acid	$C_{12}H_{18}O_3$	209.11832

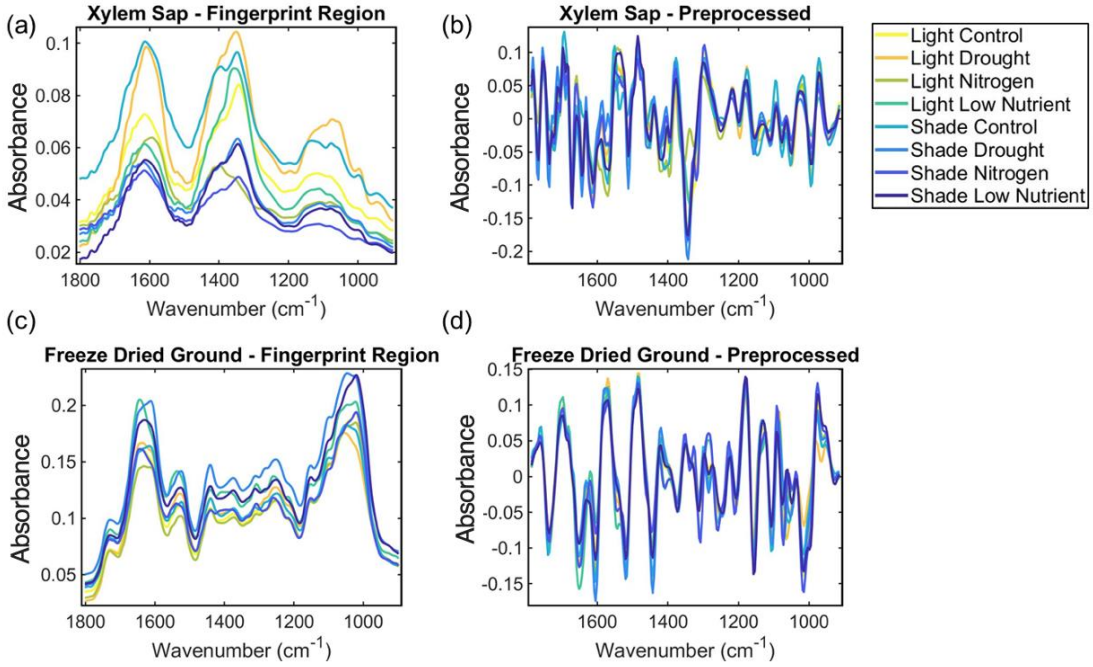

Figure S3: (a) Raw and **(b)** pre-processed class means spectra in the fingerprint region from xylem sap, **(c)** Raw and **(d)** pre-processed (Savitzky–Golay 2nd differentiation, *n*=9, and vector normalisation) class means spectra in the fingerprint region from freeze-dried ground leaves. Each class is grouped by treatment; Light Control (LC), Light Drought (LD), Light Nitrogen (LN), Light Low Nitrogen (LLN), Shade Control (SC), Shade Drought (SD), Shade Nitrogen (SN) and Shade Low Nitrogen (SLN).

Table S4: SVM parameters for classification

	Cost	**Gamma (γ)**	**Number of support vectors (N_{SV})**
Xylem Sap	31.6228	3.1623	314
Freeze-dried ground leaves	100	3.1623	194

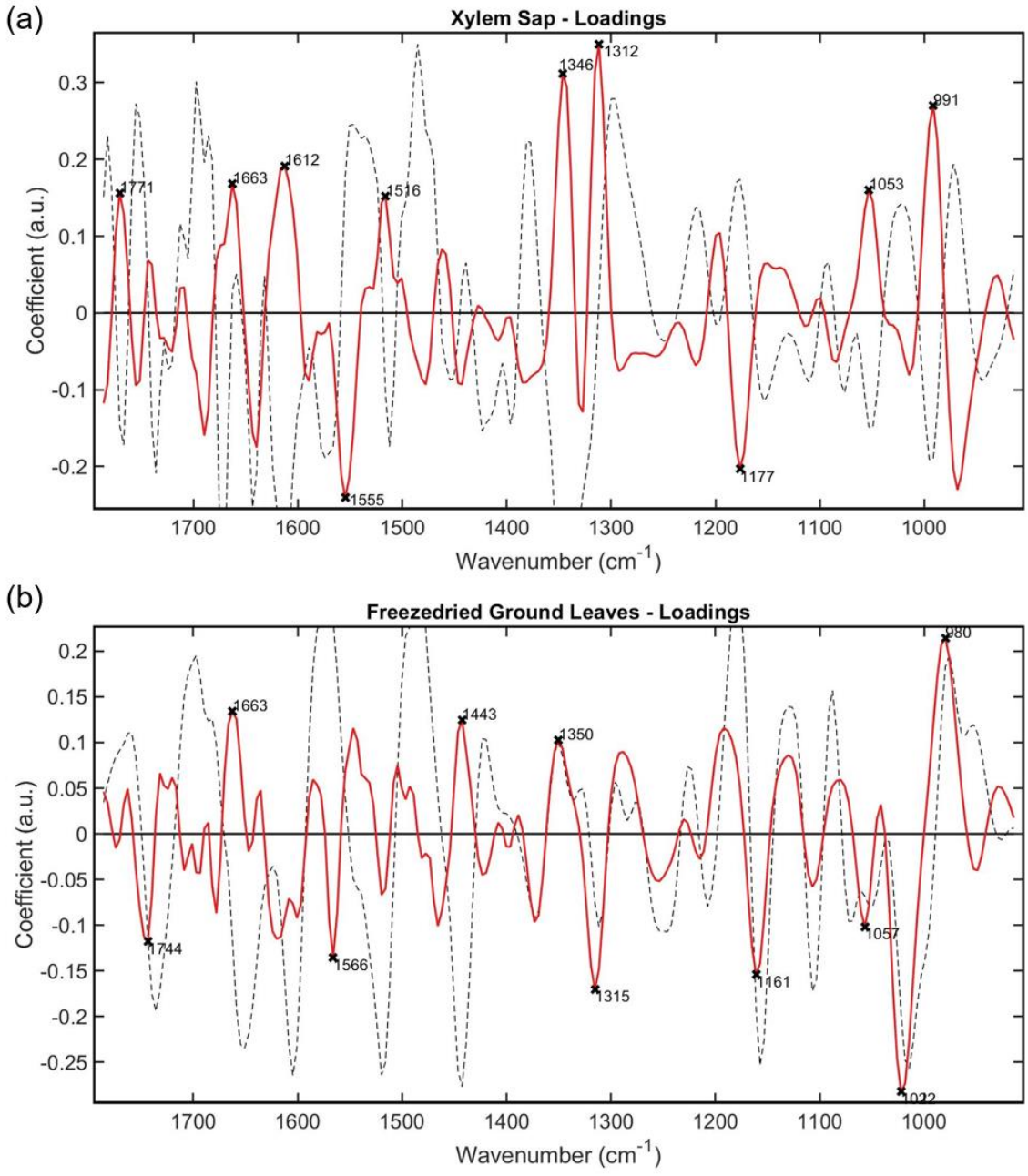

Figure S4: Loadings from spectra of a) xylem sap and b) freeze-dried ground leaf samples. These are the key wavenumbers which differentiate spectral profiles of different treatment groups from one another. The red line represents the PCA loadings and the black-dashed line represents the total mean spectrum, scaled to fit.

Table S5: PCA-loadings and biomarkers: key wavenumbers and compounds, which differentiate ATR-FTIR spectral profiles of plants from different growth conditions for both xylem sap and freeze-dried ground sample types.

Sample Type	Wavelength / cm	Tentative Molecular Assignment	Reference
Xylem sap	1770.65	v_1 symmetric stretching of C=O in the carboxylic acid of pectin or ester bond of triacylglycerol	(Nozahic and Amziane, 2012)
	1662.64	The N-C=O group of proteins. Amide I vibrations, specifically associated with disordered secondary structures or turns.	(Belfer *et al.*, 1998; Shivu *et al.*, 2013)
	1612.49	Amide I	(Jin *et al.*, 2018)
	1554.62	C-N stretching and N-H bending (Amide II vibration); C-O-O^- asymmetric stretching of proteins and glutamate	(Moskal *et al.*, 2019)
	1516.05	Amide II vibrations of proteins	(Talari *et al.*, 2017)
	1346.31	Cellulose	(Gorzsas, 2020)
	1311.59	Amide III vibrations of proteins	(Talari *et al.*, 2017)
	1176.58	C–O stretch vibration of tannins	(Falcão and Araújo, 2013)
	1053.13	Starch, *v*C–O and δC–O of carbohydrates	(Talari *et al.*, 2017; Jin *et al.*, 2018)
	991.41	C–O ribose	(Camilo L. M. Morais *et al.*, 2017)
Freeze-dried ground leaves	1743.53	Ester C=O stretch: triglycerides	(Talari *et al.*, 2017)
	1662.52	The N-C=O group of proteins. Amide I vibrations, specifically associated with disordered secondary structures or turns.	(Belfer *et al.*, 1998; Shivu *et al.*, 2013)
	1566.09	N-H bending; C-N stretching (Amide II band of proteins)	(Rana *et al.*, 2018)
	1442.65	Pectin	(Sharma and Uttam, 2018)
	1350.08	Phosphodiester stretching bands region (for absorbances due to starch)	(Talari *et al.*, 2017)
	1315.36	Cellulose	(Sharma and Uttam, 2018)
	1161.06	C–OH groups of serine, threonine and tyrosine of proteins, C-O stretching and hydrogen bonding	(Talari *et al.*, 2017)
	1056.92	Stretching C–O deoxyribose	(Talari *et al.*, 2017)
	1022.2	Starch	(Talari *et al.*, 2017)
	979.769	C-OH stretching of secondary alcohols and C-O-C vibrations of polysaccharides	(Ajitha *et al.*, 2015)

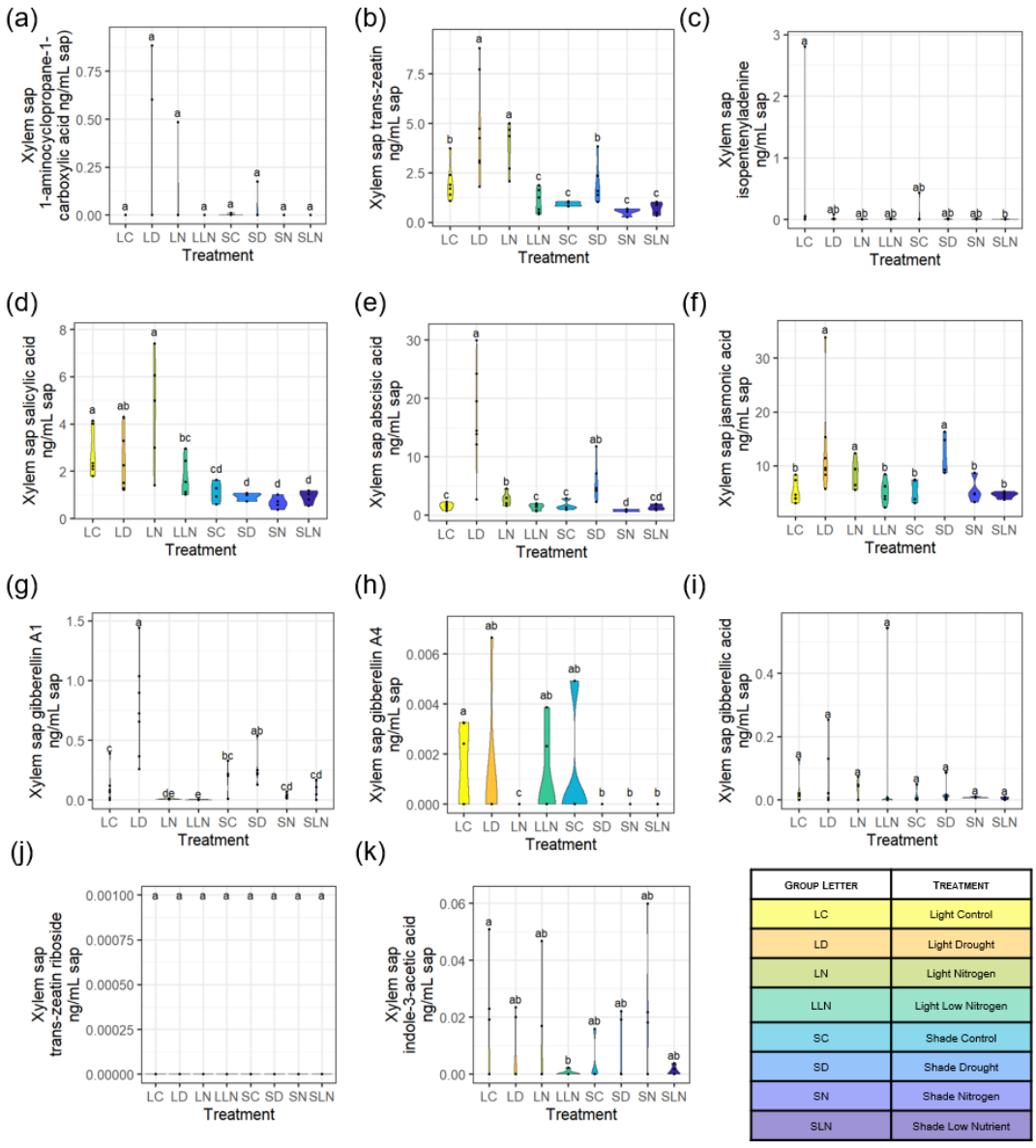

Figure S5: Hormone profiles from xylem sap measured using UHPLC– HRMS in ng·ml^{-1} sap for a) 1-amino-cyclopropanecarboxylic acid (ACC), b) *trans*-Zeatin (tZ), c) isopentyl-adenine (iP), d) salicylic acid (SA), e) abscisic acid (ABA), f) jasmonic acid (JA), g) gibberellin A1 (GA_1), gibberellin A4 (GA_4), gibberellic acid (GA_3), *trans*-zeatin riboside (tZR), and indole-3-acetic acid (IAA). ABA concentration was highest in the drought categories; LD had ~17 ng·ml^{-1} sap of ABA compared with SD which had ~7 ng·ml^{-1} sap, whilst the other categories ranged between ~1 and 3 ng·ml^{-1} sap. Shade plants had lower xylem SA levels than light ones, in the range of 0.7-1.1 ng·ml^{-1} sap compared with 1.6-4.5 ng·ml^{-1} sap respectively. Xylem sap levels of GA_1 were approximately three times higher in LD than most other treatment groups, although this was not significantly different to the other drought category, SD, due to high variation.

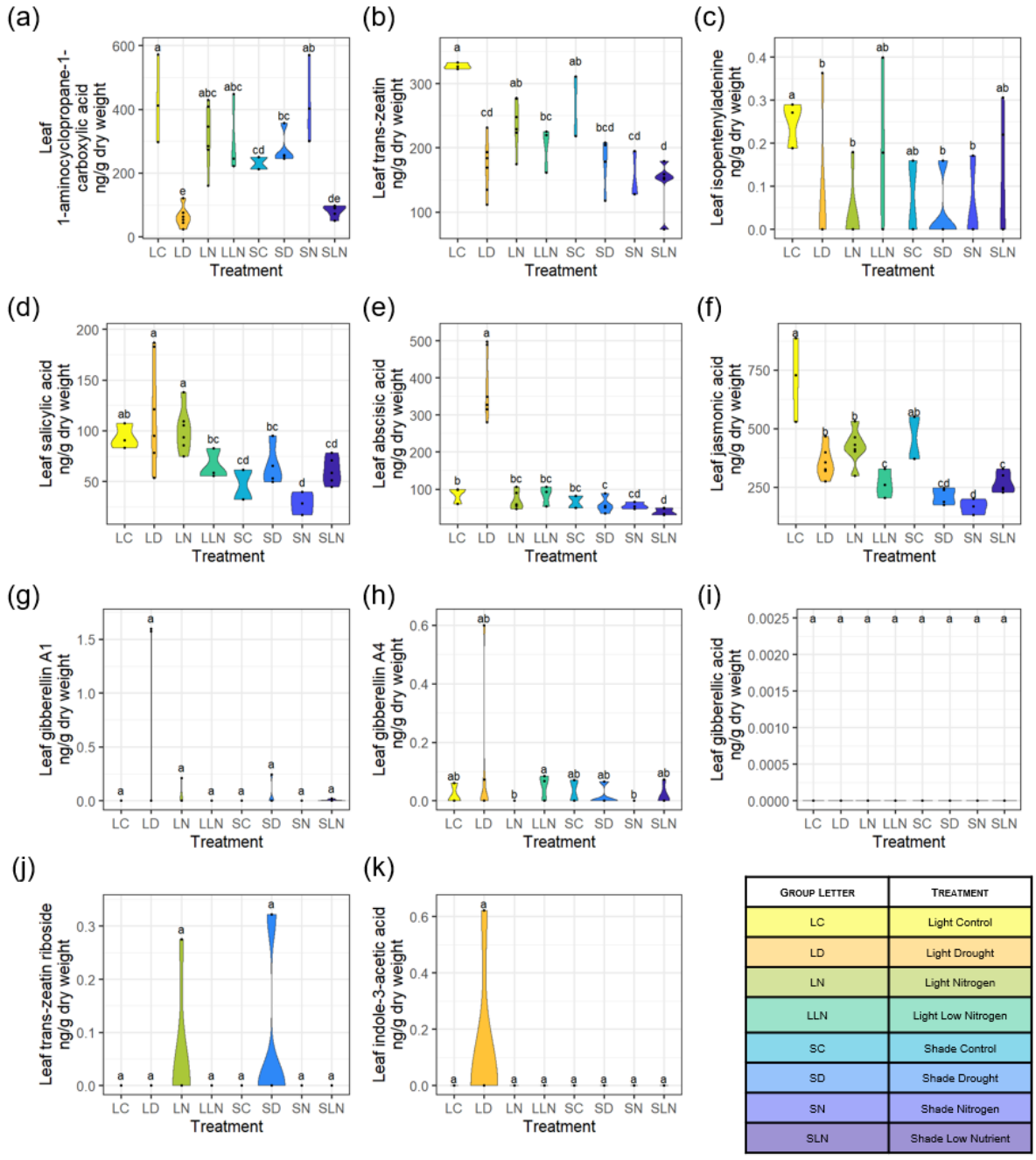

Figure S6: Hormone profiles from freeze-dried ground leaves measured using UHPLC–HRMS in ng·g^{-1} dry weight for a) 1-amino-cyclopropanecarboxylic acid (ACC), b) *trans*-Zeatin (tZ), c) isopentyl-adenine (iP), d) salicylic acid (SA), e) abscisic acid (ABA), f) jasmonic acid (JA), g) gibberellin A1 (GA_1), gibberellin A4 (GA_4), gibberellic acid (GA_3), *trans*-zeatin riboside (tZR), and indole-3-acetic acid (IAA). Leaf ABA levels (Figure S5) were approximately quadruple in LD than those of the other categories. Plants grown under LC treatment category registered approximately 4.5-fold higher of leaf tZ than those in SLN. Leaf JA concentration was significantly higher in the light control group LC (~710 ng·g^{-1} dry weight) compared to all other groups (ranging 170-420 ng·g^{-1} dry weight), except the shade control group SC (~460 ng·g^{-1} dry weight). The highest iP hormone concentration was found in leaves of category LC, at 0.25 ng·g^{-1} dry weight. This value was significantly higher compared to groups LD, LN, SD, SN (ranging 0.03-0.6 ng·g^{-1} dry weight), with the other groups falling in between.

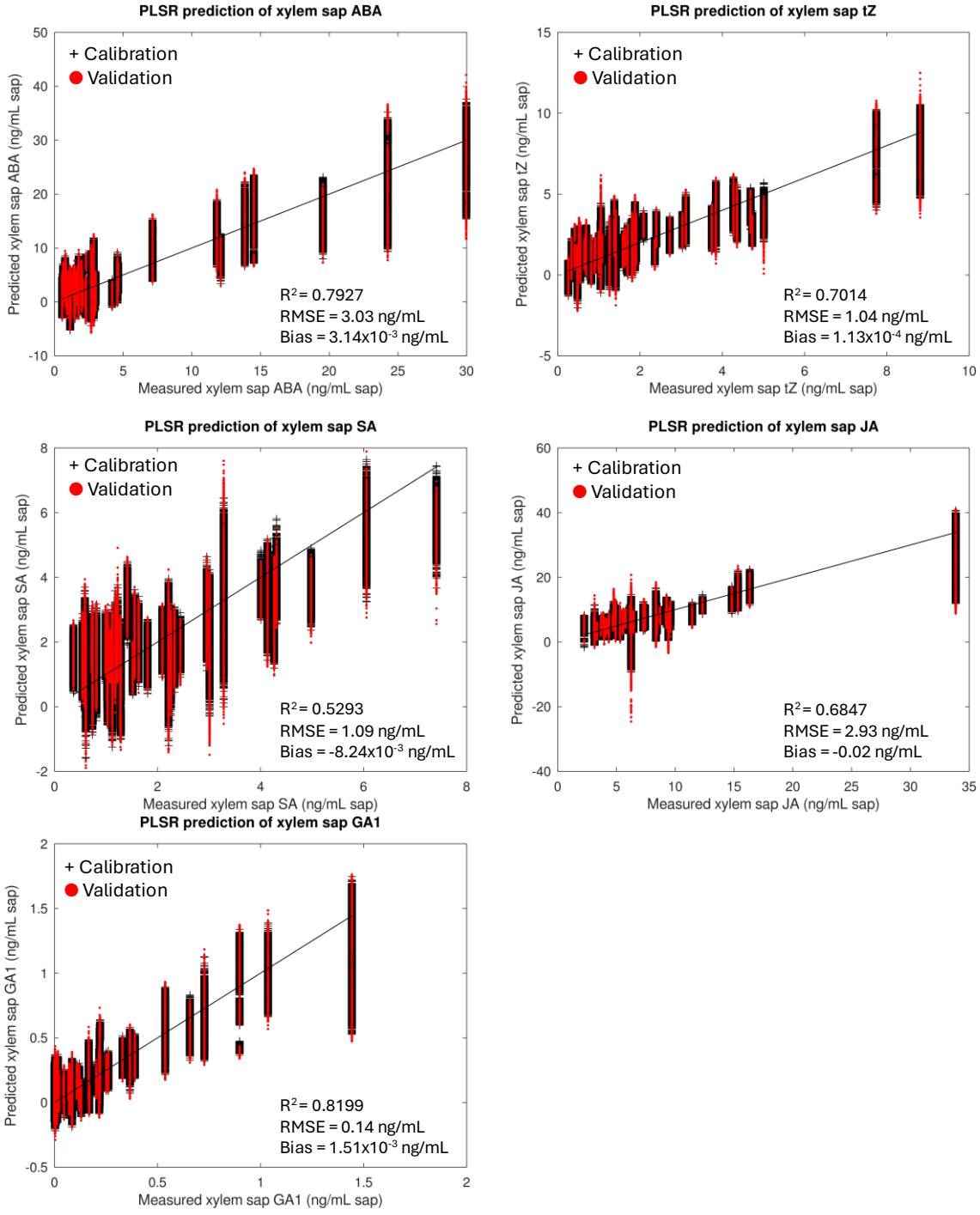

Figure S7: PLS regression graphs for prediction of plant hormones from xylem sap. Validation was performed by Monte-Carlo cross-validation with 20% of samples left-out for validation during 1000 iterations. All models were built using 10 latent variables.

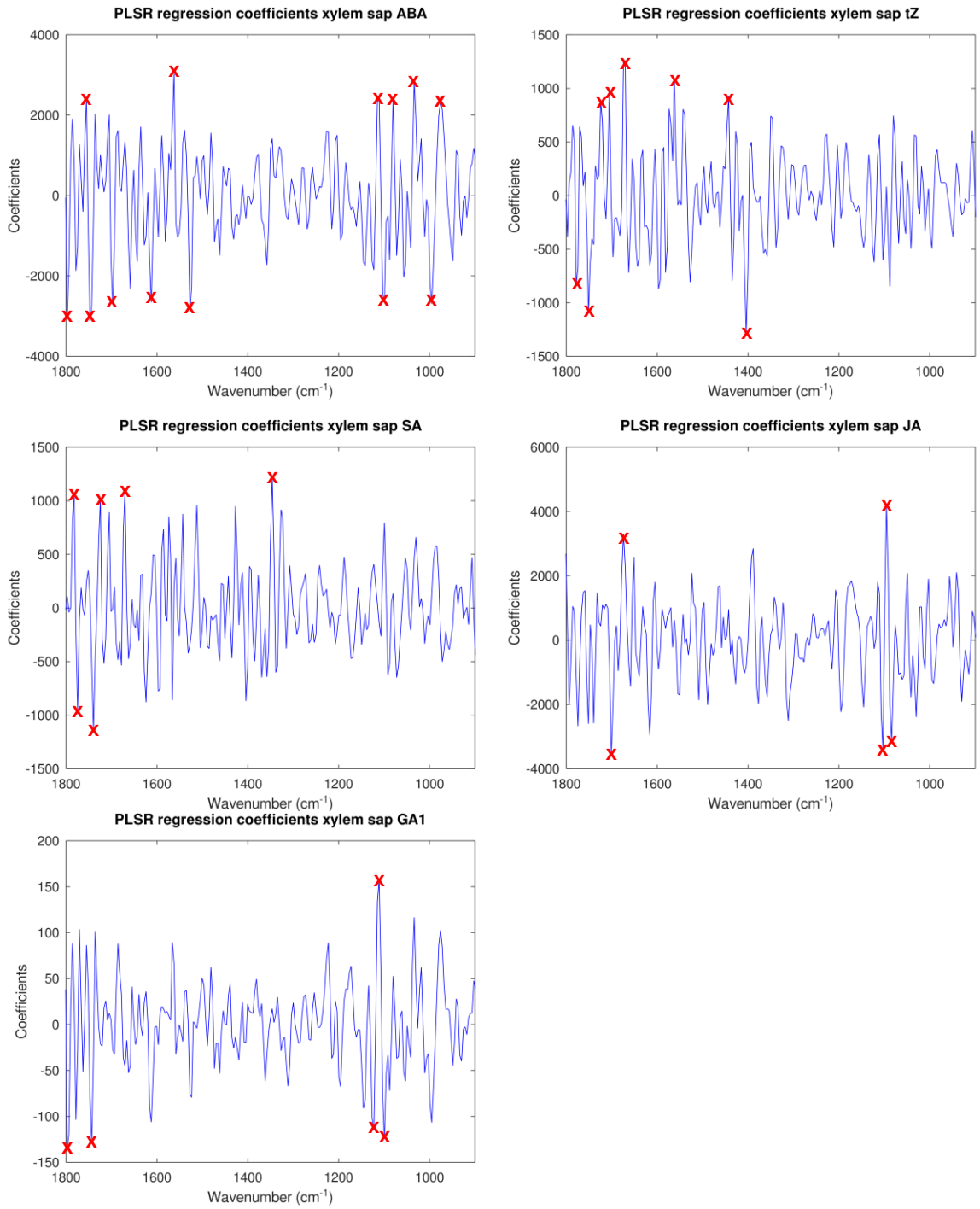

Figure S8: PLSR regression coefficients for prediction of plant hormones from xylem sap. Main wavenumbers are marked with a red X.

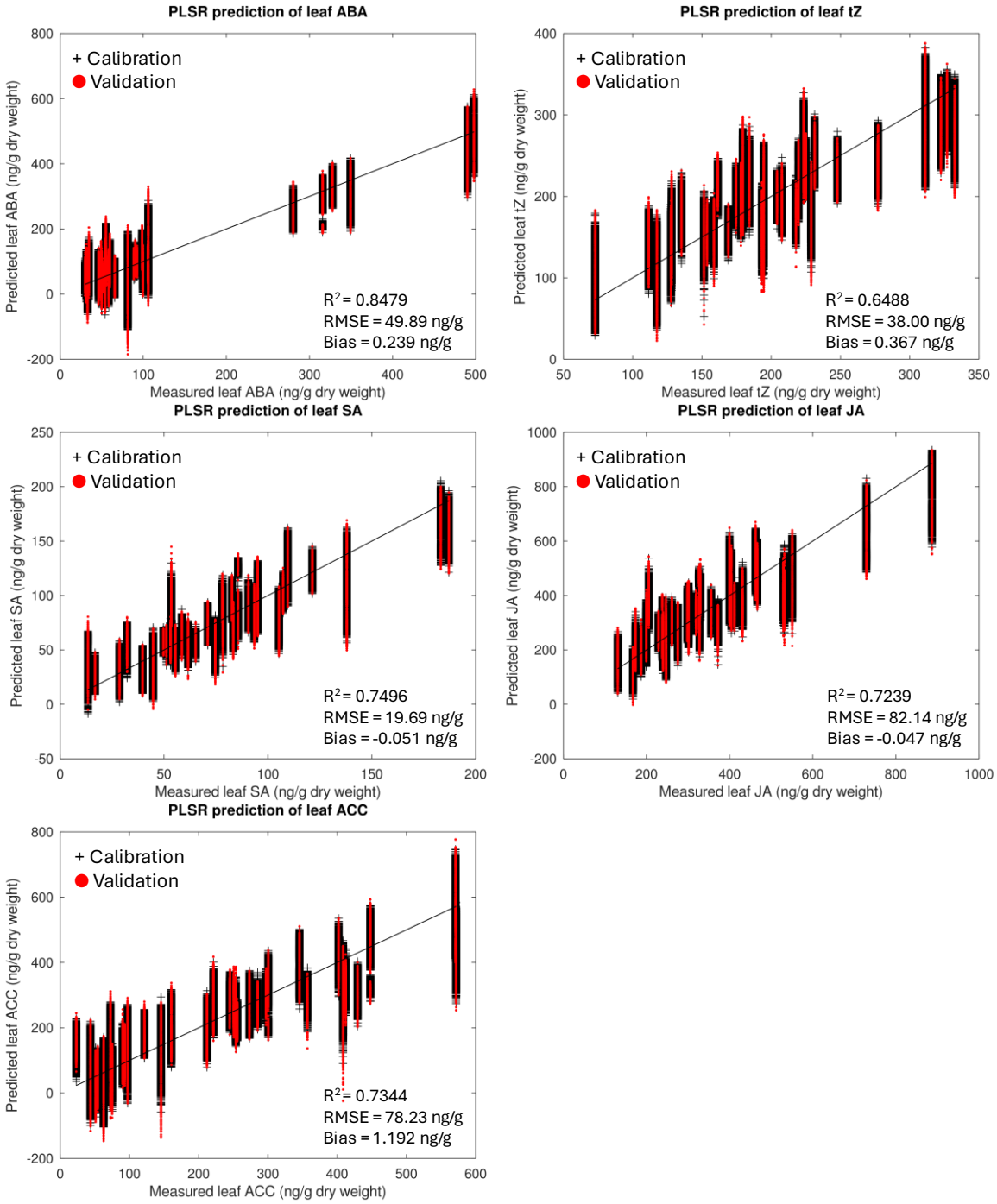

Figure S9: PLS regression graphs for prediction of plant hormones from freeze-dried ground leaves. Validation was performed by Monte-Carlo cross-validation with 20% of samples left-out for validation during 1000 iterations. All models were built using 10 latent variables.

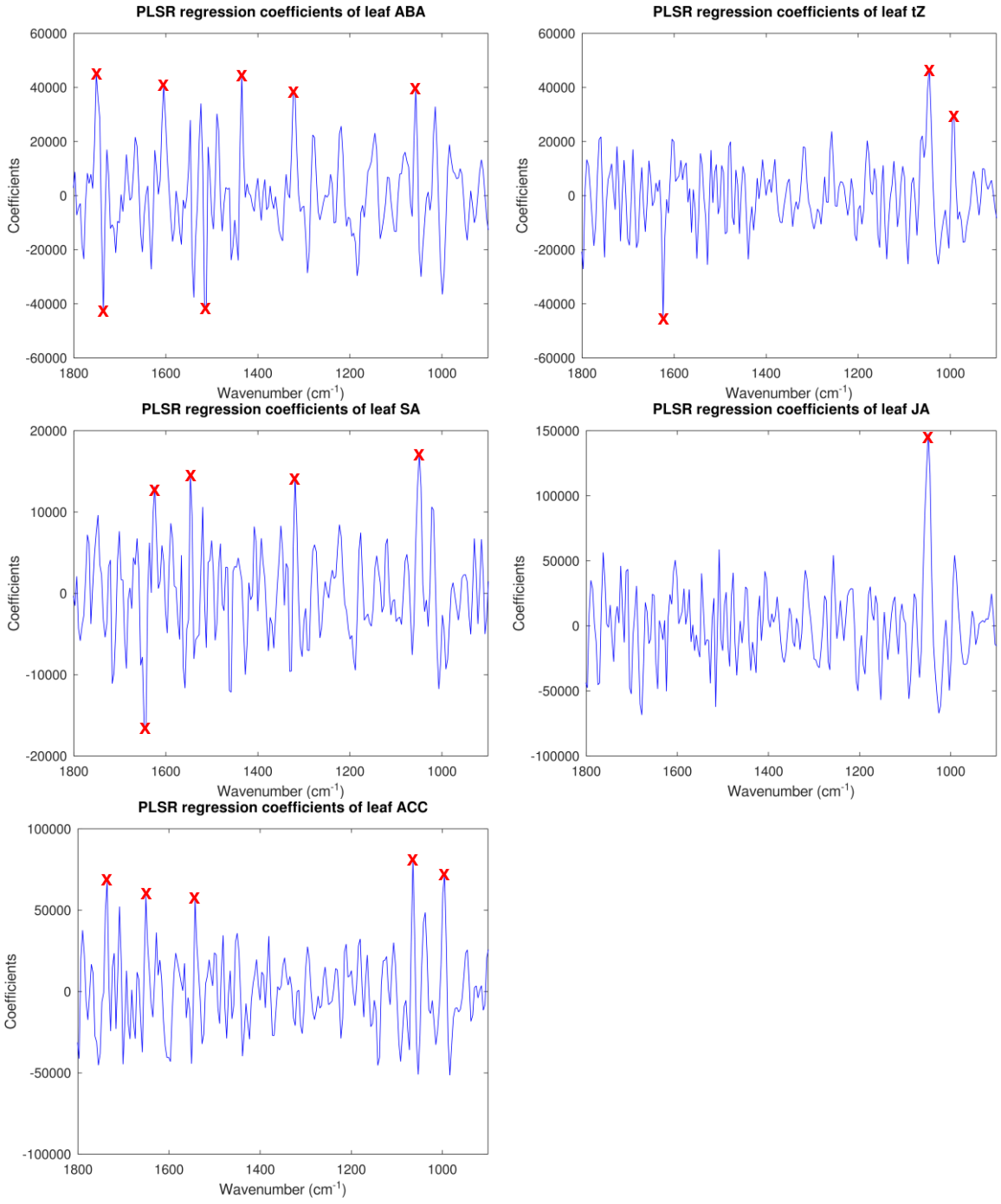

Figure S10: PLSR regression coefficients for prediction of plant hormones from freeze-dried ground leaves. Main wavenumbers are marked with a red X.

Xylem Sap Number of LVs	tz	iP	GA1	GA3	GA4	IAA	ABA	JA	SA
Light Control	6	8	9	NA	6	6	8	7	7
Light Drought	9	4	10	9	NA	NA	10	10	9
Light Nitrogen	6	4	8	5	NA	NA	6	5	5
Light Low Nutrient	7	7	9	NA	NA	NA	9	7	10
Shade Control	7	6	5	5	NA	NA	4	4	4
Shade Drought	3	NA	5	7	NA	NA	5	7	7
Shade Nitrogen	7	7	7	5	NA	6	7	6	7
Shade Low Nutrient	7	NA	7	NA	NA	NA	7	6	6

FDG Leaves Number of LVs	ACC	tz	ABA	JA	SA
Light Control	5	5	7	5	5
Light Drought	7	7	7	6	9
Light Nitrogen	8	8	9	7	7
Light Low Nutrient	5	4	4	5	5
Shade Control	3	5	2	4	4
Shade Drought	5	5	5	5	4
Shade Nitrogen	4	4	4	5	3
Shade Low Nutrient	7	6	6	8	6

Table S6: Number of latent variables (LVs) used to build the PLSR models between different types of treatment and hormone levels for xylem sap and freeze-dried ground (FDG) leaves. Higher number of LVs represents higher model complexity.