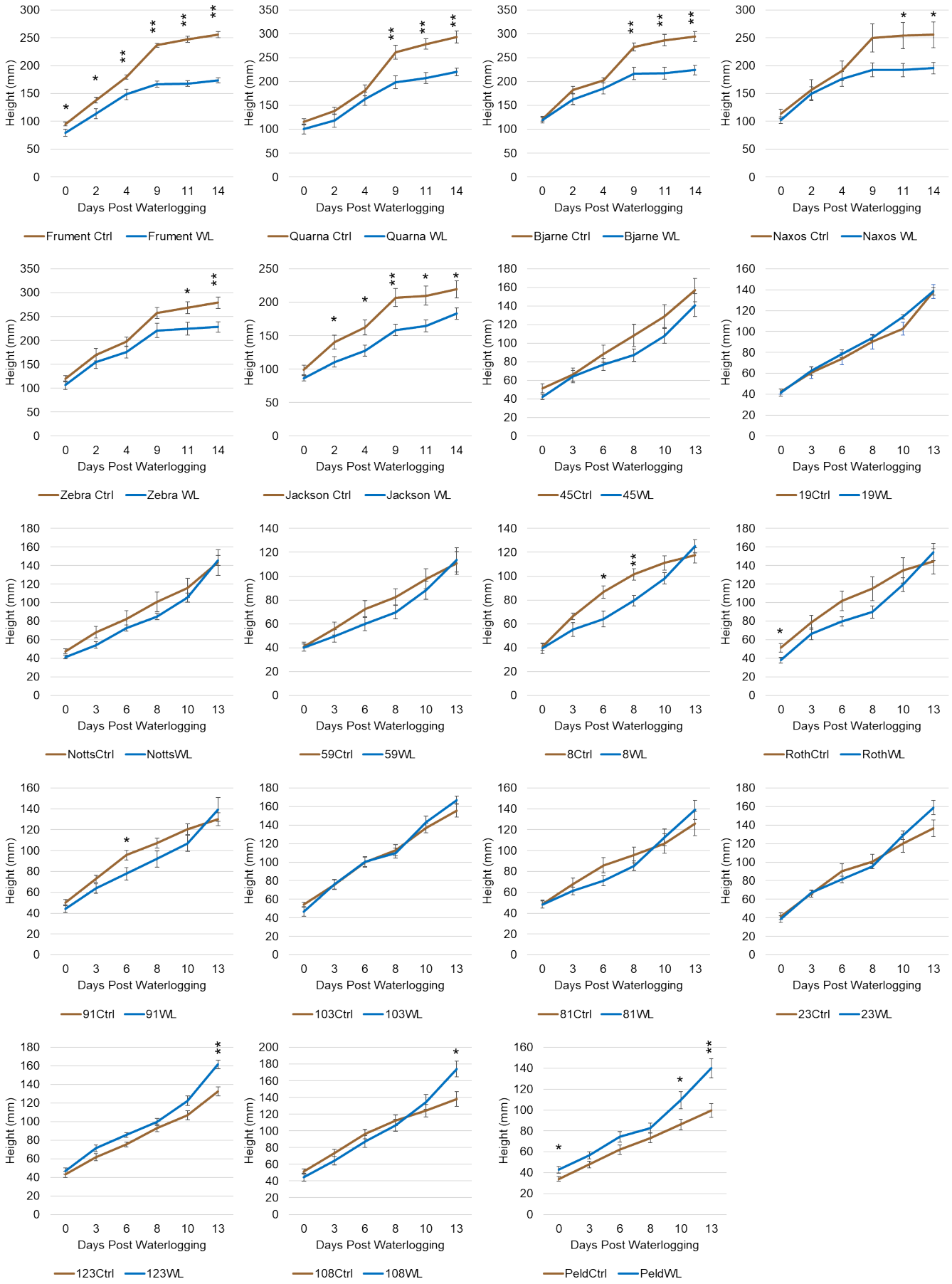
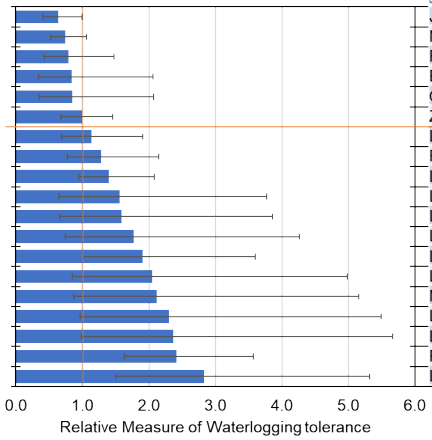


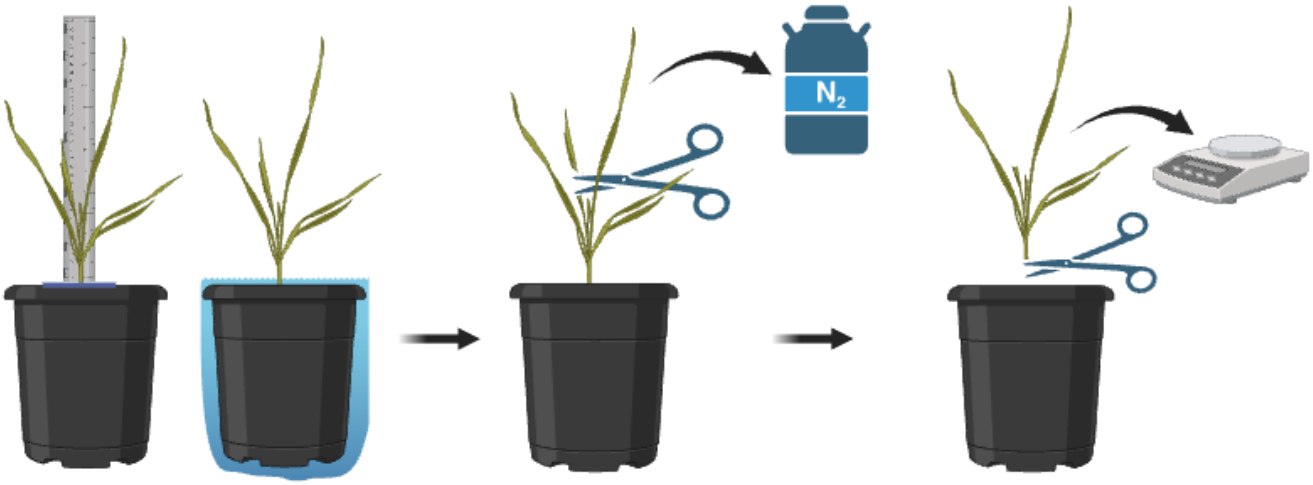
Supplementary Figure 1: Heights of longest leaf through time of wheat cultivars and black-grass biotypes grown on control (brown) or waterlogging (blue) conditions. Data shown are averages of 12 individual

plants from four pots ± standard error except for Lola23 and Notts Control, and Quarna, Lola8, Lola23, and Lola108 Waterlogged where 11 plants were measured. * indicates that a heteroscedastic, two-tailed distribution Student's T-Test gave a $P<0.05$ between control samples compared to waterlogged samples and ** indicates a $P<0.01$.

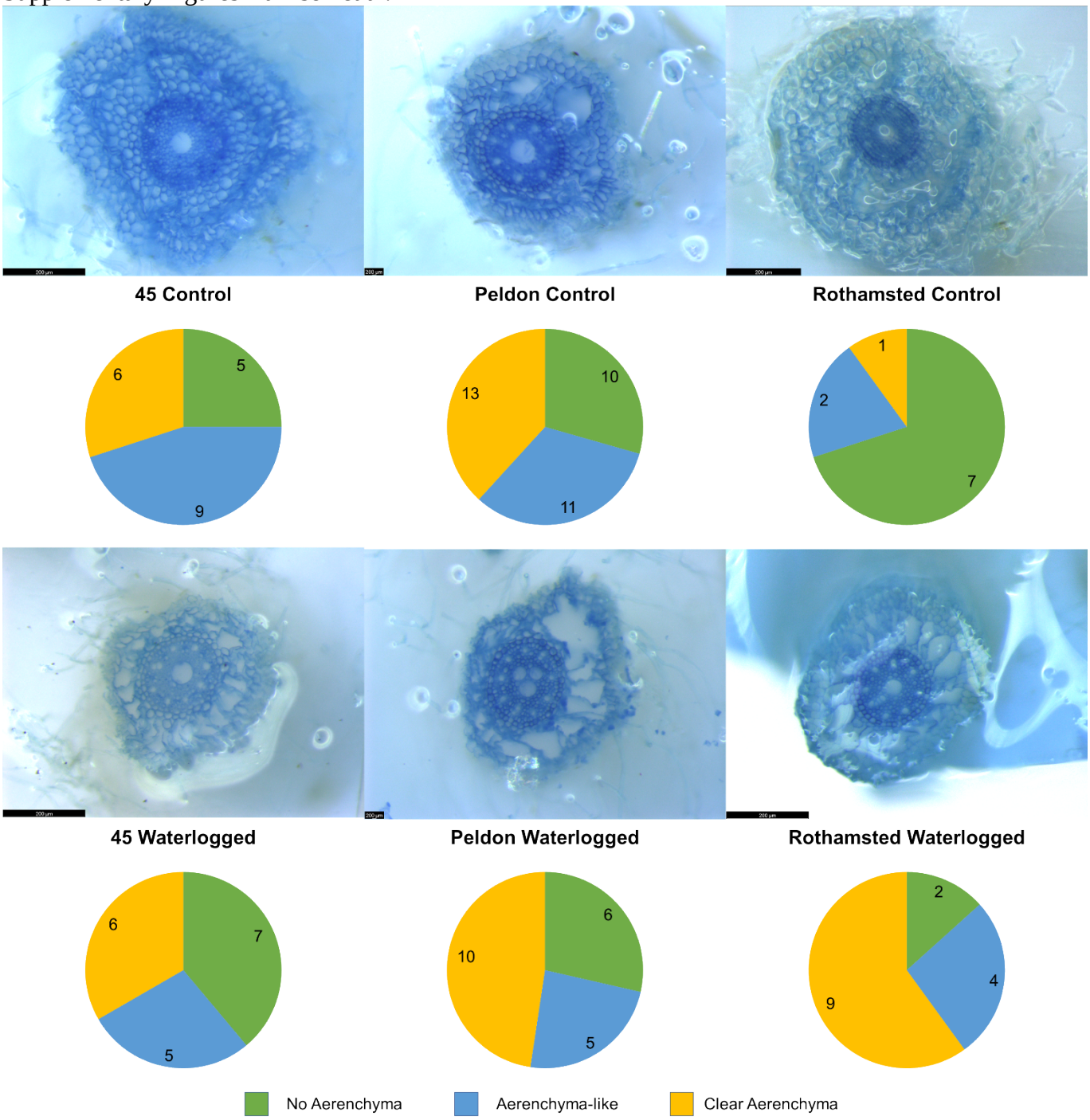


Line!	Relative Measure of Waterlogging tolerance	Lower CI bt	Upper CI bt	Control	Waterlogged	Difference (W-C)	SED	Lower CL	Upper CL	Prob
Jackson	0.642	0.414	0.996	-0.052	-0.495	-0.443	0.223	-0.882	-0.004	0.048
Naxos	0.747	0.524	1.064	0.120	-0.172	-0.292	0.180	-0.647	0.062	0.106
Frument	0.794	0.427	1.476	0.752	0.521	-0.231	0.315	-0.852	0.389	0.464
Bjarne	0.843	0.344	2.061	0.646	0.475	-0.171	0.454	-1.066	0.723	0.706
Quarna	0.853	0.351	2.075	0.205	0.046	-0.159	0.451	-1.047	0.730	0.725
Zebra	0.996	0.681	1.457	0.158	0.154	-0.004	0.193	-0.384	0.376	0.983
Lola81	1.141	0.684	1.904	-1.506	-1.374	0.132	0.260	-0.380	0.644	0.612
Roth	1.288	0.772	2.150	-1.493	-1.240	0.253	0.260	-0.259	0.766	0.331
Lola45	1.402	0.946	2.080	-1.501	-1.163	0.338	0.200	-0.056	0.732	0.092
Lola8	1.564	0.648	3.773	-2.092	-1.645	0.447	0.447	-0.433	1.328	0.318
Lola91	1.595	0.659	3.864	-1.873	-1.406	0.467	0.449	-0.417	1.352	0.299
Lola59	1.777	0.741	4.261	-2.261	-1.686	0.575	0.444	-0.300	1.450	0.197
Lola103	1.911	1.016	3.597	-1.377	-0.730	0.648	0.321	0.015	1.280	0.045
Lola19	2.057	0.849	4.982	-1.810	-1.089	0.721	0.449	-0.163	1.606	0.110
Notts	2.118	0.869	5.159	-2.086	-1.335	0.750	0.452	-0.140	1.641	0.098
Lola108	2.302	0.966	5.489	-1.725	-0.891	0.834	0.441	-0.035	1.703	0.060
Lola123	2.366	0.989	5.663	-1.740	-0.879	0.861	0.443	-0.011	1.734	0.053
Peldon	2.414	1.631	3.573	-2.015	-1.133	0.881	0.199	0.489	1.273	0.000
Lola23	2.827	1.502	5.320	-2.084	-1.045	1.039	0.321	0.407	1.671	0.001

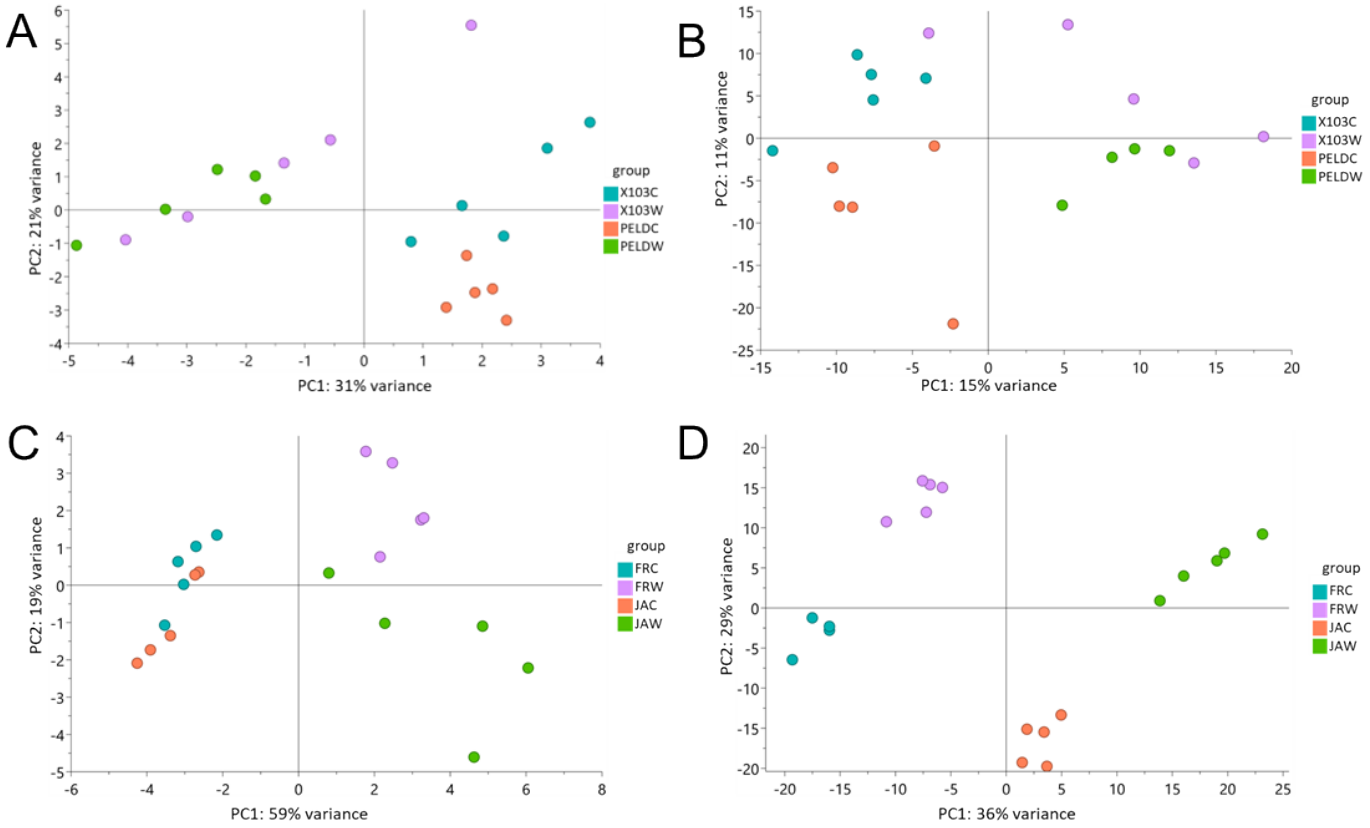
Supplementary Figure 2: Combined analysis of the natural log-transformed plant fresh weight data from a total of 294 plots from the set of waterlogging trials (ST1, ST3, ST4, ST5, ST6, ST8, ST9) calculated by fitting a linear mixed model using the REML (Restricted Maximum Likelihood) with Linear Mixed Model (LMM) algorithm. Bar chart shows the relative measure of waterlogging tolerances as determined by the back-transformed values ± lower or upper confidence limits of the log-transformed data ("Lower CL bt" and "Upper CL bt" respectively). Table columns labelled "Control" and "Waterlogged" are the fresh weight means on the log-transformed scale. Column labelled "Difference (W-C)" is the differences between these values, calculated as Waterlogged minus Control so that negative values indicate a reduction in fresh weight as a result of the waterlogging treatment and positive values indicate an increase (as described in the text in the manuscript). Column labelled "SED" is then the Standard Error of the Difference for each of these comparisons, calculated using all of the data across the 8 experiments – larger values reflect where the replication levels were lower. Column "Prob" is the significance levels for a T-Test of the null hypothesis that waterlogging has no effect on fresh weight. In summary, all wheat lines have a relative measure of waterlogging that is less than 1 and negative values for Difference (W-C) while black-grass relative measures are greater than 1 and positive differences. Jackson shows evidence of a significant reduction in fresh weight as a result of waterlogging, with three blackgrass lines (Lola23, Peldon, Lola103) showing evidence of a significant increase in fresh weight as a result of waterlogging.



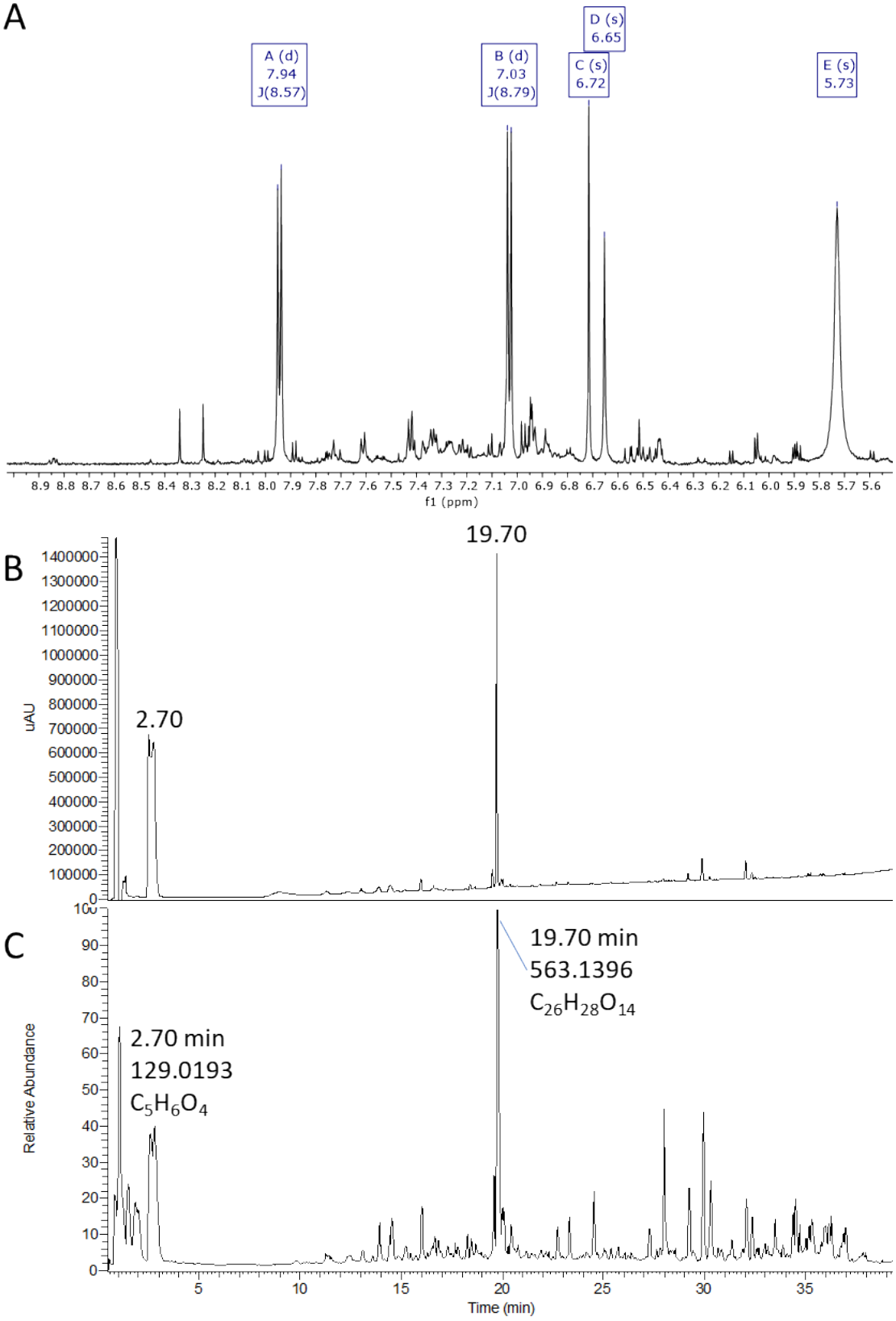
Supplementary Figure 3: Pictorial illustration of height and fresh weight measurements as well as sampling procedure for 'omics analysis. Plant height was measured with a ruler with a flat base recording the length of the longest leaf. 200-300 ng of material was taken from the youngest leaves and flash frozen in liquid nitrogen until RNA isolation, library preparation and sequenced. The remainder of the aerial tissue was removed and weighed, then flash frozen for metabolomics analysis. Figure was created in BioRender.com



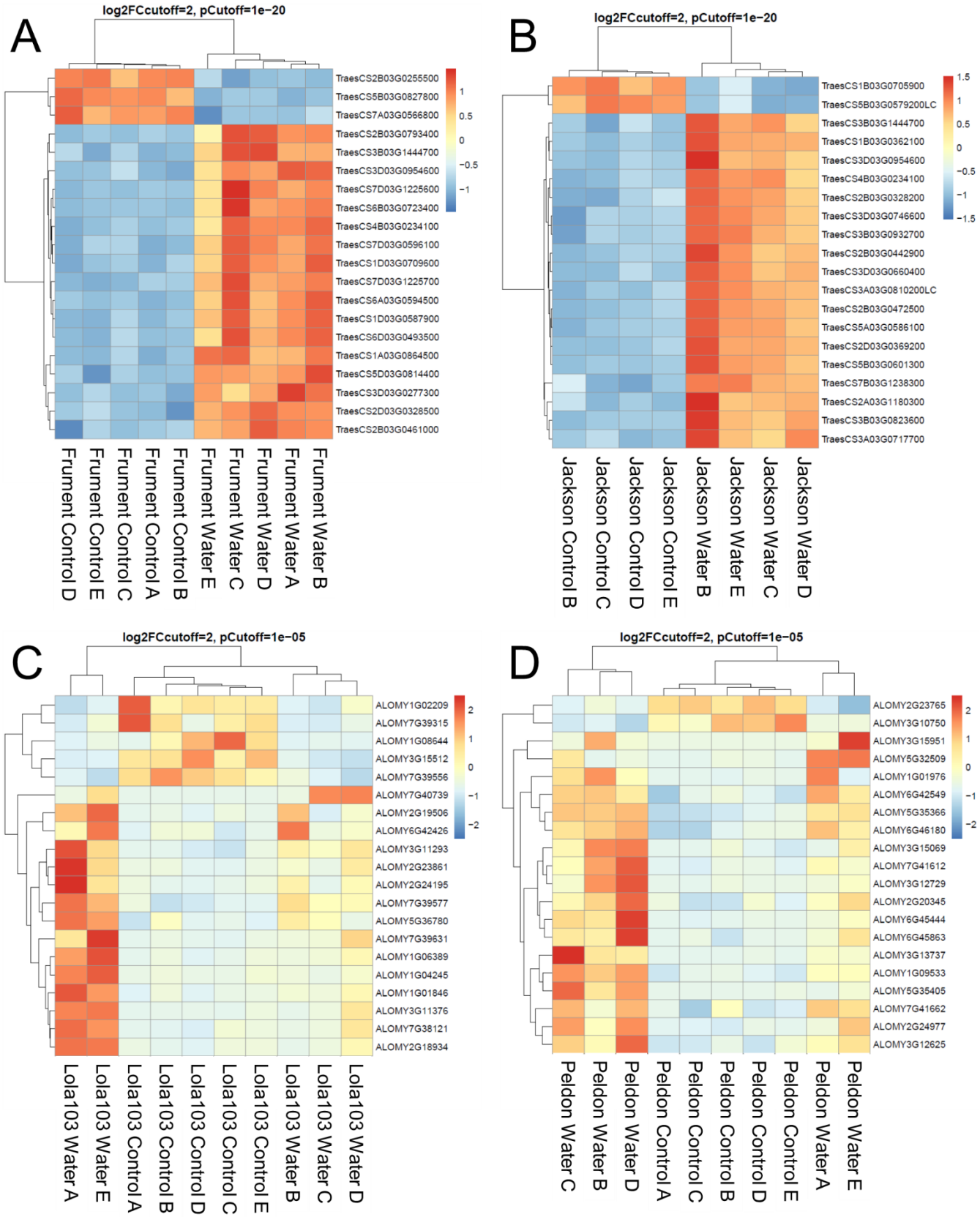
Supplementary Figure 4: Black-grass has constitutive aerenchyma in the roots. Microscopy sections showing evidence for aerenchyma formation in three different biotypes of black-grass. Scale bars represent 200 µm, Qualitative assessment for presence or absence of aerenchyma in black-grass root sections is shown below. Number of sections with no aerenchyma (green), small or ill-defined aerenchyma (blue) or clearly identifiable aerenchyma (yellow) are shown in the pie graphs.



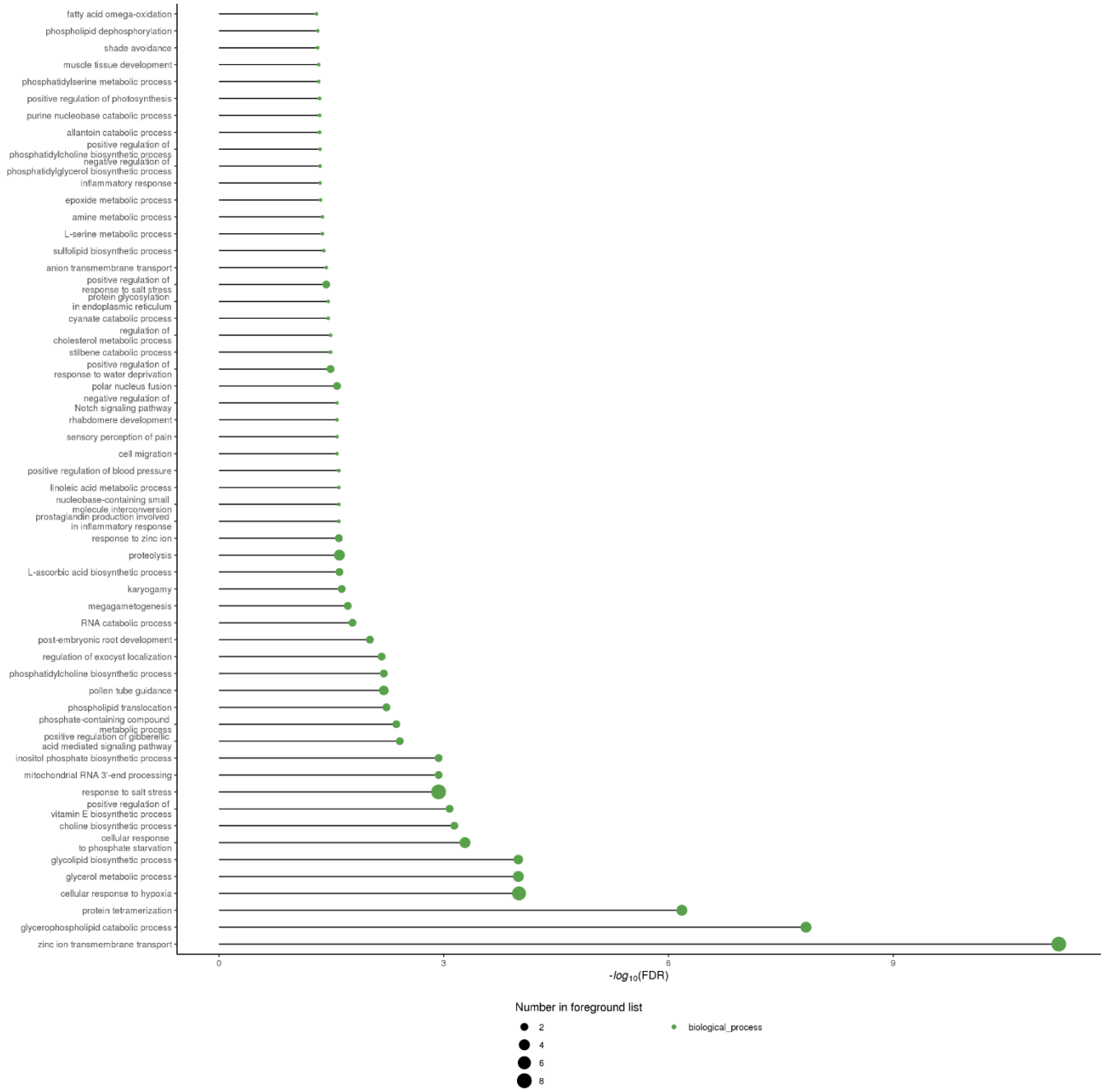
Supplementary Figure 5: Principal Component Analysis (PCA) scores plot of qNMR (A&C) and LC-MS negative ion mode (B&D) metabolomics datasets. While wheat cultivars (C&D) showed clear separation between treatments and adequate clustering of replicates, variance among black-grass samples (A&B) was less clearly explained. PELDC – Peldon control, PELDW – Peldon waterlogged, X103C – Lola103 control, X103W – Lola103 waterlogged, FRC – Frument control, FRW – Frument waterlogged, JAC – Jackson control, JAW – Jackson waterlogged.



Supplementary Figure 6. (A) Aromatic/olefinic region of ^{1}H NMR spectrum of a black-grass representative (Lola103-control) in D_2O:CD_3OD (80:20) referenced to d4-TSP (0.01% w/v) collected at 600 MHz. (B) PDA and (C) TIC (negative ion mode) traces of the water-methanol extract of Lola103-control highlighting the two major metabolites present in black-grass samples.



Supplementary Figure 7: Heat map of the top 20 DEG for Frument (A), Jackson (B), Lola103 (C) and Peldon (D) samples. Colours indicate degree to which the gene is differentially expressed within the sample with red colours indicating two-fold increase and blue colours two-fold decrease. Yellow colours indicate unchanged.



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65 **<u>Supplementary Figure 8</u>**: GO Enrichment analysis of the 129 unique DEG in Frument using online tools
66 from Chen et al. (2020). Full dataset are available in *Supplementary Table 6.*

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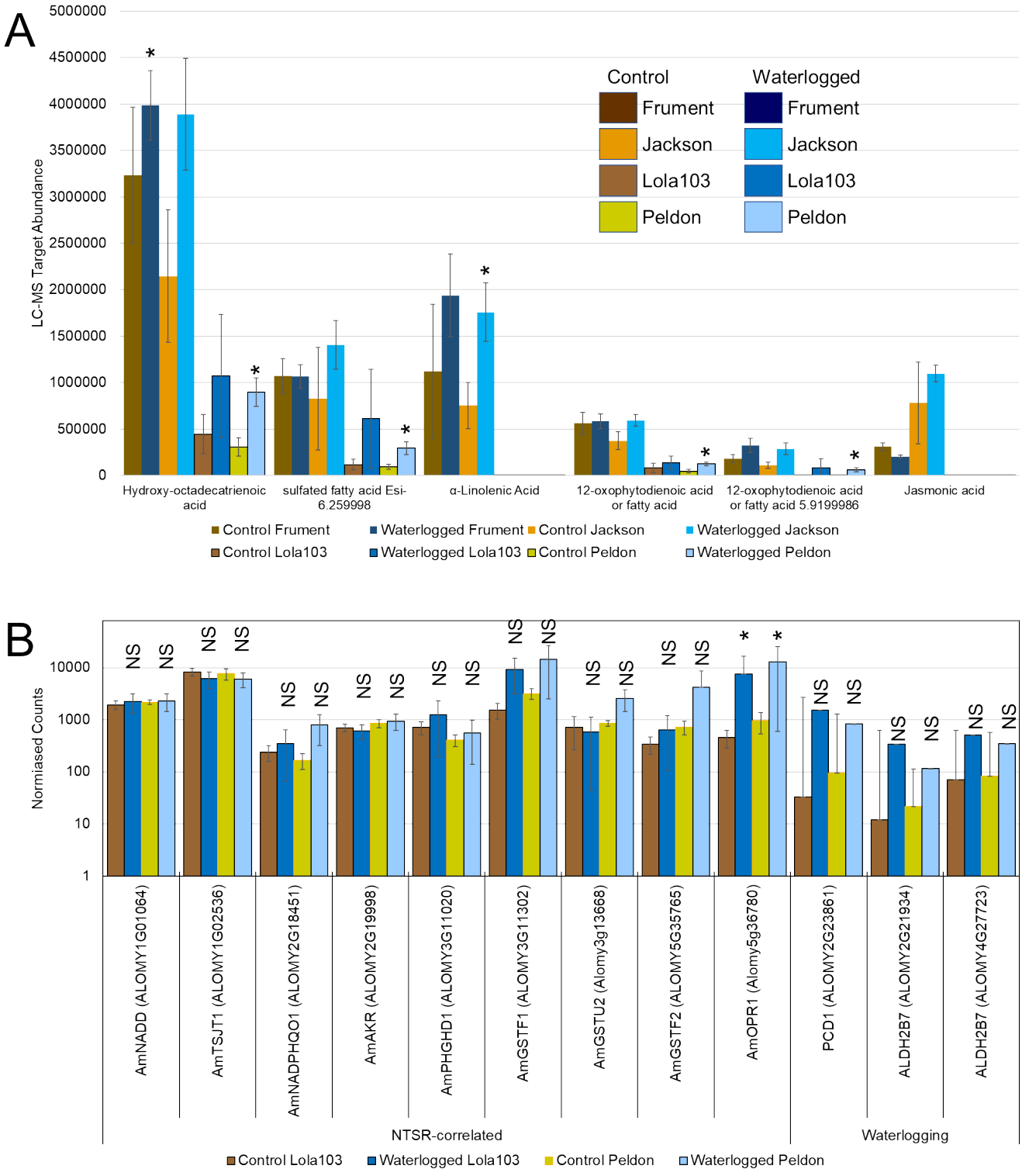

Supplementary Figure 9: Analysis of specific LC-MS identified compounds (A) and genes (B) of interest. (A) LC-MS compounds involved in biosynthesis of bioactive jasmonic acid (JA), converting 12-oxophytodienoic acid (OPDA) to 3-oxo-2-(2'-pentenyl)cyclopentane-1-octanoic acid (OPC-8:0), which is then used for synthesis of various JA derivatives, such as jasmonoyl-isoleucine (JA-Ile) were identified in Frument control (brown) or waterlogged (blue), Jackson control (dark orange) or waterlogged (light blue), Lola103 control (light brown) or waterlogged (dark purple), and Peldon control (light orange) or waterlogged (light purple) samples. Data are average values of four or five sample per treatment and

genotype ± standard deviation as described in the materials and methods section. * indicates where the significance threshold was $P<0.05$ between waterlogged and control samples using an ANOVA (B) Gene expression of targets of interest previously identified as being correlated to non-target site herbicide resistance. NS indicates no statistically significant differences or * indicates statistically significantly difference between waterlogged and control conditions using the cut-offs of log2FoldChange=+/-2 and $p<10^{-5}$.

Supplementary Tables:

Supplementary Table 1: Experimental data from the biotypes/cultivars tested over the experimental replicates with descriptions of Experimental Replicate name, days after waterlogging (DAW) when the fresh weight (FW) was taken, total number of timepoints taken and list of which Black-grass Biotypes or Wheat Cultivars were Tested.

Supplementary Table 2: Data that underpin Figure 4 reporting the absolute quantities of the major primary and secondary metabolites from 1H-NMR spectra data from wheat or black-grass cultivars/biotypes that had been exposed to waterlogging or control conditions presented as mg of target normalised by g of plant material assessed along with fold change between waterlogged and control samples within a cultivar or biotype, and the P value from ANOVA analysis between unnormalized values from waterlogged and control samples.

Supplementary Table 3: Data that underpin Figure 5 reporting the LC-MS data for a wider range of less abundant secondary metabolites giving the log of the fold change (Log FC) and corrected P value (p (Corr)) for metabolites in comparisons between Lola103 Control vs Waterlogged, Peldon Control vs Waterlogged, Frument Control vs Waterlogged or Jackson Control vs Waterlogged, Control Lola103 vs Peldon, Waterlogged Lola103 vs Peldon, Control Frument vs Jackson, and Waterlogged Frument vs Jackson.

Supplementary Table 4: Analysis of differentially expressed genes in wheat samples where ‘baseMean' is the average of the normalized count values, dividing by size factors, taken over all samples; 'log2FoldChange' is the estimate of the effect size: i.e. the change in expression due to treatment, reported on a logarithmic scale to base 2. See https://bioconductor.org/packages/devel/bioc/vignettes/DESeq2/inst/doc/DESeq2.html#differential-expression-analysis.

Supplementary Table 5: Analysis of differentially expressed genes in black-grass samples where ‘baseMean' is the average of the normalized count values, dividing by size factors, taken over all samples; 'log2FoldChange' is the estimate of the effect size: i.e. the change in expression due to treatment, reported on a logarithmic scale to base 2. See https://bioconductor.org/packages/devel/bioc/vignettes/DESeq2/inst/doc/DESeq2.html#differential-expression-analysis.

Supplementary Table 6: GO term enrichment of wheat DEG showing analysis using tools described in Chen et al. (2020) Triticeae-GeneTribe http://wheat.cau.edu.cn/TGT/m14/?navbar=GOEnrichment of the top 25 DEG identified in wheat.

Supplementary Table 7: GO term enrichment analysis of only the 19 DEG that are commonly differentially expressed in wheat showing outputs from tools described in Ashburner et al. (2000) using Gene Ontology Resource https://geneontology.org/

Supplementary Table 8: Results of gene enrichment analysis of only the 19 DEG that are commonly differentially expressed in wheat using The PANTHER (Protein ANalysis THrough Evolutionary Relationships) Classification System https://pantherdb.org/tools/compareToRefList.jsp

Supplementary Table 9: Gene Ontology terms associated with significantly differentially expressed genes (DEG) in black-grass samples. N.B. As there are not enough genes for a formal analysis, we list here all GO terms associated with all identified DEG.

Supplementary Table 10: Analysis of transcript abundance of genes previously associated with metabolism-based resistance or waterlogging in black-grass samples where ‘baseMean' is the average of the normalized count values, dividing by size factors, taken over all samples; 'log2FoldChange' is the estimate of the effect size: i.e. the change in expression due to treatment, reported on a logarithmic scale to base 2.