Takáčet al., 2013, SUPPLEMENTAL DATA:

Protein extraction for two-dimensional electrophoresis and 2-D LC MS/MS

The homogenate was resuspended in 0.5 ml of extraction buffer (0.15 M Tris-HCl pH 8.8; 0.4 M EDTA; 0.2% (v/v) 2-mercaptoethanol; 0.9 M sucrose; 25 mM-KCl), thoroughly vortexed and supplemented with 0.5 ml Tris-buffered phenol (pH 8.8). After 30 min of incubation at 4°C, the extract was centrifuged at 8,000g at 4°C for 5 min. The upper phenol phase was collected, and the residual proteins from aqueous phase were re-extracted using extraction buffer and Tris-buffered phenol. Following centrifugation, the phenol phases were combined, and additional extraction buffer was added to maximize the removal of the non-protein compounds. Next, the mixture was intensely mixed, centrifuged, and the phenol phase was collected for protein precipitation. The proteins were precipitated by addition of 5 volumes of 0.1 M ammonium acetate (in 100% methanol) to the phenol phase, and leaving at -20°C overnight. Subsequently, the precipitate was centrifuged at 16,000g at 4°C for 20 min. The supernatant was discarded and the pellet was washed twice with 0.1 M ammonium acetate (in 100% methanol), twice in 80% acetone, and twice in 75% ethanol, consequently. Between washing steps, pellet was thoroughly resuspended and incubated at -20°C for 15 min followed by centrifugation at 12,000g at 4°C for 10 min. Finally, the pellet was air-dried for 10 min.

2-D electrophoresis

Proteins (50 μg) were focused on 7 cm long IPG strips (pH range 5-8) using Protean IEF Cell (Bio-Rad) with the following conditions: 150 V for 150 VH, 500 V for 500 VH and 4000 V for 15000 VH including initial active rehydration for 12 hours at 50 V. Next, the protein strips were equilibrated in 1% (w/v) dithiothreitol (DTT) containing equilibration solution (6 M urea; 30% (v/v) glycerin; 70 mM sodium dodecyl sulfate; 0.006% (w/v) bromphenol blue) for

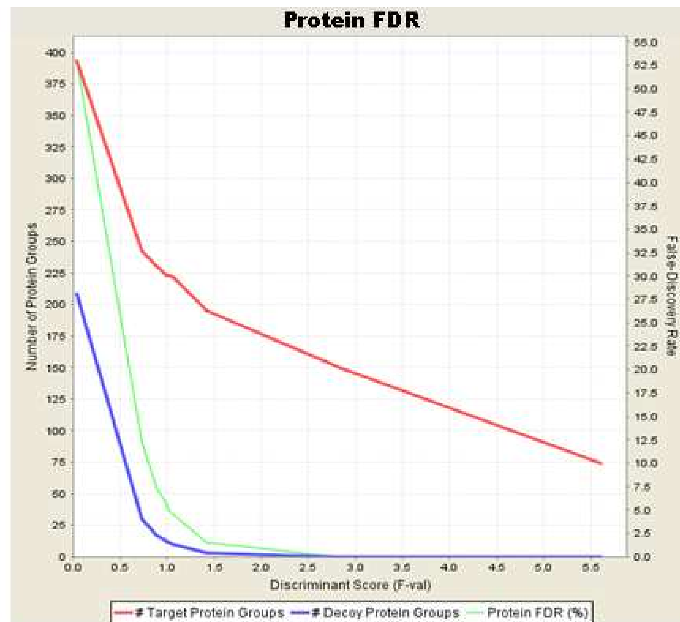
15 min, and subsequently 15 min in equilibration solution containing 2.5% (w/v) iodacetamide. The strips were applied onto the SDS-gel and sealed with agarose solution (0.5% (w/v) agarose; 0.002% bromphenol blue in 1x SDS running buffer), and separated on 10% SDS-PAGE gels at 80 V.

LCQ DecaXp Plus massspectrometer set up

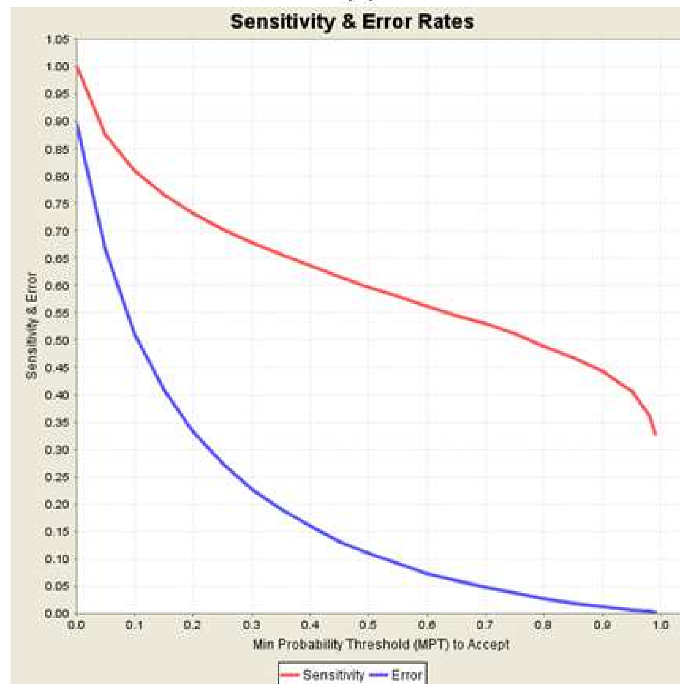
Globalsetting: Dynamic exclusion “on”, repeat count: 2, repeat duration: 0.5 minutes, exclusion duration: 2.00 minutes; exclusion mass width low = 1.00, high = 2.50; Segment setting: Reject masses: 317.0 and 444.0, Normalized collision energy: 35%, Minimum MS signal: 10 x 10^4, Minimum MS/MS: 1 x 10^4, Isolation width (m/z): 3.0. Details of instrument setting: Spray voltage 2.7 kV, Capillary temperature 170°C, AGC (automaticgaincontrol) “on” with MSn Target 4 x 10^4. Collected spectra (RAW files) were processed by Bioworks 3.1 SR1 (ThermoFinnigan, San Jose; CA) software. The subprogram TurboSequest carried out the protein identification by matching the experimentaldata (masses of parent and fragmented ions/peptides) to those in the database. Search parameters a sfollows were employed:

DTA Search: Trypsin with two internal cleavage sites, Precursor (Δ) Mass: 1.40 amu, GroupScan: 7, Minimum Group Count: 1, Minimum Ion Count: 15, Charge State: Auto, MSn level: Auto, Peptide: 2.50, Fragment Ions: 0.80, IonSeries: B and Y;

Modifications: C=57.05 (differential) for carbamidomethylation of cysteins by iodoacetamide, M= 32.0 (differential) for oxidation of methionines; Charge State Analysis: “off ”.



A



B

Figure SD 1. FDR vs. peptide scores (A), and protein probability vs. sensitivity & error (B). Data are shown for total set of identified proteins, as per ProteoIQ software (Premier Biosoft) analysis.**Supplemental Table 1.**The classification of differentially regulated proteins in Arabidopsis roots after LY294002 treatment according KEGG pathways (output of STRING web based aplication http://string-db.org)

GO_id	Term	NumberOfGenes	p-value	p-value_fdr	p-value_bonferroni
ath00290	Valine, leucine and isoleucine biosynthesis	8	1.01E-8	1.13E-6	1.13E-6
ath00710	Carbon fixation in photosynthetic organisms	7	5.61E-5	3.14E-3	6.29E-3
ath00620	Pyruvate metabolism	6	3.4E-4	1.27E-2	3.8E-2
ath00660	C5-Branched dibasic acid metabolism	2	6.98E-4	1.95E-2	7.82E-2
ath00250	Alanine, aspartate and glutamate metabolism	4	3.05E-3	6.83E-2	3.42E-1
ath00330	Arginine and proline metabolism	4	6.87E-3	1.28E-1	7.69E-1
ath00010	Glycolysis / Gluconeogenesis	5	9.69E-3	1.37E-1	1.00E+00
ath00950	Isoquinoline alkaloid biosynthesis	2	9.77E-3	1.37E-1	1.00E+00
ath00020	Citrate cycle (TCA cycle)	4	1.16E-2	1.44E-1	1.00E+00
ath00360	Phenylalanine metabolism	5	1.34E-2	1.5E-1	1.00E+00
ath00270	Cysteine and methionine metabolism	4	1.71E-2	1.74E-1	1.00E+00
ath00051	Fructose and mannose metabolism	3	2.46E-2	2.29E-1	1,00E+00
ath00966	Glucosinolate biosynthesis	2	3.4E-2	2.93E-1	1.00E+00
ath04145	Phagosome	3	3.9E-2	3.06E-1	1.00E+00
ath00650	Butanoate metabolism	2	4.09E-2	3.06E-1	1.00E+00
ath00960	Tropane, piperidine and pyridine alkaloid biosynthesis	2	4.46E-2	3.12E-1	1,00E+00
ath00350	Tyrosine metabolism	2	5.23E-2	3.44E-1	1.00E+00
ath00630	Glyoxylate and dicarboxylate metabolism	2	6.46E-2	4.02E-1	1.00E+00
ath00562	Inositol phosphate metabolism	2	1.11E-1	6.56E-1	1.00E+00
ath00910	Nitrogen metabolism	2	1.27E-1	7.09E-1	1,00E+00
ath00400	Phenylalanine, tyrosine and tryptophan biosynthesis	2	1.43E-1	7.5E-1	1.00E+00
ath03010	Ribosome	6	1.47E-1	7.5E-1	1.00E+00
ath04146	Peroxisome	2	1.59E-1	7.73E-1	1.00E+00

ath00190	Oxidative phosphorylation	4	1.82E-1	8.48E-1	1,00E+00
ath00940	Phenylpropanoid biosynthesis	3	2.29E-1	1.00E+00	1.00E+00
ath00260	Glycine, serine and threonine metabolism	1	1.00E+00	1,00E+00	1.00E+00
ath00945	Stilbenoid, diarylheptanoid and gingerol biosynthesis	1	1.00E+00	1,00E+00	1.00E+00
ath00030	Pentose phosphate pathway	1	1,00E+00	1.00E+00	1,00E+00
ath00941	Flavonoid biosynthesis	1	1.00E+00	1,00E+00	1.00E+00
ath00240	Pyrimidine metabolism	1	1.00E+00	1.00E+00	1.00E+00
ath00590	Arachidonic acid metabolism	1	1.00E+00	1.00E+00	1.00E+00
ath00480	Glutathione metabolism	1	1.00E+00	1.00E+00	1.00E+00
ath03060	Protein export	1	1.00E+00	1.00E+00	1.00E+00
ath00380	Tryptophan metabolism	1	1.00E+00	1.00E+00	1.00E+00
ath04070	Phosphatidylinositol signaling system	1	1.00E+00	1.00E+00	1.00E+00
ath00300	Lysine biosynthesis	1	1.00E+00	1.00E+00	1.00E+00
ath04140	Regulation of autophagy	1	1.00E+00	1.00E+00	1.00E+00
ath00450	Selenoamino acid metabolism	1	1.00E+00	1.00E+00	1.00E+00
ath03050	Proteasome	1	1.00E+00	1.00E+00	1.00E+00

Supplemental Table 2. The comparison of GO anotations (as asigned by Blast2GO aplication) of differentially regulated proteins after LY294002 and wortmannin treatment in *Arabidopsis* roots.in terms of biological process.

			wortmannin		LY294002	
LevelGO	Term(Acc)	Term(Name)	#Seq	Score	#Seq	Score
1	GO:0008150	biological_process	69	111.16	53	77.47
2	GO:0009987	cellular process	51	50.32	30	29.8
2	GO:0050896	response to stimulus	36	39.6	24	25.8
2	GO:0008152	metabolic process	51	37.33	31	21.25
2	GO:0032501	multicellular organismal process	13	7.8	9	5.4
2	GO:0032502	developmental process	13	7.8	9	5.4
2	GO:0071840	cellular component organization or biogenesis	11	6.6	6	3.6
2	GO:0023052	signaling	5	3	–	–
2	GO:0051179	localization	6	2.16	4	1.44
2	GO:0065007	biological regulation	5	1.08		
3	GO:0006950	response to stress	30	30	23	23
3	GO:0009628	response to abiotic stimulus	27	27	17	17
3	GO:0043170	macromolecule metabolic process	-	-	16	9.36
3	GO:0044238	primary metabolic process	-	-	16	9.22
3	GO:0007275	multicellular organismal development	13	13	9	9
3	GO:0016043	cellular component organization	11	11	6	6
3	GO:0043170	macromolecule metabolic process	17	9.96	-	-
3	GO:0044238	primary metabolic process	17	9.82	-	-
3	GO:0009607	response to biotic stimulus	6	6	3	3
3	GO:0051234	establishment of localization	6	3.6	4	2.4
3	GO:0007154	cell communication	5	3	-	-
3	GO:0051716	cellular response to stimulus	5	3	-	-
3	GO:0044237	cellular metabolic process	5	2.53	3	1.33
3	GO:0050789	regulation of biological process	5	1.8		
3	GO:0006807	nitrogen compound metabolic process	1	0.13	1	0.13
4	GO:0019538	protein metabolic process	16	16	15	15
4	GO:0006810	transport	6	6	4	4
4	GO:0007165	signal transduction	5	5	-	-

4	GO:0006091	generation of precursor metabolites and energy	4	4	2	2
4	GO:0050794	regulation of cellular process	5	3	-	-
4	GO:0044260	cellular macromolecule metabolic process	1	0.6	1	0.6
4	GO:0006139	nucleobase-containing compound metabolic process	1	0.36	1	0.36
4	GO:0034641	cellular nitrogen compound metabolic process	1	0.22	1	0.22
5	GO:0090304	nucleic acid metabolic process	1	0.6	1	0.6
6	GO:0006259	DNA metabolic process	1	1	-	-
6	GO:0006260	RNA metabolic process	-	-	1	1

Supplemental Table 3. The comparison of GO anotations (as asigned by Blast2GO aplication) of differentially regulated proteins after LY294002 and wortmannin treatment in *Arabidopsis* roots.in terms of molecular function.

			wortmannin		LY294002	
LevelGO	Term(Acc)	Term(Name)	#Seq	Score	#Seq	Score
1	GO:0003674	molecular_function	69	49.7	57	45.72
2	GO:0003824	catalytic activity	48	36.26	36	29.03
2	GO:0005488	binding	37	31.24	34	30.84
2	GO:0005198	structural molecule activity	6	6	6	6
2	GO:0005215	transporter activity	6	6	2	2
3	GO:0005515	protein binding	15	15	18	18
3	GO:0016740	transferase activity	17	14.44	7	5.72
3	GO:0016787	hydrolase activity	11	11	11	11
3	GO:0036094	small molecule binding	7	4.2	5	3
3	GO:0003676	nucleic acid binding	2	1.2	6	5.4
4	GO:0000166	nucleotide binding	7	7	5	5
4	GO:0016772	transferase activity, transferring phosphorus-containing groups	4	2.4	2	1.2
4	GO:0003677	DNA binding	1	1	3	3
4	GO:0003723	RNA binding	1	1	1	1
5	GO:0016301	kinase activity	4	4	2	2