Supporting Information

Transcriptome Analysis of Invasive Plants in Response to Mineral Toxicity of Reclaimed Coal-Mine Soil in the Appalachian Region

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Supporting figures: 3 (S1-S3) - PDF

Supporting tables: 8 (S1-S4, S6-S8) – PDF; S5 - XLSX

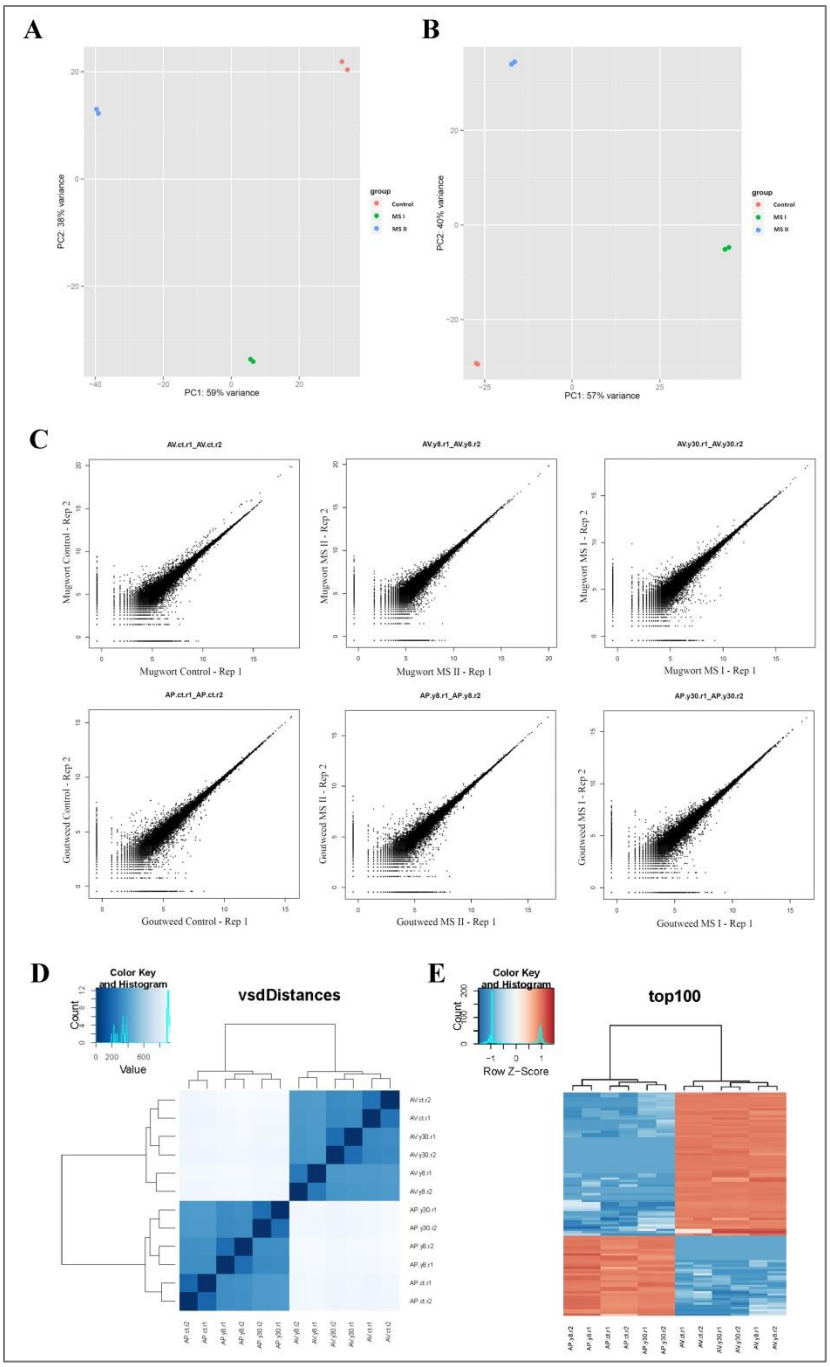

Figure S1. Scatter plots for replications of each treatment showing positive correlation (A). Principal component analysis (PCA) based on the expression of selected genes. Variance in components 1 and 2 for mugwort (B) and goutweed (C) with two replications in each treatment. Sample-wise heatmap across replications (D) and gene clustering heatmap of top 100 genes (E) between goutweed and mugwort.

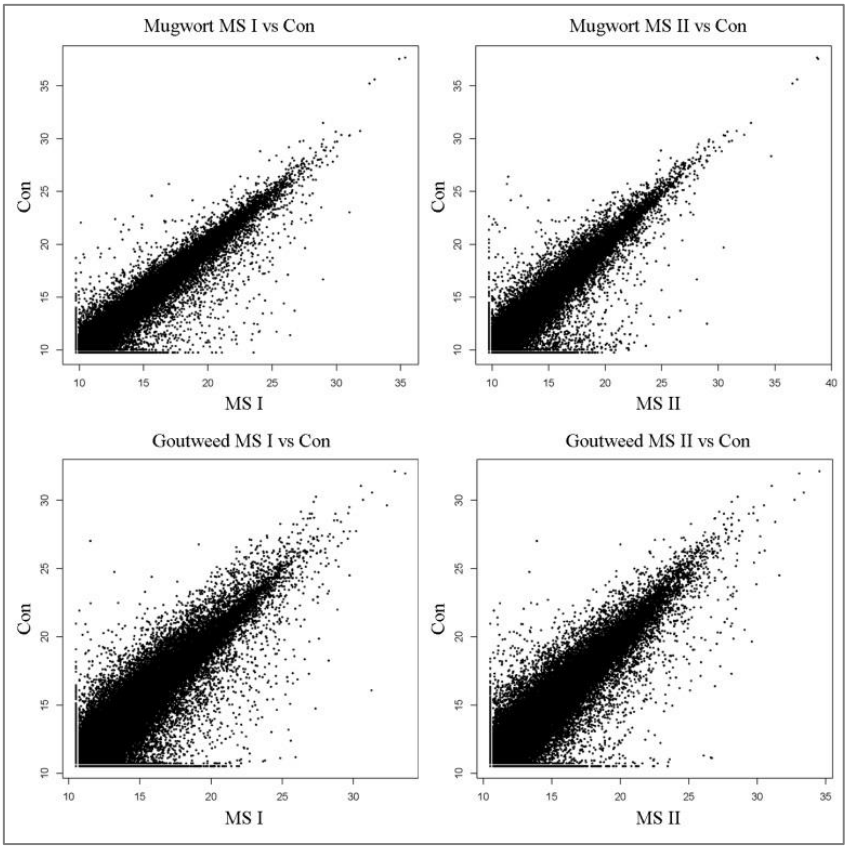

Figure S2. Scatter plots showing comparison of gene expression between control and treatments MS I and MS II for mugwort and goutweed.

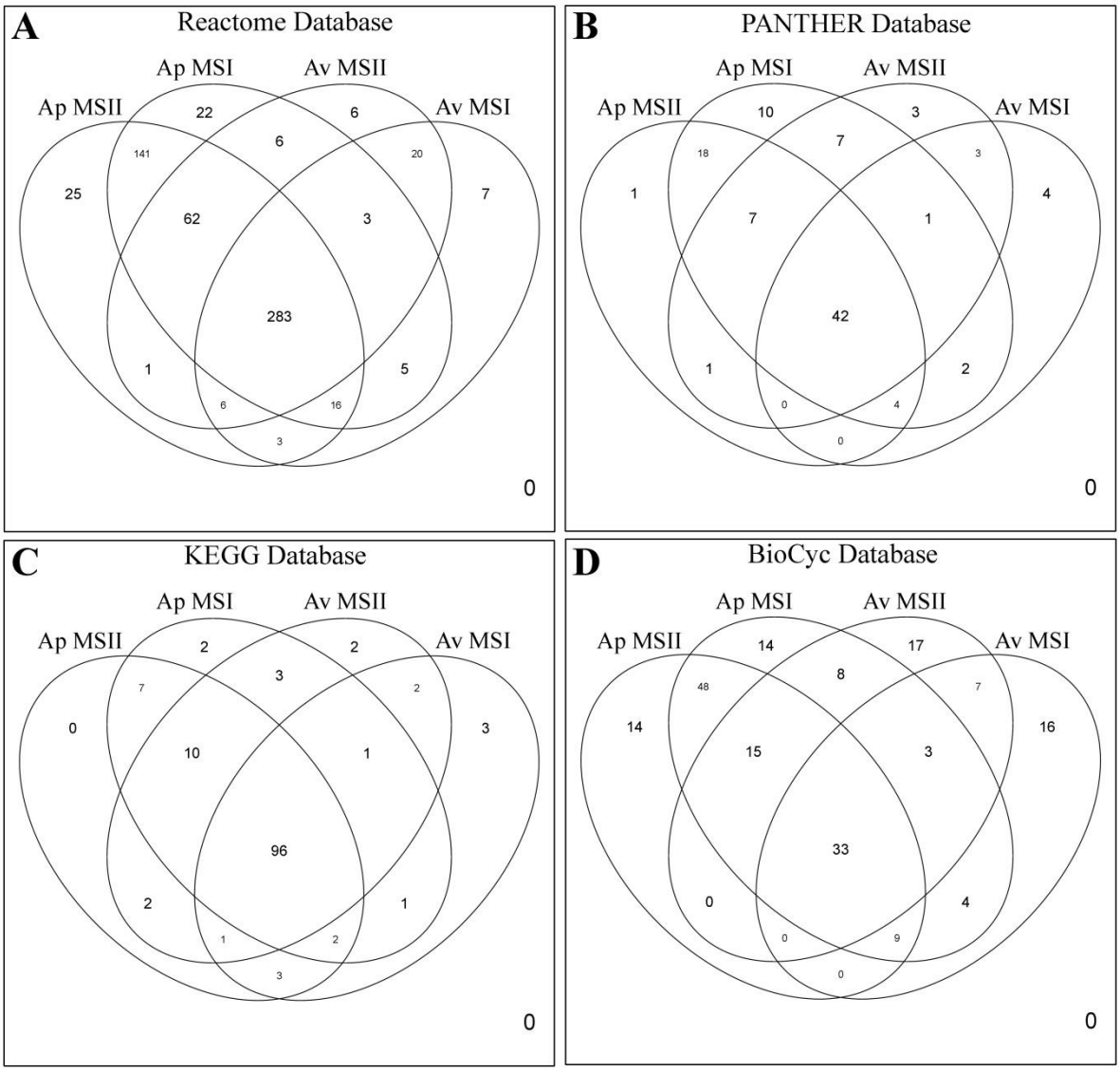

Figure S3. Four-set Venn diagram shows the distribution of genes in each pathway analysis, namely, Reactome (A), PANTHER (B), KEGG (C) and Biocyc (D). The two plant species showed similar genes for both mine sites with different metal toxicity/deficiency. A unique number of common genes appear with all four treatments.

Table S1. RNA-seq and analysis of goutweed and mugwort grown in Promix, MS-I and MS-II.

Reads	Promix		MS-I		MS-II	
	Goutweed	Mugwort	Goutweed	Mugwort	Goutweed	Mugwort
Raw	106139804	101587454	85984610	90773158	80457896	107131156
Filtered	102199362	97443914	82698950	87146415	77690738	102764379
De-duplicated	97929784	83276792	77862993	71291602	74393104	94661956

Table S2. Distribution of transcripts in each length range for goutweed and mugwort

Length of transcripts	Goutweed		Mugwort	
	Before clustering	After clustering	Before clustering	After clustering
200-300	32929	31173	32602	29311
300-500	27168	23458	25788	20516
500-1000	37728	26805	29846	20369
1000-2000	47810	28397	27522	16478
2000-3000	19909	10913	8281	4761
3000-4000	6191	3208	1932	1091
4000-5000	1491	807	394	204
>5000	465	227	145	81

Table S3: Annotations of gene clusters of top 100 genes with highly variable expression under minesite growth for mugwort

		Gene Ontology	KEGG Orthology
	Block 1		
1	comp193524_c1_seq1	uncharacterized LOC100261754 (NA)	glycerol metabolic process|glycerophosphodiester phosphodiesterase activity|plastid|GO:0006071|GO:0008889|GO:0009536
2	comp193524_c1_seq5	uncharacterized LOC100261754 (NA)	glycerol metabolic process|glycerophosphodiester phosphodiesterase activity|plastid|GO:0006071|GO:0008889|GO:0009536
3	comp189923_c1_seq4	hypothetical protein (NA)	phosphate ion transport|transferase activity, transferring glycosyl groups|nucleus|GO:0006817|-|GO:0005634
4	comp192572_c2_seq3	pyridoxal phosphate phosphatase PHOSPHO2 [EC:3.1.3.74] (Vitamin B6 metabolism(K,ko00750,ko))	cellular response to phosphate starvation|endopeptidase activity|nucleus|GO:0016036|GO:0004175|GO:0005634
5	comp191025_c0_seq1	uncharacterized LOC100242507 (NA)	metabolic process|catalytic activity|cytosol|GO:0008152|GO:0003824|-
7	comp189923_c1_seq3	hypothetical protein (NA)	phosphate ion transport|peroxidase activity|nucleus|GO:0006817|-|GO:0005634
8	comp192572_c2_seq6	pyridoxal phosphate phosphatase PHOSPHO2 [EC:3.1.3.74] (Vitamin B6 metabolism(K,ko00750,k	cellular response to phosphate starvation|inorganic diphosphatase activity|nucleus|GO:0016036|GO:0004427|GO:0005634

		o))	
9	comp192788_c1_seq7	uncharacterized LOC100243295 (NA)	protein glycosylation|catalytic activity|mitochondrion|GO:0006486|-|GO:0005739
11	comp180438_c0_seq1	ribonuclease T2 [EC:3.1.27.1] (NA)	aging|RNA binding|extracellular region|GO:0007568|GO:0003723|GO:0005576
13	comp192920_c4_seq1	Tartrate-resistant acid phosphatase type 5 precursor, putative (EC:3.1.3.2) (NA)	regulation of transcription, DNA-templated|DNA binding|extracellular space|GO:0006355|GO:0003677|GO:0005615
14	comp192920_c4_seq2	uncharacterized protein LOC100261163 (NA)	regulation of transcription, DNA-templated|DNA binding|extracellular space|GO:0006355|GO:0003677|GO:0005615
15	comp191333_c0_seq3	uncharacterized LOC100252537 (NA)	tryptophan catabolic process|acid phosphatase activity|extracellular region|GO:0006569|GO:0003993|GO:0005576
17	comp189590_c5_seq1	transcription factor ORG2 (NA)	cellular response to iron ion starvation|protein binding|plasmodesma|GO:0010106|GO:0005515|-
19	comp192572_c2_seq1	pyridoxal phosphate phosphatase PHOSPHO2 [EC:3.1.3.74] (Vitamin B6 metabolism(K,ko00750,ko))	dephosphorylation|pyrophosphatase activity|nucleus|GO:0016311|GO:0016462|-
20	comp191065_c4_seq1	hypothetical protein (NA)	response to arsenic-containing substance|electron carrier activity|Golgi apparatus|GO:0046685|-|GO:0005794
22	comp189037_c0_seq2	glutathione reductase (NA)	glutathione metabolic process|glutathione-disulfide reductase activity|nucleus|GO:0006749|GO:0004362|GO:0005634
	Block2		

1	comp183272_c1_seq2	similar to zinc finger (C3HC4-type RING finger) family protein (NA)	NA
2	comp163599_c0_seq1	uncharacterized LOC100242734 (NA)	response to stress|catalytic activity|cell part|GO:0006950|GO:0003824|GO:0044464
3	comp185258_c0_seq1	uncharacterized protein LOC100255353 (NA)	oxidation-reduction process|superoxide dismutase activity|apoplast|GO:0055114|GO:0004784|GO:0048046
4	comp195903_c3_seq18	DNA-damage-inducible protein f, putative (NA)	drug transmembrane transport|citrate transmembrane transporter activity|plasma membrane|GO:0006855|GO:0015137|GO:0005886
5	comp192726_c0_seq2	uncharacterized LOC100255855 (NA)	response to stress|carboxypeptidase activity|cytoplasm|-|GO:0004180|-
6	comp194700_c0_seq4	Gamma-glutamyl-gamma-aminobutyrate hydrolase, putative (NA)	glutamine metabolic process|hydrolase activity|nucleus|GO:0006541|GO:0016787|GO:0005634
9	comp184225_c0_seq3	Abscisic stress ripening protein, putative (NA)	NA
10	comp194475_c0_seq1	legumain [EC:3.4.22.34] (Lysosome(K,ko04142,ko))	proteolysis|cysteine-type endopeptidase activity|nucleus|GO:0006508|GO:0004197|-
	Block 3		
1	comp187629_c0_seq1	uncharacterized protein LOC100266052 (NA)	ion transport|transporter activity|integral component of membrane|GO:0006811|GO:0005215|GO:0016021
2	comp195724_c0_seq8	nicotianamine synthase [EC:2.5.1.43] (NA)	pollen development|nicotianamine synthase activity|nucleus|GO:0009555|GO:0030410|GO:0005634
3	comp194437_c1	solute carrier family 39	cellular iron ion homeostasis|iron ion

	_seq5	(zinc transporter), member 1/2/3 (NA)	transmembrane transporter activity|plasma membrane|GO:0006879|GO:0005381|GO:0005886
4	comp195724_c0_seq9	nicotianamine synthase [EC:2.5.1.43] (NA)	pollen development|nicotianamine synthase activity|nucleus|GO:0009555|GO:0030410|-
5	comp194437_c1_seq7	solute carrier family 39 (zinc transporter), member 1/2/3 (NA)	manganese ion transport|iron ion transmembrane transporter activity|trans-Golgi network|GO:0006828|GO:0005381|GO:0005802
6	comp195750_c4_seq2	phenylalanine ammonia-lyase [EC:4.3.1.24] (Phenylpropanoid biosynthesis(K,ko00940, ko))	L-phenylalanine catabolic process|phenylalanine ammonia-lyase activity|cytoplasm|GO:0006559|GO:0045548|GO:0005737
7	comp195848_c0_seq3	LL-diaminopimelate aminotransferase [EC:2.6.1.83] (Lysine biosynthesis(K,ko00300, ko))	methylation-dependent chromatin silencing|L-aspartate:2-oxoglutarate aminotransferase activity|mitochondrion|GO:0006346|GO:0004069|GO:0005739
8	comp195848_c0_seq5	LL-diaminopimelate aminotransferase [EC:2.6.1.83] (Lysine biosynthesis(K,ko00300, ko))	methylation-dependent chromatin silencing|L-aspartate:2-oxoglutarate aminotransferase activity|mitochondrion|GO:0006346|GO:0004069|GO:0005739
9	comp194121_c0_seq5	hypothetical protein (NA)	toxin catabolic process|oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen|cytoplasm|GO:0009407|GO:0016702|GO:0005737
10	comp195468_c0_seq1	ferric-chelate reductase, putative (EC:1.6.3.1) (NA)	oxidation-reduction process|NAD(P)H oxidase activity|integral component of membrane|GO:0055114|GO:0016174|GO:0016021
11	comp195848_c0_seq13	LL-diaminopimelate aminotransferase [EC:2.6.1.83] (Lysine	methylation-dependent chromatin silencing|L-aspartate:2-oxoglutarate aminotransferase activity|mitochondrion|GO:0006346|GO:00040

		biosynthesis(K,ko00300, ko))	69|GO:0005739
12	comp186475_c0_seq2	aldo-keto reductase, putative (EC:2.3.1.170) (NA)	response to salt stress|transferase activity, transferring acyl groups|cytoplasm|GO:0009651|GO:0016746|GO:0005737
	Block 4		
3	comp176284_c0_seq1	cell wall serine-threonine-rich galactomannoprotein Mp1 (NA)	NA
4	comp184968_c2_seq1	ATP-dependent helicase HrpB [EC:3.6.4.13] (NA)	microtubule-based movement|hydrolase activity|cytoplasm|-|GO:0016787|-
8	comp118368_c0_seq1	chaperone/heat shock protein Hsp12, putative (NA)	response to stress|DNA binding|nucleus|GO:0006950|-|-
13	comp194236_c1_seq1	Tar1p (NA)	NA
15	comp194214_c0_seq4	hypothetical protein (NA)	single-multicellular organism process|monooxygenase activity|cytoskeleton|-|GO:0004497|-
17	comp196113_c3_seq5	hypothetical protein (NA)	metabolic process|hydrolase activity|mitochondrion|-|GO:0016787|GO:0005739
19	comp174588_c0_seq1	hypothetical protein (NA)	primary metabolic process|L-tyrosine:2-oxoglutarate aminotransferase activity|chloroplast|GO:0044238|-|-
27	comp196970_c0_seq1	hypothetical protein (NA)	carbohydrate transport|substrate-specific transmembrane transporter activity|integral component of membrane|GO:0008643|GO:0022891|GO:0016021

29	comp193262_c0_seq22	solute carrier family 25 (mitochondrial adenine nucleotide translocator), member 4/5/6/31 (Calcium signaling pathway(K,ko04020,ko))	transmembrane transport|transporter activity|mitochondrial inner membrane|GO:0055085|GO:0005215|GO:0005743
31	comp192409_c0_seq1	uncharacterized LOC100249173 (NA)	ammonium transmembrane transport|ammonium transmembrane transporter activity|integral component of membrane|GO:0072488|GO:0008519|GO:0016021
33	comp192409_c0_seq2	uncharacterized LOC100249173 (NA)	ammonium transmembrane transport|ammonium transmembrane transporter activity|integral component of membrane|GO:0072488|GO:0008519|GO:0016021
38	comp118631_c0_seq1	Pc12g09320 (NA)	transmembrane transport|galactosylxylosylprotein 3-beta-galactosyltransferase activity|integral component of membrane|GO:0055085|-|GO:0016021
	Block 5		
1	comp194437_c1_seq15	solute carrier family 39 (zinc transporter), member 1/2/3 (NA)	water transport|iron ion transmembrane transporter activity|plasma membrane|GO:0006833|GO:0005381|GO:0005886
2	comp194866_c3_seq6	delta8-fatty-acid desaturase [EC:1.14.19.4] (NA)	fatty acid biosynthetic process|iron ion binding|integral component of membrane|GO:0006633|GO:0005506|GO:0016021
3	comp189138_c0_seq5	hypothetical protein (NA)	amino acid transport|poly(A)-specific ribonuclease activity|nucleus|GO:0006865|-|-
4	comp194437_c1_seq12	solute carrier family 39 (zinc transporter), member 1/2/3 (NA)	iron ion transport|iron ion transmembrane transporter activity|membrane|GO:0006826|GO:0005381|GO:0016020

5	comp194379_c0_seq35	oligopeptide transporter, putative (NA)	oligopeptide transport|oligopeptide transporter activity|cytoplasm|GO:0006857|GO:0015198|GO:0005737
	Block 6		
2	comp195903_c3_seq1	uncharacterized LOC100267144 (NA)	drug transmembrane transport|citrate transmembrane transporter activity|plasma membrane|GO:0006855|GO:0015137|GO:0005886
3	comp191998_c4_seq7	uncharacterized LOC100249012 (NA)	NA
4	comp195729_c1_seq6	hypothetical protein (NA)	protein retention in ER lumen|catalytic activity|mitochondrion|-|GO:0003824|-
5	comp195543_c0_seq3	phosphoenolpyruvate carboxykinase (ATP) [EC:4.1.1.49] (Glycolysis / Gluconeogenesis(K,ko00010,ko))	gluconeogenesis|DNA binding|nucleolus|GO:0006094|GO:0003677|GO:0005730

Table S4: Annotations of gene clusters of top 100 genes with highly variable expression under minesite growth for goutweed

		Gene Ontology	KEGG Orthology
	Block 1		
6	comp164739_c11_seq1	F-type H+-transporting ATPase subunit c [EC:3.6.3.14] (Oxidative phosphorylation(K,ko00190,ko))	ATP synthesis coupled proton transport|lipid binding|mitochondrion|GO:0015986|GO:0008289|GO:0005739
13	comp106951_c0_seq1	hypothetical protein (NA)	carbohydrate metabolic process|aldehyde-lyase activity|nucleus|GO:0005975|GO:0016832|-
15	comp159495_c0_seq1	hypothetical protein (NA)	recognition of pollen|RNA binding|cell periphery|GO:0048544|-|GO:0071944
18	comp119129_c0_seq1	acetolactate synthase I/II/III large subunit [EC:2.2.1.6] (Valine, leucine and isoleucine biosynthesis(K,ko00290,ko))	butanediol metabolic process|magnesium ion binding|nucleus|GO:0034077|GO:0000287|-
21	comp165191_c0_seq1	similar to PhiA protein (NA)	NA
22	comp148474_c0_seq1	NADH-ubiquinone oxidoreductase chain 5 [EC:1.6.5.3] (Parkinson's disease(K,ko05012,ko))	intron homing|DNA binding|mitochondrial inner membrane|GO:0006314|GO:0003677|GO:0005743
23	comp165148_c0_seq1	IgE-binding protein (NA)	NA
25	comp165441_c0_seq1	hypothetical protein (NA)	pentose-phosphate shunt|serine-type endopeptidase activity|extracellular region|-|-|GO:0005576

29	comp164677_c0_seq1	uncharacterized protein LOC100267335 (NA)	SRP-dependent cotranslational protein targeting to membrane|7S RNA binding|integral component of membrane|-|-|GO:0016021
32	comp163225_c6_seq1	ubiquinol-cytochrome c reductase cytochrome b subunit (Oxidative phosphorylation(K,ko00190,ko))	respiratory electron transport chain|endonuclease activity|mitochondrial inner membrane|GO:0022904|GO:0004519|GO:0005743
35	comp153368_c4_seq2	cytochrome c oxidase subunit 1 [EC:1.9.3.1] (Cardiac muscle contraction(K,ko04260,ko))	oxidative phosphorylation|DNA binding|mitochondrial inner membrane|GO:0006119|GO:0003677|GO:0005743
36	comp165255_c0_seq1	choline oxidase (CodA) (EC:1.1.3.17) (NA)	alcohol metabolic process|choline dehydrogenase activity|membrane|GO:0006066|GO:0008812|-
38	comp165266_c0_seq1	stearoyl-CoA desaturase (delta-9 desaturase) [EC:1.14.19.1] (Biosynthesis of unsaturated fatty acids(K,ko01040,ko))	fatty acid biosynthetic process|stearoyl-CoA 9-desaturase activity|endoplasmic reticulum|GO:0006633|GO:0004768|GO:0005783
40	comp161826_c0_seq18	elongation factor 1-alpha (RNA transport(K,ko03013,ko))	GTP catabolic process|translation elongation factor activity|cytoplasm|GO:0006184|GO:0003746|GO:0005737
41	comp159543_c0_seq5	H+-transporting ATPase [EC:3.6.3.6] (Oxidative phosphorylation(K,ko00190,ko))	ATP catabolic process|ATP binding|integral component of membrane|GO:0006200|GO:0005524|GO:0016021
43	comp165422_c0_seq1	amino acid transporter, AAT family (NA)	amino acid transmembrane transport|amino acid transmembrane transporter activity|integral component of membrane|GO:0003333|GO:0015171|GO:0016021
48	comp165439_c	hypothetical protein	signal transduction|nucleotide

	0_seq1	(NA)	binding|nucleus|GO:0007165|GO:0000166|-
50	comp162291_c8_seq5	uncharacterized LOC100245897 (NA)	NA
52	comp149830_c0_seq2	phosphoglycerate mutase family protein, putative (NA)	NA
55	comp155770_c0_seq1	hypothetical protein (NA)	nodulation|zinc ion binding|integral component of membrane|GO:0009877|-|GO:0016021
58	comp151220_c0_seq2	similar to NADP-dependent mannitol dehydrogenase (NA)	oxidation-reduction process|carbonyl reductase (NADPH) activity|endosome|GO:0055114|GO:0004090|-
59	comp155770_c0_seq4	Early nodulin, putative (NA)	nodulation|NADH dehydrogenase (ubiquinone) activity|integral component of membrane|GO:0009877|-|GO:0016021
	Block 2		
1	comp160633_c0_seq1	Tar1p (NA)	NA
2	comp149222_c0_seq1	Nonspecific lipid-transfer protein precursor, putative (EC:1.3.1.74) (NA)	lipid transport|lipid binding|mitochondrion|GO:0006869|GO:0008289|-
4	comp164935_c3_seq8	beta-fructofuranosidase [EC:3.2.1.26] (Starch and sucrose metabolism(K,ko00500, ko))	sucrose metabolic process|sucrose alpha-glucosidase activity|vacuole|GO:0005985|GO:0004575|GO:0005773
5	comp135307_c1_seq1	hypothetical protein (NA)	transport|translation initiation factor activity|cytoplasm|GO:0006810|-|-
	Block 3		
2	comp160629_c0_seq1	ATP-binding cassette, subfamily F, member 3 (NA)	translation|RNA binding|mitochondrion|-|-|GO:0005739

3	comp158446_c1_seq1	transmembrane protein, putative (NA)	response to stress|ligase activity|membrane|GO:0006950|GO:0016874|GO:0016020
5	comp155809_c0_seq2	sphingolipid delta-4 desaturase [EC:1.14.-.-] (Sphingolipid metabolism(K,ko00600,ko))	lipid biosynthetic process|oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen|extracellular region|GO:0008610|GO:0016705|-
8	comp156568_c0_seq1	elongation factor 1-alpha (RNA transport(K,ko03013,ko))	GTP catabolic process|translation elongation factor activity|plastid|GO:0006184|GO:0003746|-
9	comp161970_c0_seq5	uncharacterized LOC100256632 (NA)	positive regulation of seed germination|o-succinylbenzoate-CoA ligase activity|mitochondrion|GO:0010030|GO:0008756|GO:0005739
10	comp154950_c0_seq1	solute carrier family 25 (mitochondrial adenine nucleotide translocator), member 4/5/6/31 (Calcium signaling pathway(K,ko04020,ko))	gene silencing|ATP binding|membrane|-|-|GO:0016020
12	comp159802_c0_seq1	cathepsin X [EC:3.4.18.1] (Lysosome(K,ko04142,ko))	cell wall macromolecule catabolic process|peptidase activity, acting on L-amino acid peptides|cytoplasm|-|GO:0070011|-
13	comp164663_c1_seq10	H+-transporting ATPase [EC:3.6.3.6] (Oxidative phosphorylation(K,ko00190,ko))	ATP catabolic process|ATP binding|nucleus|GO:0006200|GO:0005524|GO:0005634
16	comp154187_c0_seq1	cathepsin L [EC:3.4.22.15] (Rheumatoid arthritis(K,ko05323,ko))	response to salt stress|protein binding|vacuole|GO:0009651|GO:0005515|GO:0005773
17	comp160996_c	Delta(5) fatty acid desaturase, putative	lipid metabolic process|binding|mitochondrion|GO:0006629|G

	1_seq3	(NA)	O:0005488|-
18	comp156207_c0_seq1	cathepsin B [EC:3.4.22.1] (Lysosome(K,ko04142,ko))	proteolysis|cysteine-type endopeptidase activity|extracellular space|GO:0006508|GO:0004197|GO:0005615
20	comp157219_c0_seq1	phosphatidylserine decarboxylase [EC:4.1.1.65] (Glycerophospholipid metabolism(K,ko00564,ko))	cellular protein modification process|carboxy-lyase activity|mitochondrion|-|GO:0016831|GO:0005739
21	comp158982_c0_seq1	similar to predicted protein (NA)	response to oxidative stress|peptidase activity|membrane|-|GO:0008233|GO:0016020
22	comp158687_c0_seq1	polyadenylate-binding protein (RNA transport(K,ko03013,ko))	phospholipid metabolic process|nucleic acid binding|plasma membrane|-|GO:0003676|-
23	comp157670_c0_seq1	similar to Proton-coupled amino acid transporter 3 (Proton/amino acid transporter 3) (Solute carrier family 36 member 3) (Tramdorin-2) (NA)	NA
26	comp164799_c1_seq10	alcohol dehydrogenase, putative (EC:1.6.5.5) (NA)	sulfur amino acid metabolic process|NADPH:quinone reductase activity|chloroplast stroma|GO:0000096|GO:0003960|GO:0009570
27	comp153982_c0_seq1	uncharacterized LOC100261203 (NA)	nitrate transport|nitrate transmembrane transporter activity|plasma membrane|GO:0015706|GO:0015112|GO:0005886
28	comp160285_c0_seq2	Ran BP2/NZF zinc finger-like protein (NA)	MAPK cascade|UDP-glucosyltransferase activity|nucleus|-|-|GO:0005634

29	comp146253_c0_seq1	LOC100193963 (NA)	NA
	Block 4		
1	comp158247_c1_seq1	uncharacterized protein LOC100266052 (NA)	ion transport|transporter activity|integral component of membrane|GO:0006811|GO:0005215|GO:0016021
2	comp159145_c0_seq1	solute carrier family 39 (zinc transporter), member 1/2/3 (NA)	manganese ion transport|iron ion transmembrane transporter activity|trans-Golgi network|GO:0006828|GO:0005381|GO:0005802
3	comp163515_c0_seq34	hypothetical protein (NA)	cellular amino acid metabolic process|oxidoreductase activity|chloroplast|-|GO:0016491|-
4	comp155719_c0_seq2	riboflavin synthase [EC:2.5.1.9] (Riboflavin metabolism(K,ko00740, ko))	riboflavin biosynthetic process|riboflavin synthase activity|chloroplast|GO:0009231|GO:0004746|GO:0009507
5	comp155859_c0_seq2	uncharacterized protein LOC100254863 (NA)	single-organism metabolic process|oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors|cytoplasm|GO:0044710|GO:0016706|GO:0005737
6	comp150935_c0_seq1	uncharacterized LOC100246375 (NA)	photorespiration|oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors|cytoplasm|-|GO:0016706|GO:0005737

Table S5. Excel file (separately attached)

Table S6. Common genes for goutweed and mugwort grown on MS-I and MS-II soil

Upregulated with MS-I	Goutweed	Mugwort
2og-fe oxygenase family protein	4.17	6.23
Ca2+ antiporter cation exchanger	2.14	2.74
Cytochrome p450	9.35	2.76
High affinity nitrate transporter	7.48	6.65
Nadh dehydrogenase subunit 4	9.58	8.15
Nitrate transmembrane transporter	2.12	8.32
Nitrate transporter	6.69	2.39
Laccase	3.66	4.77
Downregulated with MS-I		
Abc transporter c family member 5-like	-2.82	-2.44
Bacterial-induced peroxidase	-2.51	-4.40
Chalcone synthase	-3.27	-6.12
Cytochrome p450	-7.30	-4.38
Early light-inducible protein	-9.19	-3.32
Germin-like protein	-2.27	-8.92
Glutathione s-transferase	-3.82	-2.86
Glycerophosphoryl diester phosphodiesterase family protein	-3.85	-9.60
Guanine nucleotide-binding protein subunit beta-2-like	-6.77	-3.76
Heat shock protein 90	-9.81	-3.15
Mate efflux family protein dtx1-like	-7.41	-2.02
Nitrate transporter -like	-2.24	-3.47
Phosphate transporter pho1 homolog 3-like	-6.41	-5.52
Rhicadhesin receptor	-4.85	-4.16
Upregulated with MS-II		
2og-fe oxygenase family protein	3.04	8.13
4-coumarate- ligase	5.78	6.64
Abc transporter f family member 1-like	12.29	2.47
Ap2 erf domain-containing transcription factor	3.99	5.02
Aspartic proteinase nepenthesin-2	4.98	2.25
Atp binding	3.58	2.09
Auxin response factor 17	3.55	2.23
Beta- -glucanase	4.37	2.35
Calmodulin-binding protein	6.59	2.35
Cellulose synthase-like protein e1	2.45	2.96
Cytochrome p450	6.93	2.31
E3 ubiquitin-protein ligase rglg2	3.93	5.98
Ethylene-responsive transcription factor 5-like	2.86	3.09
Ferric reductase-like transmembrane component	2.24	3.02
Glutathione peroxidase	8.01	3.90
High affinity nitrate transporter	10.39	10.06
Light-regulated zinc finger protein 1	5.68	2.48

Myb transcription factor	3.04	3.01
Nac domain protein	2.93	4.68
Peroxidase 52	6.22	3.59
Phosphoenolpyruvate carboxykinase	7.79	1.92
Peptide nitrate transporter at2g37900-like	3.63	7.56
Leucine-rich repeat receptor-like protein kinase at5g49770-like	5.73	4.05
Lrr receptor-like serine threonine-protein kinase at4g08850-like	4.79	7.28
Protein phosphatase 2c	5.07	2.91
Wrky transcription	8.02	6.22
Downregulated with MS-II		
2-oxoglutarate-dependent dioxygenase	-6.98	-5.79
Abc transporter c family member 5-like	-3.22	-2.36
Abscisic acid receptor pyl4	-2.19	-1.70
Ammonium transporter	-1.84	-10.62
Beta expansin precursor	-3.86	-4.49
Calcium dependent protein kinase	-3.06	-6.41
Cytochrome p450	-7.07	-7.43
Ethylene-responsive transcription factor	-3.74	-4.52
Germin-like protein	-3.61	-12.31
Glutathione s-transferase	-7.07	-7.51
Molybdenum cofactor sulfurase	-3.83	-3.03
Nitrate transporter -like	-2.89	-1.74
Rhicadhesin receptor	-3.94	-5.73
Serine threonine-protein kinase sapk2-like	-4.74	-3.35

Table S7. Differentially expressed genes unique to goutweed and mugwort grown on MS-I and MS-II soil

Annotated genes	Upregulated genes in goutweed on MS-I
Abc transporter c family member 3-like	4.01
Aldehyde dehydrogenase	9.34
Ap2 erf domain-containing transcription factor	7.41
Basic-leucine zipper transcription factor	6.94
Bzip transcription factor	2.79
Calmodulin-binding protein	4.17
Cytochrome b	11.38
Early nodulin 93 protein	5.77
Ferric reductase defective 3b	6.94
Glutathione reductase	8.66
Glutathione s-transferase	4.15
Heavy metal transport detoxification domain-containing protein	2.56
Heavy-metal-associated domain--containing protein	3.52
Metallothionein-like protein	8.14
Mitogen-activated protein kinase kinase kinase 1-like	4.65
Myb family transcription factor-like protein	5.58
Pho1-like protein	8.71
Phosphate transporter pho1 homolog 3-like	2.03
Plasma membrane h+-atpase pma1	8.45
Plasma membrane h+-atpase pma1	6.92
Potassium channel	2.60
Protein aluminum sensitive 3	4.40
Sodium proton exchanger	3.14
Sucrose transporter	5.20
Sulfate transporter	3.70
Sulfite exporter family protein	4.18
Transcription factor bhlh130-like	5.77
Transcription factor bhlh137	6.34
Wrky transcription factor 12	3.82
Zinc finger ser thr protein kinase-like protein	8.16
Annotated genes	Upregulated genes in mugwort on MS-I
Copper transporter	5.14
Ferric reductase-like transmembrane component	3.09

Ferric-chelate reductase	4.93
Metal-nicotianamine transporter ysl3	7.59
Na+ h+ antiporter	2.52
Nitrate and chloride transporter	2.28
Sodium transporter hkt1-like	2.78
Transcription factor bhlh47	3.17
Zinc transporter	2.58
Zip transporter	10.28
Annotated genes	Downregulated genes for goutweed on MS-I
Aluminum-activated malate transporter 10	-4.59
Auxin efflux carrier component	-4.08
Bhlh transcriptional regulator	-6.48
Ca2+-transporting atpase	-6.36
Calcium-dependent protein kinase	-3.41
Calcium-transporting atpase plasma membrane-type-like	-4.54
Cell division control protein 50	-6.17
F-box family protein	-4.99
Fe transport protein	-11.87
Ferric-chelate reductase	-8.29
Metal transporter	-11.10
Sodium calcium exchanger family protein	-4.97
Wrky transcription factor 22	-5.62
Zinc ion binding protein	-7.00
Zinc transporter	-2.14
Zip transporter	-7.82
Annotated genes	Downregulated genes in mugwort on MS-I
Ammonium transporter 3 member 1-like	-5.66
Beta- -glucanase	-6.54
Chalcone isomerase	-5.90
Cytochrome c peroxidase	-7.22
Dna-damage-inducible protein	-11.83
F-box lrr-repeat protein 3-like	-6.23

Ferredoxin i	-4.38
Ferric reductase defective 3b	-6.96
Glutaredoxin	-9.28
Metal ion binding	-4.97
Nitrate chlorate transporter	-6.34
Nitrate transmembrane transporter	-7.12
Phosphorus starvation-induced protein	-9.66
Proline transporter 1	-7.71
Protein aluminum sensitive 3	-4.74
Sugar porter family mfs transporter	-6.87
Sulfate transporter	-4.55
Vacuolar-processing enzyme precursor	-8.94
Annotated genes	Upregulated genes in goutweed on MS-II
Adenine nucleotide translocase	11.49
Aspartic proteinase nepenthesin-2	2.61
Magnesium transporter	2.87
Mitochondrial phosphate transporter	5.42
Nitrate transporter	2.84
Nitrate transporter	3.13
Nitrate transporter	3.46
Plasma membrane h+-atpase	4.92
Potassium channel	2.44
Protein aluminum sensitive 3	4.67
Sodium proton exchanger	3.85
Sulfate transporter	5.96
Annotated genes	Upregulated genes in mugwort on MS-II
Ammonium transporter 3 member 1-like	6.60
Cyclin t1	6.51
High affinity inorganic phosphate transporter	5.42
Metal transporter	9.82

Nitrate transmembrane transporter	7.90
Sodium calcium exchanger family protein	3.33
Symbiotic ammonium transporter	2.00
Transcription factor bhlh25-like	6.58
Zinc transporter	5.77
Zip transporter	9.35
Annotated genes	Downregulated genes in goutweed on MS-II
Adenine phosphoribosyltransferase	-9.70
Aluminum-activated malate transporter 10	-3.98
Aquaporin sip1-2	-2.70
Brassinosteroid-regulated protein bru1	-2.86
Calcineurin-like metallo-phosphoesterase-like protein	-3.53
Copper transporter	-7.71
Fe transport protein	-11.53
Ferric reductase-like transmembrane component	-2.43
Ferric reduction oxidase 4	-7.71
Ferric-chelate reductase	-8.22
Gata transcription factor	-2.99
High-affinity nitrate transporter	-2.36
Metal transporter	-7.59
Nac domain-containing protein	-7.77
Phosphate transporter pho1 homolog 3-like isoform 1	-6.86
Potassium channel	-8.18
Potassium transporter 4-like	-6.56
Protein phosphatase	-7.81
Sulfite exporter family protein	-2.48
Transcription factor bhlh135-like	-5.69
Wrky transcription factor 5	-4.63
Zinc finger family protein	-3.27
Zinc ion binding protein	-7.31

Zinc transporter	-2.43
Zip family metal transporter	-2.06
Zip transporter	-3.51
Annotated genes	Downregulated genes in mugwort on MS-II
Acyl carrier protein	-9.03
Aquaporin nip1-2	-8.56
Blue copper protein	-6.68
Chalcone isomerase	-5.92
Chalcone synthase	-6.31
Phosphate transporter	-8.11
Probable nitrite transporter at1g68570-like	-3.17
Sulfate transporter	-4.41

Table S8. Primers used for RT-qPCR in this study

Transcript/Gene/Protein	Forward (5'→3')	Reverse (5'→3')
Goutweed MS-I Up		
Lipid transfer protein	CCGGCTGACCGCAAAC	ACGGAAGAAGCAAGGCTCTTAA
Cytochrome b	CGGTAACTTAGAGATTTCTGTTCCAA	CCCGACTTGTTTAGACACTTTTTCT
NADH dehydrogenase subunit 5	CCCCCCGTATCTTCTGAAGTC	ATTCTTAGGAGCGGGAGCAGTA
ATP synthase subunit 9	CAATTCCTACACCGGCTCCTAT	CAAGCAGCAAAAATAATTGGAACA
Phosphatidyl synthase	CCGCAAGGTGGCACTTTT	TGCGCCACTGCTGCAA
Transaldolase	TGGCCTTGTACCAGTCGAGAA	GGGCGCTTTCCTCATCTCT
Actin cytoskeleton protein	TCAGACCCGGTCCCAAAAC	CGCGACATTGCCATGATG
6-phosphogluconate dehydrogenase	CCATGTCGAGCGCGTTAAT	TATGGATGCTGCGGGACAA
o-acyltransferase WSD1	CACGGCTACAAGTCAGTGAGTGA	GGTTACCCCATGGCATGCT
Peptidase inhibitor	TCGAGAAGGATGGCGTTGTC	TGTCATGCCGCGTTGATC
Goutweed MS-I Down		
Metal transporter	TCAGTCGACCAGCTCCTGATG	TGATTGCTATCACGCCAAGTCT
Fe transport protein	TTCGTCATCGTTAAGGCATTTG	CGTGCATAAATCCGGTTCCT
Early light-inducible protein	GTGGACCTGCACCAGAAAGAA	GGCTGCTACGAATCCGATCA
Heat shock protein 90	TTAGCAGTCCAGCCGTATTCAC	TGTCTGACCGCGTTGTTGAT
Copper transporter	CCCCAAAAGAAAGTCATATGCAT	AAACGGCACCGGTGGTAA
Gibberellin 20 oxidase	TCCATTTCCGCTACTAACTTCGT	CCCGAGGGTAATGAAGATTTCA
Ferric-chelate reductase	GCTCAACAGTTTCGCATGGA	AATCACGACAAGGCGTTCGT
Oxidoreductase	TCACATTCTTGGTTGGCGATT	CAAATGCCTGCAGAGGAGAAG
Polygalacturonase	CAAATTTTGAACACCAGGTTCTTG	CCCCGGTCATGGTATTAGCA
Allene oxide cyclase	CAGCCCTGCTTACCTTCGTTT	GGAACGAGATCGCCCAAA
Mugwort MS-I Up		

ZIP transporter	ATCGTTCCGGTGAGAAATGAA	CGGCGGGATGTCTCTGAT
Nitrate transmembrane transporter	CCACCTCGAAAGGCAAGCT	GTCATTCCGGCCCATTTG
NADH dehydrogenase	TGGGACTCGGCCTAAAAATAATC	TGCATACGGGTGCTGCTAGA
Metal-nicotianamine transporter YSL3	AGGCAAGAAGCCCATGCA	TCTTCCGGCCTCAGTCCTT
Serine carboxypeptidase-like	CATGGGACCAATTTCGAAAAA	CGCTGGAGGACCTGGTTGT
Histone-arginine methyltransferase CARM1	CCGTTGTCAGCCACCTTTGT	GGTTTGGCATGTTGGTTTGAT
High affinity nitrate transporter	GCTTCATCTTGCCGGGATAG	CGAGCTAGAAGGTTTGCCATTC
Glucan-beta-glucosidase	CCGAAGGATCGTCGTTTCC	TTCTTGTTACTGCCGATTCCAA
Zinc iron specific protein	GCAATCTCTTGATCTCCAACGA	CAACGTGATAGCGGCTGAAG
2OG-Fe oxygenase family protein	CCACCAATCCCATCTTGAAGA	GTGTCGGGAGGCACTCAGATA
Mugwort MS-I Down		
DNA-damage-inducible protein	TGGGAAAGAACGTGTGCAATAG	CCAATTCTTATCTTTGTCTGCAAGTT
Phosphorus starvation-induced protein	TCGACCCAAAGTCATTTCTCACT	TTGACATGATCGGATAAGCTCAGTA
Glycerophosphoryl diester phosphodiesterase	CGGCAGCCGGATTTCTAA	GGGCGGATTGGATGGAA
Transcription factor ORG2	TTTTGATCACTTGATATGGGTAGCA	CGCAAGCGGGCTAATGATT
Pyridoxal phosphate phosphatase phospho2	TCATGAACCCCGGCCTTA	AATTTTCCGGTTTGGAACTTGA
SPX domain-containing protein	AGAGTAAGTCTGTGGTGAAGAATGGTT	ACCGTTCTTCGTTTACCATACCTT
Glutaredoxin	GGACCGCCAGGTTATGAAATT	AGACGTGTACGCGGAATGG
Inorganic pyrophosphatase 2	GGTCTTCTCCATCGGTCCATT	ATATGCAAAAACCGAGAGCTTGT
Vacuolar-processing enzyme precursor	GCCAAGAAGCACAGCATAGAAA	CCAAAGGATTACACCGGAGAAT
Germin-like protein	GGATGAGAGCACTTGTCAATGC	CATGGCTGCTTTTGTGACCTT
Goutweed MS-II Up		
ABC transporter f family	ACAAAGCAACACGCATCTTCA	TCGGCCAGAACAAGTTTGTG
Cysteine proteinase	CGATTGGCCCGAGGAATC	CAATAGGGTATGAGGGCTGGTT
High affinity nitrate transporter	CCCATCAAATCCGAAAGCA	CTAGCTTTGGGTTGGCCAATAT

Cytochrome p450	TTGTTGAGTGGCTATGGTTTTCA	GAGCCCGGACGCCAAT
Vacuolar H^+-pyrophosphatase	ACAGCCGCTTTGTGAGCAT	CATAGAGGGCGGCAACATG
Zinc finger ser/thr protein kinase	AAATCCAGTCACCACATTTCCAA	GGCATCAGGAGGGTATGCAT
Calreticulin precursor	CACCAGCTTCTCCAGAGTCCTT	TGCTGATTGGCAGTCTCGTT
Glutathione peroxidase	CGGCGGTAGGTTGAGCAA	CACCTCCTCCTCCGTCAAGA
V-type H^+-transporting ATPase	TCCGTCGACGAGGATCAAA	CCAACTGCAAGAGGACCATTC
Lipid transfer protein precursor	AACACCGCCCGTCTTCAG	TGTGGACAAGTGACAAGCTCAA
Goutweed MS-II Down		
Fe transport protein	TTCGTCATCGTTAAGGCATTTG	CGTGCATAAATCCGGTTCCT
Riboflavin synthase alpha chain	GAGAGCGGTGACACCATCAA	CGTCCACGTGTCCTTGCA
Probable polyol transporter	TCCTGGGATCGCGCTTAC	TGCTTCCAAGAATTCCTTGTCTT
Heat shock protein 90	TTAGCAGTCCAGCCGTATTCAC	TGTCTGACCGCGTTGTTGAT
Ferric-chelate reductase	GCATGGAAGAGGCCAATGA	GAAAAAACATAGCTCAATTCCAGAAA
Potassium channel	GGGTTCGATGTGCGAAGCT	AAGCAGCCAGAACAATGAACTG
Metal transporter	CATCCAGGCTTCTTTTGGTTCTT	GCCCTTGACGTCGAAAACA
bHLH transcriptional regulator	GGCTGAAGTTGCAAGCCTAGA	CATGTAAGCCTCCTTGTTGTTTGT
NAC domain-containing protein	TGTTTCCCAGGTGTGGTCCTA	GCGGAGAATGTTTCTGGATTG
Copper transporter	CCCCAAAAGAAAGTCATATGCAT	AAACGGCACCGGTGGTAA
Mugwort MS-II Up		
High-affinity nitrate transporter	GGCCCCTCTTGTTCCGATAA	TGTCGGAGCGGGTGAGAT
Metal transporter	TTTCCATGTTGCCAGGATCA	CATTTGTTGGCCCTGGTTTC
Zip transporter	TGGTGTCCATGGAAGTGCAT	CGCTGGAGACCAGGAGATTG
2og-Fe oxygenase	TCCACCAATCCCATCTTGAAG	GTGTCGGGAGGCACTCAGATA
Pollen-specific protein	CCGGTGACGCATCCGTAT	GAAACCAGCACGGCAGGTA
Nicotianamine synthase	TCGGCCTGACCACAAAGTCT	GCCAATCTTCCAGCGAATATACA

Nitrate transmembrane transporter	CCACCTCGAAAGGCAAGCT	GTCATTCCGGCCCATTTG
Polyphenol oxidase	CGTGTGGAACCTGTGCAAAG	AGGACCTGCCAACACCATGT
Phytochrome A-associated F-box protein	GCTCACCGTCATCATGAAACC	GGATATGCATTCCGCGTTTT
LRR receptor-ser/thr kinase	CCACCCTCTCCGAGCAAA	CTATTCGGAACTAAAGGCTGCAA
Mugwort MS-II Down		
Germin-like protein	GGATGAGAGCACTTGTCAATGC	CATGGCTGCTTTTGTGACCTT
DNA-damage-inducible protein	TGGGAAAGAACGTGTGCAATAG	CCAATTCTTATCTTTGTCTGCAAGTT
Vacuolar-processing enzyme precursor	GCCAAGAAGCACAGCATAGAAA	CCAAAGGATTACACCGGAGAAT
Serine carboxypeptidase II	GTCACTGCATACATCCCATGATG	CAACTCCGCGGTACAAAAGG
Ammonium transporter	CCTTTCACCTTGTCCCCAAA	GGAGACGAGGCGGCTCAT
Acyl carrier protein	CAACTCGCTAGTGTCTCGATGATC	TCGGCCTTGATGGTCACA
Nitrate chlorate transporter	AACCATCCTCGGCTTTTCG	GCGTCATGGTTCAGACTGTGA
MYB transcription factor	GGGCGAGTGGCAATAAGCTA	GAACACCTTTGATTCCCAAGACA
Leucine rich protein	CGGCTATGCATCACCTCTCA	GCCCGGTGTGTAGGCAAAT
Fatty acid desaturase	TCCCTGGTCCGTTGTTCAAA	GTACATCCCAAAACGCAAGTCA