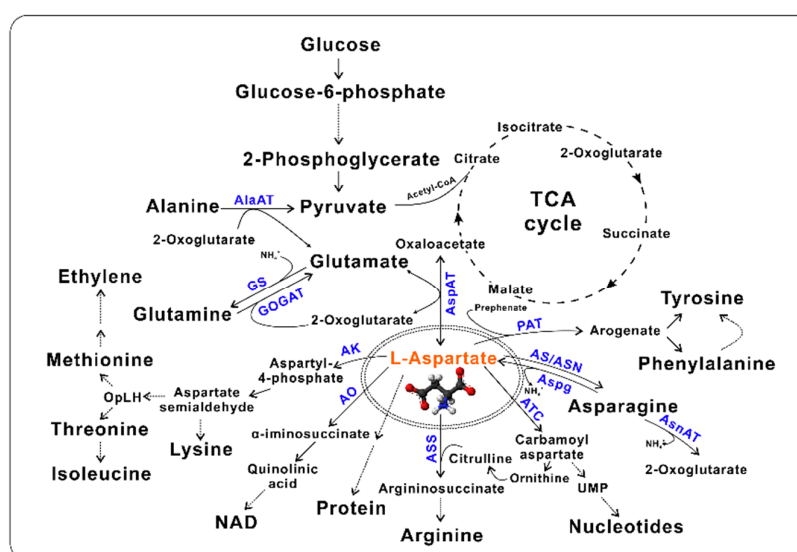


# New Insight into Aspartate Metabolic Pathways in *Populus*: Linking the Root Responsive Isoenzymes with Amino Acid Biosynthesis during Incompatible Interactions of *Fusarium solani*

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## Supplementary Material



**Figure S1.** Schematic model representing the central metabolites intermediated by Asp metabolic pathways in plants. PAT, prephenate aminotransferase; TCA cycle, tricarboxylic acid cycle; NAD, nicotinamide adenine dinucleotide; UMP, uridine monophosphate.



**Figure S2.** Genomic structures and *in silico* prediction of the *cis*-regulatory elements in gene promoters. (a) Genomic structures show the patterns of the exon/intron organization. The exon lengths are displayed proportionally to the scale on the bottom. (b) The various *cis*-regulatory elements were boxed in colors.

**Table S1.** List of isoenzymes involved in aspartate (Asp) metabolic pathways in *P. trichocarpa*.

Enzyme <sup>1</sup>	Gene (v3.1)	Name	Gene (bp)	ORF (bp)	Size (a.a)	MW (kDa)	PI	Target <sup>2</sup>	<i>A.thaliana</i>	Name
GLN/GS	Potri.004G085400	<i>PtGS1/PtGS1.1a</i>	3321	1071	356	39.26	5.52	Cyto	AT1G48470	<i>GLN1;5</i>
	Potri.005G093200	<i>PtGS2/PtGS1.2a</i>	4777	1071	356	39.06	5.14	Cyto	AT1G66200	<i>GLN1;2</i>
	Potri.007G069600	<i>PtGS3/PtGS1.2b</i>	5537	1071	356	38.99	5.53	Cyto	AT3G17820	<i>GLN1;3</i>
	Potri.008G200100	<i>PtGS4/PtGS2a</i>	5169	1299	432	47.75	6.48	Plastid	AT5G16570	<i>GLN1;4</i>
	Potri.010G029100	<i>PtGS5/PtGS2b</i>	5029	1299	432	47.89	6.48	Plastid	AT5G35630	<i>GLN2</i>
	Potri.012G043900	<i>PtGS6/PtGS1.3a</i>	3227	1071	356	39.12	5.86	Cyto	AT5G37600	<i>GLN1;1</i>
	Potri.015G034700	<i>PtGS7/PtGS1.3b</i>	2964	1071	356	39.22	5.81	Cyto		
	Potri.017G127300*	<i>PtGS8</i>	1942	492	163	18.04	8.35	Cyto		
GLU/GOGAT	Potri.017G131100	<i>PtGS9/PtGS1.1b</i>	3485	1071	356	39.22	5.95	Cyto		
	Potri.006G038400	<i>PtGOGAT1</i>	15954	4476	1491	162.70	5.85	Plastid	AT2G41220	<i>GLU2</i>
	Potri.012G011700	<i>PtGOGAT2</i>	11095	6687	2228	243.90	6.48	Plastid	AT5G04140	<i>GLU1</i>
	Potri.015G017500	<i>PtGOGAT3</i>	11684	6687	2228	244.10	6.15	Plastid	AT5G53460	<i>GLT1</i>
	Potri.016G036900	<i>PtGOGAT4</i>	17137	4863	1620	176.53	6.41	Plastid		
AspAT	Potri.005G079200	<i>PtAspAT1/PAT1</i>	4021	1443	480	51.59	7.11	Plastid	AT1G62800	<i>ASP4</i>
	Potri.006G107100	<i>PtAspAT2</i>	4501	1269	422	46.96	7.19	Mito	AT2G22250	<i>PAT</i>
	Potri.006G241500	<i>PtAspAT3</i>	3832	1266	421	46.56	6.50	Cyto	AT2G30970	<i>ASP1</i>
	Potri.006G241600	<i>PtAspAT4</i>	3829	1350	449	49.18	9.16	Cyto	AT4G31990	<i>ASP5</i>
	Potri.006G260200	<i>PtAspAT5</i>	4211	1401	466	51.03	8.67	Plastid	AT5G11520	<i>ASP3</i>
	Potri.T079800**	<i>PtAspAT6/PAT2</i>	4159	1446	481	51.89	8.06	Plastid	AT5G19550	<i>ASP2</i>

	Potri.014G143300	<i>PtAspAT7</i>	9230	1287	428	47.79	8.35	Mito		
	Potri.016G133000*	<i>PtAspAT8</i>	2806	915	304	33.11	5.66	Cyto		
	Potri.018G022200	<i>PtAspAT9</i>	5060	1401	466	50.92	8.67	Plastid		
	Potri.018G082500	<i>PtAspAT10</i>	5417	1224	407	44.47	7.72	Cyto		
AS/ASN	Potri.001G278400	<i>PtAS1</i>	2272	1338	445	49.77	6.58	Cyto	AT3G47340	ASN1
	Potri.005G075700	<i>PtAS2</i>	6490	1755	584	65.64	6.39	Cyto	AT5G10240	ASN3
	Potri.009G072900	<i>PtAS3</i>	3969	1770	589	66.07	6.00	Cyto	AT5G65010	ASN2
Aspg	Potri.002G122900	<i>PtAspg1</i>	3038	987	328	34.68	4.69	Cyto	AT3G16150	ASPGB1
	Potri.014G022900	<i>PtAspg2</i>	1967	987	328	34.60	4.81	Per	AT5G08100	ASPGA1
	Potri.014G080600	<i>PtAspg3</i>	3622	1260	419	43.71	6.03	Cyto		
	Potri.014G191600	<i>PtAspg4</i>	2919	1077	358	37.90	6.14	ER		
	Potri.014G191900	<i>PtAspg5</i>	2705	1077	358	37.93	6.14	Extra		
AsnAT	Potri.001G253300	<i>PtAsnAT1</i>	5120	1206	401	44.08	8.59	Per	AT2G13360	AGT1
AlaAT	Potri.001G162800	<i>PtAlaAT1</i>	5087	1446	481	52.98	6.25	Per	AT1G17290	ALAAT1
	Potri.003G072600	<i>PtAlaAT2</i>	5155	1602	533	58.27	5.77	Mito	AT1G72330	ALAAT2
	Potri.008G187400	<i>PtAlaAT3</i>	6013	1446	481	53.37	5.99	Per	AT1G23310	GGT1
	Potri.010G045100	<i>PtAlaAT4</i>	5953	1446	481	53.48	6.92	Per	AT1G70580	GGT2
AO	Potri.001G348600	<i>PtAO1</i>	5603	1953	650	71.70	6.40	Plastid	AT5G14760	AO
AK/AK-HSDH	Potri.002G236800	<i>PtAK1</i>	6690	1674	557	61.05	5.72	Plastid	AT3G02020	AK3
	Potri.014G145700	<i>PtAK2</i>	6860	1686	561	61.71	5.69	Plastid	AT5G13280	AK1
	Potri.013G099500	<i>PtHSDH.1</i>	9727	2769	922	100.80	6.94	Plastid	AT5G14060	AK2
	Potri.019G071700	<i>PtHSDH.2</i>	9535	2751	916	100.33	6.70	Plastid	AT1G31230	HSDH1
									AT4G19710	HSDH2
ASS	Potri.008G020200	<i>PtASS1</i>	4278	1485	494	54.25	6.31	Plastid	AT4G24830	ASS
	Potri.010G239000	<i>PtASS2</i>	4140	1485	494	54.35	6.45	Plastid		
ATC	Potri.001G357200	<i>PtATC</i>	5200	1155	384	42.94	6.78	Plastid	AT3G20330	ATC

<sup>1</sup> glutamine synthetase (GLN/GS), glutamate synthase (GLU/GOGAT), Asp aminotransferase (AspAT), asparagine synthetase (AS/ASN), asparaginase (Aspg), asparagine aminotransferase (AsnAT), alanine aminotransferase (AlaAT), Asp oxidase (AO), aspartokinase/aspartokinase-homoserine dehydrogenases (AK/AK-HSDH), argininosuccinate synthase (ASS), Asp transcarbamoylase/carbamoyltransferase (ATC).

<sup>2</sup> cytoplasm (Cyto), extracellular (Extra), mitochondrion (Mito), plastid, endoplasmic reticulum (ER), and peroxisome (Per). \* Genes ID only present in v3.0, \*\* The ID is updated to Potri.007G088426 in v4.1.

**Table S2.** The number of annotated compounds classified by families upon *F. solani* infection in roots.

Chemical Family	Number of annotated compounds		
	48/0 hpi	72/0 hpi	72/48 hpi
Carbohydrates and carbohydrate conjugates	70	56	15
Flavonoid glycosides	61	38	7
Triterpenoids	45	13	11
Terpenoids (mono, di, and sesqui)	28	21	4
Amino acids, peptides, and analogues	26	24	9
Flavans	18	8	5
Fatty acid-related	13	16	5
Stilbene glycosides	12	10	1
Biflavonoids and polyflavonoids	11	12	3
Furanocoumarins	9	5	1
Hydroxycinnamic acids and derivatives	8	8	1
Amines	6	6	1

**Table S3.** Statistical list of variable annotated amino acids and derivatives.

Compound	KEGG/PubChem ID	log2FC (72/0 hpi)	log2FC (48/0 hpi)	log2FC (72/48hpi)
3-amino-2-piperidone		-0.959242085	-1.287624277	ND
Acetylhomoserine		0.413502763	0.289123839	ND
Citrulline	C00327	ND	-0.965678271	0.72762098
gamma-Glutamylaspartic acid		0.811968258	0.980476886	ND
Glycine		0.93688711	0.9762432	ND
Glycylglycylglycine		12.07483783	ND	3.245442267
L-2-Amino-5-hydroxypentanoic acid		1.31739572	1.134765593	ND
L-alpha-Aminobutyric acid	C02356	2.484660714	1.96971639	ND
L-Asparagine	C00152	-1.39960787	-1.040911987	ND
L-asparagine		-2.243842063	-1.406524372	ND
L-Glutamic acid	C00025	0.871826199	0.70855538	ND
L-Isoleucine	C00407	ND	0.347996561	-0.695894603
L-Oxalylalibizziine		0.580167883	0.415950772	ND
L-proline		1.334475436	1.356375227	ND
L-threonine		ND	0.82296079	-0.535254662
Lycoperdic acid		1.381155267	1.455909502	ND
Ornithine		ND	-1.234841524	0.559956343
S-Methylmethionine		5.031482626	ND	1.952267173

**Table S4.** List of primers used for qRT-PCR analyses.

Primer Name	Sequence	Access number/Gene
<i>β-Actin_qF</i>	GACCTTCAATGTGCCTGCAA	Potri.019G006700.1/ <i>Ptβ-Actin</i>
<i>β-Actin_qR</i>	ACCATCACCAGAATCCAGCA	
<i>EF1α_qF</i>	TCCGTCTTCCACTTCAGGATGTCT	Potri.006G130900.1/ <i>PtEF1α</i>
<i>EF1α_qR</i>	GTCACGACCATAACCAGGCTTCAG	
<i>UBIC_qF2</i>	TTGCTTGAGGAACCTGAAC	Potri.006G205700.7/ <i>PtUBIC</i>
<i>UBIC_qR2</i>	GTGAGGACCGATTACAGT	
<i>qFW113</i>	CGCATCAGCTATAAAAGAAGCAC	Potri.004G085400/ <i>PtGS1</i>
<i>qRV113</i>	TCTCAGTGGAGTCTGAGAGGTT	
<i>qFW114</i>	AAATGCCAGTGTGGCTGTTG	Potri.005G093200/ <i>PtGS2</i>
<i>qRV114</i>	TGAAAAATGCAGAGAATTGTGACT	
<i>qFW116</i>	CTCCTTGACTTTGGGGATGC	Potri.007G069600/ <i>PtGS3</i>
<i>qRV116</i>	TCCAAGTCAATGCCACCTTAT	
<i>qFW117</i>	CCCGTGGATGAAAAGGCTCT	

<i>qRV117</i>	TTCCACACAAGGCCAAGGAA	Potri.008G200100/ <i>PtGS4</i>
<i>qFW118</i>	ATCAGCATAACAAGGGCGACA	Potri.010G029100/ <i>PtGS5</i>
<i>qRV118</i>	CCCCGTGTTTGGGGATTAAGA	
<i>qFW119</i>	TTCAGCTGTACCTTCGAGCC	Potri.012G043900/ <i>PtGS6</i>
<i>qRV119</i>	TCGACGGTGAAGGCGTTTA	
<i>qFW120</i>	ATGATTGTCCTCCGTTCCCTG	Potri.015G034700/ <i>PtGS7</i>
<i>qRV120</i>	CTACCAAACACACCCACCCTT	
<i>qFW121</i>	AGCGTGGTCTGAGGCATATC	Potri.017G127300*/ <i>PtGS8</i>
<i>qRV121</i>	CGATTAGCAACCCCCGTTTG	
<i>qFW122</i>	TCTTCAACAATAATCCGCCCT	Potri.017G131100/ <i>PtGS9</i>
<i>qRV122</i>	AAGGTCTGAAAGCAACGACAT	
<i>qFW123</i>	GTGCATCATTGAGGCAACC	Potri.006G038400/ <i>PtGOGAT1</i>
<i>qRV123</i>	CGAATTCGGGTTTTCCGGC	
<i>qFW124</i>	TGCTTATGAGCGTGAAGGTGT	Potri.012G011700/ <i>PtGOGAT2</i>
<i>qRV124</i>	AGGGCATCCCCAGTTTTCTC	
<i>qFW125</i>	GCTCATTTGGTAGTTGTTGAGGA	Potri.015G017500/ <i>PtGOGAT3</i>
<i>qRV125</i>	GCTAAACCACAAGGCACAACG	
<i>qFW126</i>	ACATCACCCAAGAGCATCCC	Potri.016G036900/ <i>PtGOGAT4</i>
<i>qRV126</i>	GTCCTGGGTGTGTTCAGCAT	
<i>PtAspAT1_qFw</i>	GCAGAGGTTGAAGGGTTTGG	Potri.005G079200/ <i>PtAspAT1/PAT1</i>
<i>PtAspAT1_qRv</i>	TGTAGGGTGGTCAGAGAAGC	
<i>PtAspAT2_qFw</i>	TCCAACCACCATAACATA	Potri.006G107100/ <i>PtAspAT2</i>
<i>PtAspAT2_qRv</i>	CATTCTTGACATCATCCAT	
<i>PtAspAT3_qFw</i>	GGACAATGAGCCCACGAATTG	Potri.006G241500/ <i>PtAspAT3</i>
<i>PtAspAT3_qRv</i>	TCCTCTATTCTCTCCACCACTT	
<i>PtAspAT4_qFw</i>	GTTGCAGGCAGGGTTGAAAG	Potri.006G241600/ <i>PtAspAT4</i>
<i>PtAspAT4_qRv</i>	GGCTTCAAAGAGTTTCTGGCG	
<i>PtAspAT5_qFw</i>	TGCACGTGGTATGGAGCTTT	Potri.006G260200/ <i>PtAspAT5</i>
<i>PtAspAT5_qRv</i>	TCGGGCAATCCTTTTCAGCT	
<i>PtAspAT6_qFw</i>	TAGCAATGGTGCCAAGCAGA	Potri.T079800/ <i>PtAspAT6/PAT2</i>
<i>PtAspAT6_qRv</i>	TCACCCAAAATGGTGCAGGA	
<i>PtAspAT7_qFw</i>	AGCAATCCTCTGTTCAT	Potri.014G143300/ <i>PtAspAT7</i>
<i>PtAspAT7_qRv</i>	CAGCCATTACCTTCACTTC	
<i>PtAspAT8_qFw</i>	GAAGATGGGCACCTGGTAGG	Potri.016G133000*/ <i>PtAspAT8</i>
<i>PtAspAT8_qRv</i>	GCTTTCCCGCAAAGTTGTCC	
<i>PtAspAT9_qFw</i>	TCCTCCTGTTCATGGGGCTA	Potri.018G022200/ <i>PtAspAT9</i>
<i>PtAspAT9_qRv</i>	CCTCCCAGCCATCATTCCA	
<i>PtAspAT10_qFw</i>	CACTGGCTTGAACACTGAACA	Potri.018G082500/ <i>PtAspAT10</i>
<i>PtAspAT10_qRv</i>	AAAAGATCCCCAGAAGCGGA	
<i>qFW127</i>	CCGTTCTCTTGATTTTTCAGGGTC	Potri.001G278400/ <i>PtAS1</i>
<i>qRV127</i>	GTGGGTCATATGGGGTCGAG	
<i>qFW128</i>	GTGCCCTCTCTGTGTTTT	Potri.005G075700/ <i>PtAS2</i>
<i>qRV128</i>	AGATGGGTGAATGATGCTCGT	
<i>qFW129</i>	AAGCCTTTGACGACGAGGAG	Potri.009G072900/ <i>PtAS3</i>
<i>qRV129</i>	TGTCAGTCACATGTTGGGCA	
<i>qFR130</i>	TGTCAAGTCGGAGCACAATCT	

<i>qRV130</i>	AAATTCTTTCCTTGTCGCGGG	Potri.002G122900/ <i>PtAspg1</i>
<i>qFW132</i>	GATGGGCTATAGCGGTGCAT	Potri.014G022900/ <i>PtAspg2</i>
<i>qRV132</i>	CGAAGAGCAGAGATGCCGAT	
<i>qFW133</i>	GAAGCCAAAAGTCCCAAGCG	Potri.014G080600/ <i>PtAspg3</i>
<i>qRV133</i>	CCAGGACCCTTGCGAAGAAT	
<i>qFW134</i>	TTGGAGGTCCCACTGGAATG	Potri.014G191600/ <i>PtAspg4</i>
<i>qRV134</i>	CTTATCGAAGTTTTAGTCGTGCT	
<i>qFW135</i>	GACCAGAGCAGAACGGAACA	Potri.014G191900/ <i>PtAspg5</i>
<i>qRV135</i>	AAAGGCCAGGTGCTCACTAC	
<i>qFW136</i>	TGAGACTCCCAAGGTCTCGT	Potri.001G253300/ <i>AsnAT1</i>
<i>qRV136</i>	TCTGCATCAGAAGTACCTGTC	
<i>qFW213</i>	ACGCATGGTTTGTGGCATTG	Potri.001G162800/ <i>PtAlaAT1</i>
<i>qRV213</i>	TCCTACCACCTTCGAAAGCC	
<i>qFW214</i>	AATAATACACGCCCCCAGGC	Potri.003G072600/ <i>PtAlaAT2</i>
<i>qRV214</i>	TTCAAGGAACTGACGAGCCG	
<i>qFW215</i>	AGTTGCCGATGGACCTAAGC	Potri.008G187400/ <i>PtAlaAT3</i>
<i>qRV215</i>	AAGCCCTTAGAGGAGGTCGT	
<i>qFW216</i>	TGCAAGCTGTTTCGTGATGC	Potri.010G045100/ <i>PtAlaAT4</i>
<i>qRV216</i>	AAGCTTGAGCTTCTTCACCT	
<i>qFW217</i>	GGGGGCAAGAAAAAGGCAC	Potri.001G348600/ <i>PtAO1</i>
<i>qRV217</i>	TGATCTCGAGGAGCAACGG	
<i>qFW218</i>	AGCCAAGTTTGGACTGATCG	Potri.002G236800/ <i>PtAK1</i>
<i>qRV218</i>	CGGACCAGTAAAGTGACCCA	
<i>qFW219</i>	TGATACAGCCATCTCATCGTG	Potri.014G145700/ <i>PtAK2</i>
<i>qRV219</i>	AACAATGGCCCAAAGGTTAGA	
<i>qFW220</i>	TCAAAGCAAGGGCTGTCTGC	Potri.013G099500/ <i>PtHSDH.1</i>
<i>qRV220</i>	TAGGAGAGGTTCCGCTAGGA	
<i>qFW221</i>	CGCGAGCATTTAGTGACGTG	Potri.019G071700/ <i>PtHSDH.2</i>
<i>qRV221</i>	GCTCTCAGTGGTTGAGGGAC	
<i>qFW222</i>	CAGCGGATGTTTTGTCAAGCA	Potri.008G020200/ <i>PtASS1</i>
<i>qRV222</i>	GGCCTCCTGGTGAGAGATTG	
<i>qFW223</i>	TGACAAGCAGGGTACTTGGG	Potri.010G239000/ <i>PtASS2</i>
<i>qRV223</i>	CCACATGCACAAGAATCCGA	
<i>qFWATC</i>	GGTCCTGGACAACATCCAC	Potri.001G357200/ <i>PtATC</i>
<i>qRVATC</i>	GCGGACTGTCTCCCATTAG	