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Supporting information

A metabolomic study in oats (*Avena sativa*) highlights a drought tolerance mechanism based on salicylate signalling pathways and the modulation of carbon, antioxidant and photo-oxidative metabolism.

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Supporting information Table S1. Significant compounds highlighted by the general model (modelled with all residuals) and related metabolic pathways.

Supporting information Table S2. Significant compounds from the analysis of each of the specific pathways arisen from the general analysis but that did not appeared significant in the general analysis.

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Supporting information Table S1: Significant compounds highlighted by the general model (modelled with all residuals) and related metabolic pathways

m/z	Compound	Pathway ^a	Code ^b	Also significant from individual pathway analysis
109	Catechol	Phenylp	a	YES
110	Pyrrole-2-carboxylate	Arg-Pro	a	YES
111	Uracil	β-alanine	a	YES
112	1-Pyrroline-5-carboxylate	Ala-Asp-Glu / Arg-Pro	a	YES
	Thiosulfate	Cys-Met	a	YES
	Creatinine / 1-Pyrroline-2-carboxylate/ 1-Pyrroline-5-carboxylate	Arg-Pro	a	YES
149	Arabinose	Asc-Ald	a*	YES
		Pentoses	a*	YES
	Ribulose / Xylulose	Pentoses	a*	YES
	Xylose	Starch / Pentoses	a*	YES
	Guanidinobutanoate	Arg-Pro	a*	NO
	Tartrate / Mesotartrate	Glyox-dicarb	a*	YES
155	2-Phosphoglycolate	Glyox-dicarb	a*	YES
	Indole-3-acetonitrile	Tryptoph	a*	YES
169	Glyceraldehyde 3-phosphate	Galact	a	YES
		Gly-Glu	a	YES
		Fruct-Mann	a	YES
		Pentoses	a	YES
		Phenylp	a	YES
	Dihydroxyacetone phosphate	Galact	a	YES
		Gly-Glu	a	YES
		Fruct-Mann	a	YES
Pentoses		a	YES	
171	Acetylglutamine	Arg-Pro	a	YES
	3-Dehydroshikimate	Phenylp	a	YES
173	Arginine	Arg-Pro	a	YES
	Dehydroascorbate	Asc-Ald	a	YES
		Glutathione	a	YES
	Indol-3-acetamide / Indol-3-acetaldoxime	Tryptoph	a	YES
	N-Acetylornithine	Arg-Pro	a	YES
	Shikimic acid	Phenylp	a	YES
	Cis-aconitate	Phenylp	a	YES
TCA		a	YES	
Glyox-dicarb		a	YES	

175	N-Carbamoyl-aspartate	Ala-Asp-Glu	a	YES
	4,6-Dihydroxy-2,5-dioxohexanoate / 5-dehydro-4-deoxy-glucuronate	Pentoses	a	YES
	Ascorbate	Glutathione	a	YES
		Asc-Ald	a	NO
	N-Hydroxyl-tryptamine / Serotonin	Tryptoph	a	YES
Glucuronolactone	Asc-Ald	a	NO	
177	2-Dehydro-3-deoxy-gluconate	Pentoses	a*	NO
	Galactono-1,4-lactone / 2-Dehydro-3-deoxy-galactonate	Galact	a*	YES
	Gulono-1,4-lactone / Galactone- 1,4 lactone	Asc-Ald	a*	NO
186	Acetylspermidine	Arg-Pro	a*	YES
195	Altronate	Pentoses	a*	NO
	Galactonate	Galact	a*	YES
	Gulonate	Asc-Ald / Pentoses	a*	NO
115	Fumarate	Ala-Asp-Glu / Arg-Pro / Phenylp / TCA	b	YES
129	Agmatine	Arg-Pro	b	YES
	2,5-dioxo-pentanoate	Asc-Ald / Arg-Pro	b	YES
		Pentoses	b	NO
133	3-Dehydro-threonate	Asc-Ald	b	YES
	Malate	TCA	b	NO
		Glyox-dicarb	b	NO
		Phenylp	b	YES
137	Salicylate	Phenylp	b	YES
167	Phosphoenolpyruvate	Gly-Glu	b	YES
		Phenylp	b	YES
		TCA	b	YES
168	3-Sulfo-acetate / Cysteate	Cyst-Met	b	YES
181	Sorbitol	Fruct-Mann / Galact	b*	YES
	Galactitol	Galact	b*	YES
	Mannitol	Fruct-Mann	b*	YES
210	5-(2'-Formylethyl)-4,6-dihydroxypicolinate	Tryptoph	b*	NO
	Creatine phosphate	Arg-Pro	b*	YES
226	5-(2'-Formylethyl)-4,6-dihydroxypicolinate	Tryptoph	b	YES
	Glutamyl 5-phosphate	Arg-Pro	b	NO
440	Folate	Phenylp	b*	YES
119	3-Methylthiopropionate	Cys-Met	c	YES
130	Creatine	Arg-Pro	c	YES
	2-Oxosuccinamate	Ala-Asp-Glu	c	YES
	N-Acetyl-beta-alanine	β -alanine	c	YES
	Glutamate 5-semialdehyde	Arg-Pro	c	YES
	5-Amino-2-oxopentanoic acid / Norspermidine / N-carbamoylputrescine	Arg-Pro	c	YES

146	Glutamate	Arg-Pro / Glutathione / Ala-Asp-Glu / Glyox-	c	NO
	Acetylserine	Cyst-Met	c	YES
	2-Oxo-4-hydroxy-5-aminovalerate	Arg-Pro	c	YES
156	2-Aminomuconate	Tryptoph	c*	YES
185	3-Phospho-glycerate	Glyox-dicarb	c	YES
		Gly-Glu	c	YES
		Glyox-dicarb	c	YES
		Phenylp	c	YES
	Glycerate-2-phosphate	Gly-Glu	c	YES
		Pentoses	c	NO
Phenylp		c	YES	
188	1-Acetylisatin	Tryptoph	c	YES
	N-Acetyl-glutamate	Arg-Pro	c	NO
196	Carbamoyl phosphate	Ala-Asp-Glu / Asc-Ald	c	YES
198	Phosphoserine	Cys-Met	c	NO
201	Spermine	β -alanine / Glutathione	c	YES
		Arg-Pro	c	NO
204	6-hydroxy-kynurenate / Xanthurenate / Indolelactate	Tryptoph	c	NO
206	4-(2-aminophenyl)-2,4-dioxobutanoate / 2-Formaminobenzoylacetate	Tryptoph	c	YES
221	Cystathionine	Cys-Met	c	YES
244	beta-Alanyl-arginine	β -alanine	c	YES
116	Indole	Tryptoph	d	YES
	Aspartate-4-semialdehyde	Arg-Pro / Cys-Met	d	YES
	Guanidinoacetate	Arg-Pro	d	YES
134	Homocysteine	Cys-Met	d	YES
136	Anthranilate	Tryptoph	d	YES
		Phenylp	d	YES
147	Xylonolactone	Pentoses	d	YES
	4-Methylthio-2-oxobutanoate	Cys-Met	d	YES
	2-Dehydro-3-deoxyarabinonate/Arabinono-1,4-lactone	Asc-Ald	d	YES
	Oxaloglycolate	Glyox-dicarb	d	YES
163	Fucose / Fuculose	Fruct-Mann	d	YES
	Rhamnofuranose / Rhamnose	Fruct-Mann	d	YES
564	CDP-glucose	Starch	d	YES
565	UDP-glucose	Asc-Ald	d	YES
		Galact	d	YES
		Pentoses	d	YES
		Starch	d	NO
	UDP-galactose	Galact	d	YES
579	UDP-glucuronate	Asc-Ald	d	YES
		Pentoses	d	YES
		Starch	d	YES

135	Threonate	Asc-Ald	e	YES
148	Methionine	Cys-Met	e	NO
	Dihydroxyindole / 2-formylaminobenzaldehyde	Tryptoph	e	YES
153	Protocatechuate / 2,3-Dihydroxybenzoate	Phenylp	e	YES
154	Histidine	β -alanine	e	YES
165	Arabinonate	Asc-Ald	e	YES
	Xylonate	Pentoses	e	YES
	Lyxonate	Asc-Ald / Pentoses	e	YES
174	Monodehydroascorbate	Asc-Ald	e	YES
	Carboxy-norspermidine	Arg-Pro	e	YES
	Citrulline	Arg-Pro	e	YES
	N-acetylaspartate	Ala-Asp-Glu	e	YES
193	Glucuronate / Galacturonate Pinitol/ononitol	Asc-Ald	e	NO
		Pentoses	e	YES
		Starch	e	NO
211	Isophenoxazine	Tryptoph	e	YES
216	gamma-glutamylputrescine	Arg-Pro	e	YES
	beta-Alanyl-lysine	β -alanine	e	YES
229	Ribulose-5-phosphate	Glyox-dicarb	e	YES
261	Mannitol 1-phosphate / Sorbitol 6-phosphate	Fruct-Mann	e	YES
338	Fructose 1,6-bisphosphate	Fruct-Mann	e	YES
343	Melibiitol	Galact	e	YES

Pathways refer to; Gly-Glu:

Glycolysis/Gluconeogenesis (Ko00010); TCA: TCA Cycle (Ko00020); Pentoses: Pentose phosphate pathway and Pentoses and glucuronate interconversions (Ko00030 / Ko00040); Fruct-Mann: Fructose and Mannose metabolism (Ko00051); Galact: Galactose metabolism (Ko00052); Asc-Ald: Ascorbate and aldarate metabolism (Ko00053); Ala-Asp-Glu: Alanine, Aspartate and Glutamate metabolism (Ko00250); Gly-Ser: Glycine, serine and threonine metabolism (Ko00260); Cys-Met: Cysteine and Methionine metabolism (Ko00270); Arg-Pro: Arginine and Proline metabolism (Ko00330); Tryptoph: Thryptophane metabolism (Ko00380); β -alanine: β -alanine metabolism (Ko00410); Glutathione: Glutathione metabolism (Ko00480); Starch: Starch and sucrose metabolism (Ko00500); Glyox-dicarb: Glyoxylate-dicarboxylate metabolism (Ko00630); Phenylp: Main core phenylpropanoid metabolism (Map01061); according to KEEG database.

b) Code legend

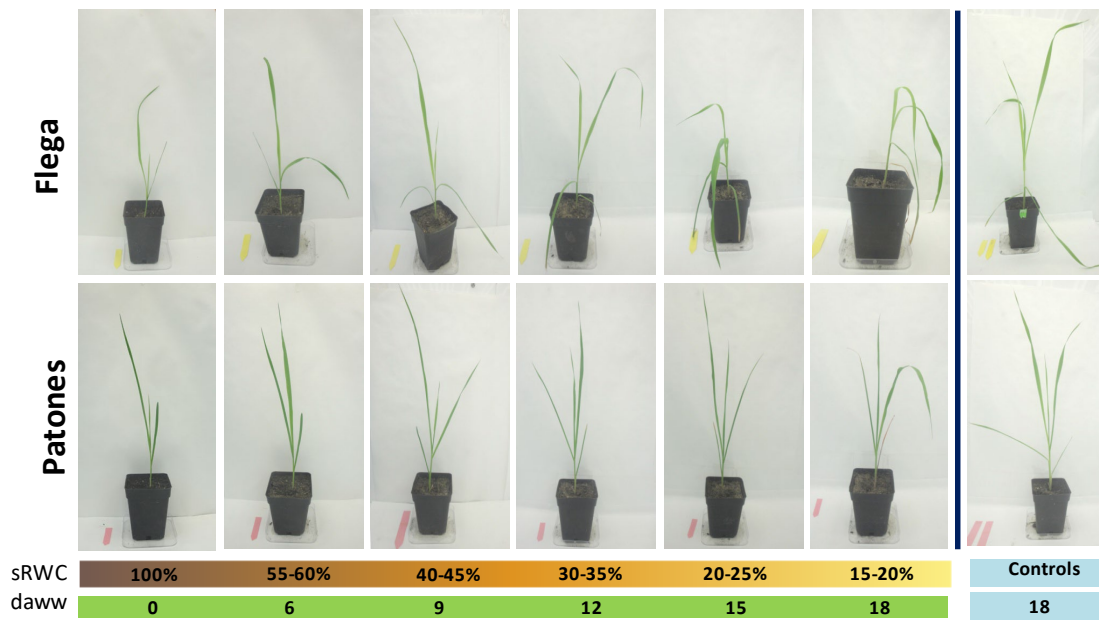
- a -> Significant in discriminating between susceptible and resistant genotypes subjected to drought stress but not in watered controls, in at least three sampling times .
- b -> Significant discriminating susceptible and resistant genotypes subjected to drought stress but not in watered controls, in two sampling times
- c -> Significant discriminating susceptible and resistant genotypes subjected to drought stress but not in watered controls, in one sampling time
- d -> Significant discriminating susceptible and resistant genotypes subjected to drought stress but also watered controls, in at least 4 of the 5 sampling times
- e -> Significant only in specific time points and/or comparisons
- *Significant discriminating susceptible and resistant genotypes subjected to drought stress but not in watered controls also in the general model (all time points)

Supporting information Table S2: Significant compounds from the analysis of each of the specific pathways arisen from the general analysis but that did not appeared significant in the general analysis.

<i>m/z</i>	Compound	Pathway
101	Cadaverine	Glutathione
103	Malonate	β -alanine
	Hydroxy pyruvate	Glyox-dicarb
117	Succinate	Ala-Asp-Glu / Glyox-dicarb
120	Cysteine	Glutathione
131	Oxaloacetate	Gly-Glu / Glyox-dicarb
	Ornithine	Arg-Pro/ Glutathione
	N-carbamoyl- β -alanine	β -alanine
	N-carbamoylsarcosine	Arg-Pro
140	2-Aminomuconate-semialdehyde	Tryptoph
179	Glucose	Gly-Glu
180	3-hydroxy-kyrenamine	Tryptoph
212	4-Aspartyl-Phosphate	Cys-Met
239	Anserine	β -alanine
247	5- Hydroxyindoleacetyl glycine	Thryptoph
258	Galactosamine 6-phophate	Galact
259	Glucose 1-phosphate	Gly-Glu
264	3-Phospho-glyceroyl phosphate	Gly-Glu
271	Arbutin	Gly-Glu
300	N-Acetyl-Galactosamine-6P	Galact
309	Ribulose-1,5-bisphosphate	Glyox-dicarb
365	Salicin-6P	Gly-Glu
586	GDP-4-oxo-6-deoxy-mannose	Fruct-Mann

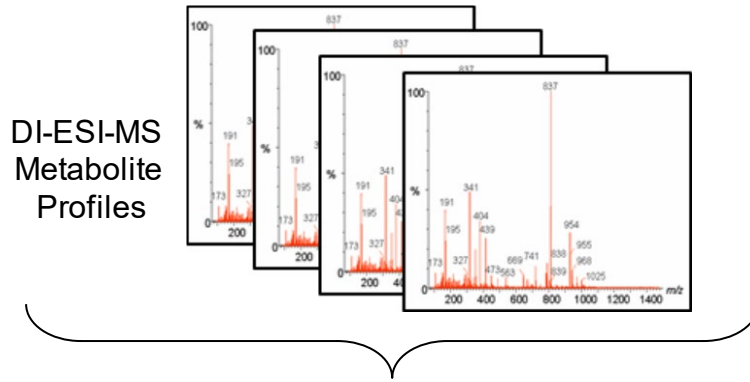
Pathways refer to; Gly-Glu: Glycolysis/Gluconeogenesis (Ko00010); Fruct-Mann: Fructose and Mannose metabolism (Ko00051); Galact: Galactose metabolism (Ko00052); Ala-Asp-Glu: Alanine, Aspartate and Glutamate metabolism (Ko00250); Arg-Pro: Arginine and Proline metabolism (Ko00330); Tryptoph: Thryptophane metabolism (Ko00380); β -alanine: β -alanine metabolism (Ko00410); Glutathione: Glutathione metabolism (Ko00480); Glyox-dicarb: Glyoxylate-dicarboxylate metabolism (Ko00630) according to KEEG database

SUPPORTING INFORMATION FIGURES

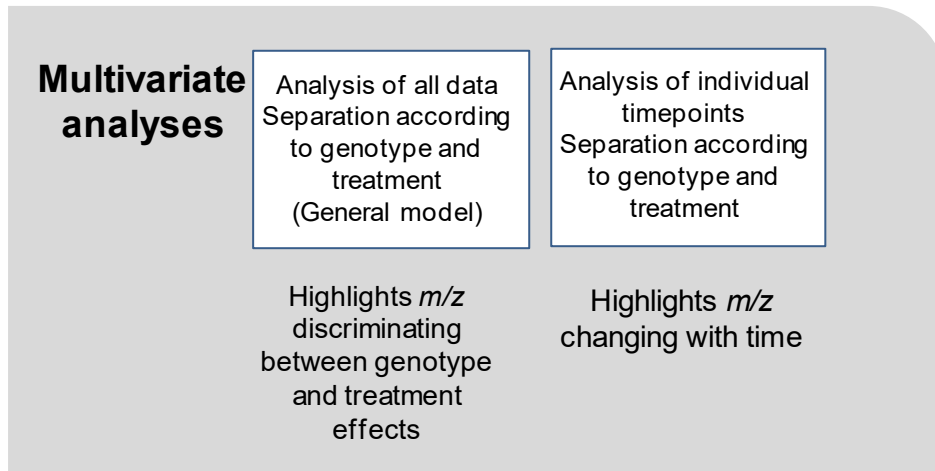


Supporting information Figure S1. Pictures of Flega a Patones plants at key time points of the water deficit time course experiment.

A

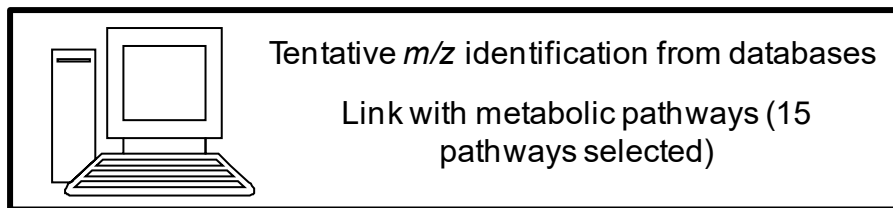


B

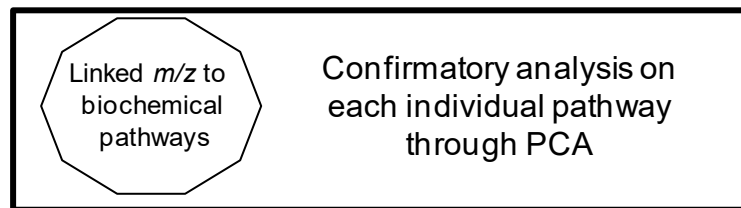


Identification of key *m/z* based on loading vectors

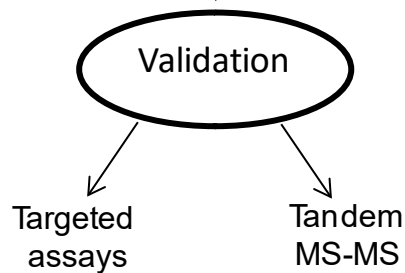
C



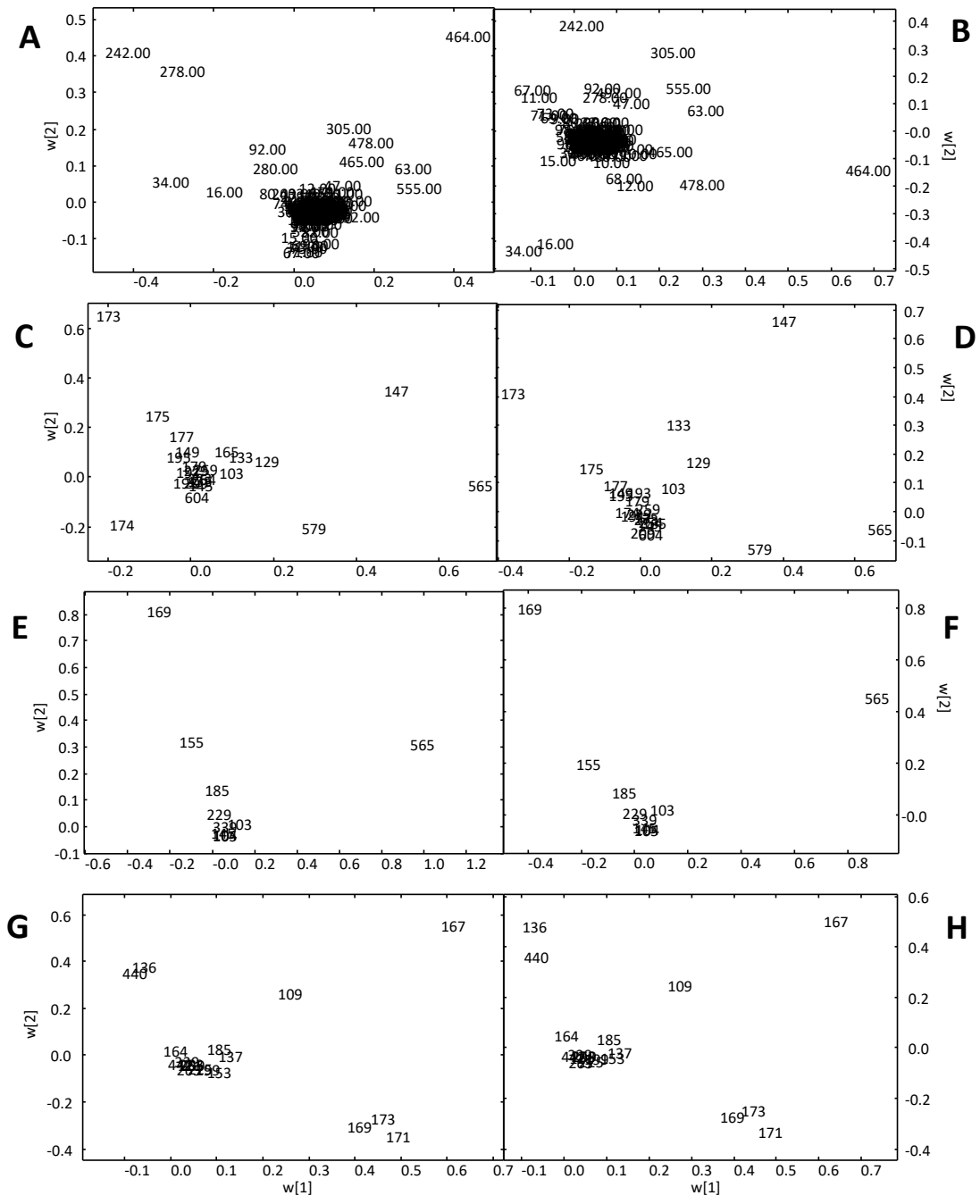
D



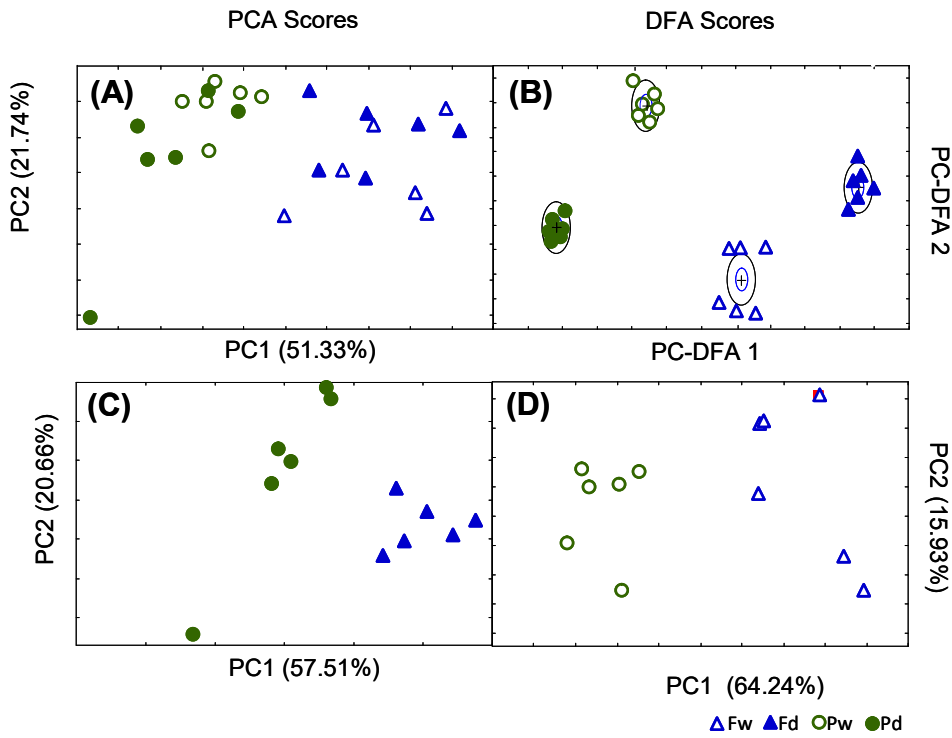
E



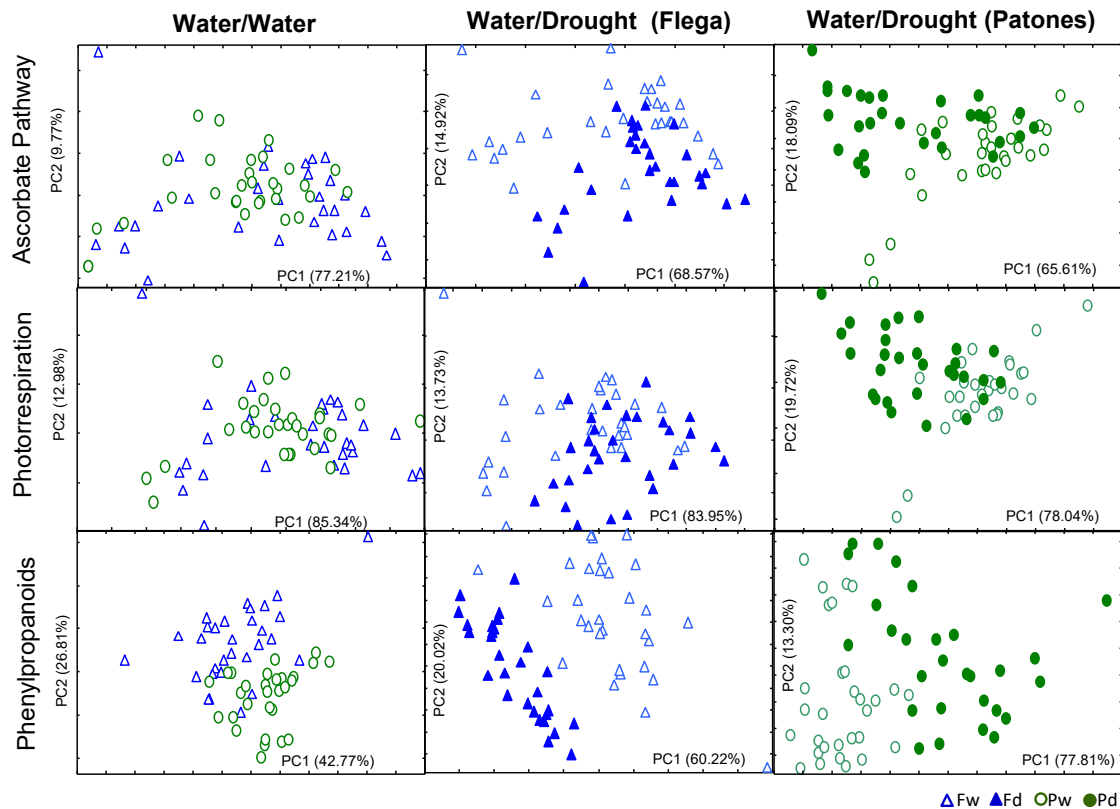
Supporting information Figure S2: Analysis Pipeline. A: Experimental samples were profiled using Direct-Infusion-Electrospray Ionisation Mass Spectroscopy (DI-ESI-MS). **B:** The multiple spectra were analysed using multivariate approaches. Data were first interrogated using Principal Component Analysis (PCA; Causton 1987) followed by Discriminant Function Analysis (DFA) which is a supervised projection method (Manly 1994) which attempts to designate groups on the basis of the retained PCs. PC-DFA models were created to identify the key metabolites differentially produced in the susceptible and tolerant cultivars based on deriving robust models where differences between classes were separated along a particular PC-DF axis. This allowed plotting the contributions of individual metabolites measured to the model (“loading vectors”). Those metabolites that appeared $> \pm 1$ standard deviation (STD) from the mean value loading were recorded as significant metabolites associated with the differences (Supporting information Table 1). To maximise the information obtained from these analyses, PC-DFA were employed in a two-step manner 1) analysis of all data using classes of genotype and treatment (4 classes) or by treatment grouped according to the sampling times (10 groups per cultivar); 2) analysis of each specific sampling time by genotype and treatment. This identified the key time points and m/z to discriminate the susceptible and resistant cultivars. **C:** Each m/z was tentatively identified according to predicted true mass ($m/z + 1$) and interrogation of ESI-MS metabolite libraries constructed from the analysis of tomato plants and olive oils (Goodacre *et al.* 2002) and of the Kyoto Encyclopaedia of Genes and Genomes (KEGG) metabolic pathways. **D** Predicted metabolites (based on m/z) were grouped according to their biochemical pathways and tested by ANOVA for significant differences between the experimental groups. Analysis of each individually targeted pathway according to genotypes, treatment and time point classes were performed to confirm the significance of metabolites. **E.** The importance of the targeted metabolites and pathways in discriminating between the experimental classes was validated through targeted assays and tandem MS.



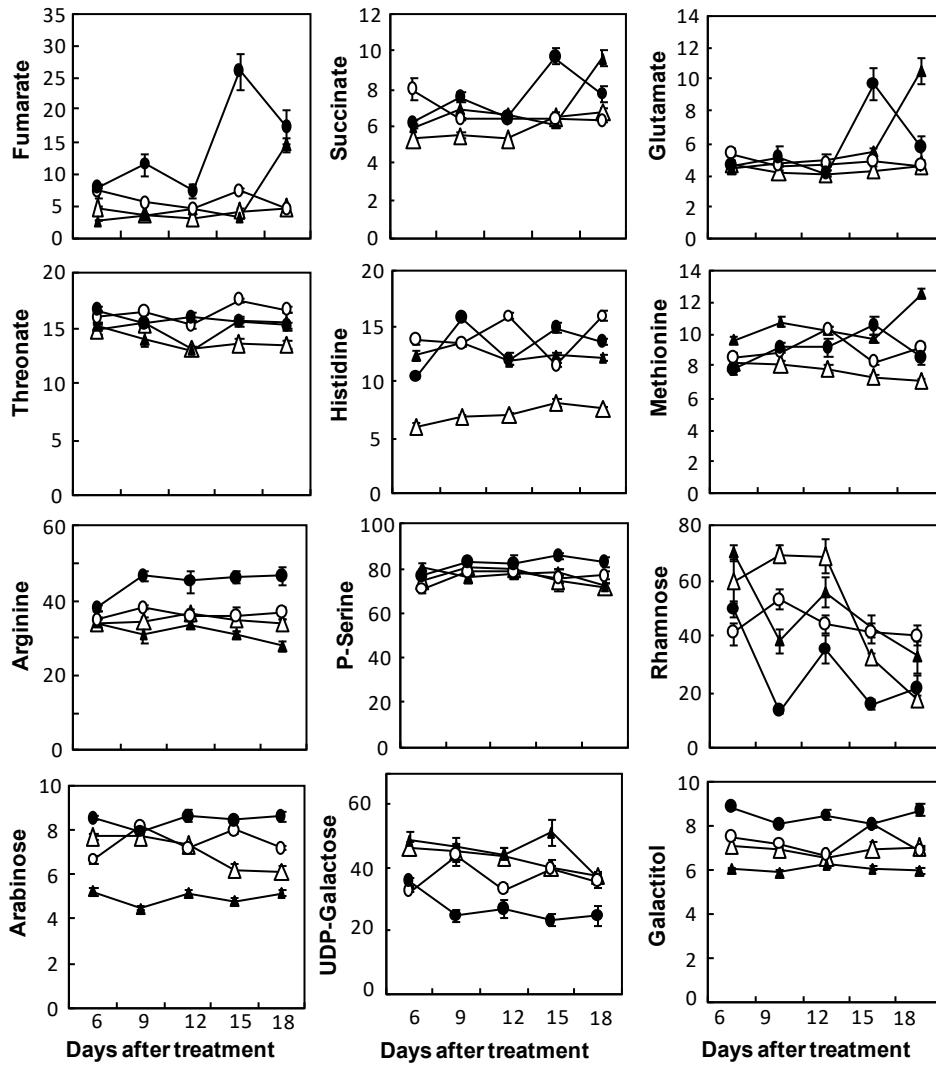
Supporting information Figure S3. PCA loadings of the variables on the first two axes. A, B. PCA loadings corresponding to Figure 2A, for all samples and droughted samples respectively. **C, D.** PCA loadings corresponding to Figure 3A, B. **E, F.** PCA loadings corresponding to Figure 5A, B. **G, H.** PCA loadings corresponding to Figure 7A, B.



Supporting information Figure S4. Example sequential analyses of Mass Spectrometry profiles from a single experimental time point. **A.** Principal Component Analysis (PCA) and **B.** Discriminant Functional Analysis (DFA) of metabolite profiles from drought susceptible Flega (F) and tolerant Patones (P) droughted (d) or control well watered plants at a given time point. In DFA crosses indicate mean value for each class and the circles 90 and 95% confidence intervals; **C.** PCA model based only on samples subjected to drought treatment. **D.** PCA model based on control well watered samples. Significant metabolites explaining clustering from these three different models were compared to determine the importance of these metabolites at this specific sampling time and their constitutive or inducible condition



Supporting information Figure S5. Principal Component analysis of pathways profiles from oat cultivar Flega (triangles) and Patones (circles) well watered plants (open symbols) and during a time course of water stress (solid symbols).



Supporting information Figure S6. Exemplary metabolic changes in susceptible oat Flega and tolerant Patones under drought. Data represent normalized responses of metabolite pools, that is, mass detector signals in arbitrary units normalized to internal standard and sample dry weight in Flega (triangles) and Patones (circles) under well-watered (open symbols) or drought conditions (solid symbols). Data indicates mean \pm standard error of 6 replicates which were taken from four pooled plants.