

**Table S1** List of primers used for qRT-PCR analysis

<b>Primers used for tissue, N perturbation and leaf senescence analysis</b>		
<b>Name</b>	<b>Sequence</b>	<b>Access number</b>
PtAspAT-1_qF	GCAGAGGTTGAAGGGTTTGG	Potri.005G079200
PtAspAT-1_qR	TGTAGGGTGGTCAGAGAAGC	
PtAspAT-2_qF	TCCAACCACCATAACATA	Potri.006G107100
PtAspAT-2_qR	CATTCTTGACATCATCCAT	
PtAspAT-3_qF	GGACAATGAGCCCACGAATTG	Potri.006G241500
PtAspAT-3_qR	TCCTCTATTCTCTCCACCACTT	
PtAspAT-4_qF	GTTGCAGGCAGGGTTGAAAG	Potri.006G241600
PtAspAT-4_qR	GGCTCAAAGAGTTTCTGGCG	
PtAspAT5-qFW	TGCACGTGGTATGGAGCTTT	Potri.006G260200
PtAspAT5-qRV	TCGGCAATCCTTTTCAGCT	
PtAspAT-6_qF	TAGCAATGGTGCCAAGCAGA	Potri.007G088400
PtAspAT-6_qR	TCACCCAAAATGGTGCAGGA	
PtAspAT-7_qF	AGCAATCCTCCTGTTTCAT	Potri.014G143300
PtAspAT-7_qR	CAGCCATTACCTTCACTTC	
PtAspAT-9_qF	TCCTCCTGTTTCATGGGGCTA	Potri.018G022200
PtAspAT-9_qR	CCTCCAGCCATCATTCCA	
PtAspAT-10_qF	CACTGGCTTGAACACTGAACA	Potri.018G082500
PtAspAT-10_qR	AAAAGATCCCCAGAAGCGGA	
UBIC_qF	TTGCTTGAGGAACTTGAAC	Potri.006G205700.1
UBIC_qR	GTGAGGACCGATTACAGT	
$\beta$ -Actin_qF	GACCTTCAATGTGCCTGCAA	Potri.019G006700.1
$\beta$ -Actin_qR	ACCATCACCAGAATCCAGCA	
EF1 $\alpha$ _qF	TCCGTCTTCCACTTCAGGATGTCT	Potri.006G130900.1
EF1 $\alpha$ _qR	GTCACGACCATAACCAGGCTTCAG	

<b>Primers used for alternative splicing analysis</b>		
<b>Name</b>	<b>Sequence</b>	<b>Access number</b>
PtAspAT-1.1_qF	ATCAGGCCACAGGTTGCATTA	Potri.005G079200.1
PtAspAT-1.1_qR	TACATTACACAGGGACGGCA	
PtAspAT-1.2_qF	ATCAGGCCACAGGGGTTG	Potri.005G079200.2
PtAspAT-1.2_qR	TACATTACACAGGGACGGCA	
PtAspAT-1.3_qF	AGGCCTTTGCAATGACTGG	Potri.005G079200.3
PtAspAT-1.3_qR	AGTGACGCTTTGGATAAACGA	
PtAspAT-9.1_qF	AGCCAACGGTTTTTCAAGTGT	Potri.018G022200.1
PtAspAT-9.1_qR	AGAGGGATTGCCTTTCTCCT	
PtAspAT-9.2_qF	GTACATCGCACAAGTGGCTC	Potri.018G022200.2
PtAspAT-9.2_qR	TCCAAGCCTCATCTTTCCCT	
PtAspAT-9.3_qF	CCAACGGTTTTTCAAGATGGCT	Potri.018G022200.3
PtAspAT-9.3_qR	TCCAAGCCTCATCTTTCCCT	
PtAspAT-9.4_qF	CCAACGGTTTTTCAAGATGGCT	Potri.018G022200.4
PtAspAT-9.4_qR	ACTTCCAAGCCTCATCTTGAGA	

**Table S2.** List of *AspAT* candidate genes in *Populus*

Gene	Locus v3.0	NCBI accession ID	Chromosome location	ORF (aa)	MW (kDa)	pI	CDS (bp)	Trps	Target
PtAspAT1	Potri.005G079200.1	XP_002307109.2	5829792-5834408	480	51.59	7.11	1443	3	Cp
PtAspAT2	Potri.006G107100.1	XP_024458570.1	8334612-8338470	422	46.96	7.19	1269	2	Mt
PtAspAT3	Potri.006G241500.1	PNT3347.1	25025032-25029627	479	53.31	8.01	1440	2	Cy
PtAspAT4	Potri.006G241600.1	XP_002308618.1	25030792-25035161	449	49.18	9.16	1350	4	Cy
PtAspAT5	Potri.006G260200.1	XP_024459097.1	26353269-26357505	466	51.03	8.67	1401	3	Cp
PtAspAT6	Potri.T079800*	XP_002310582.2	11464519-11467816	481	51.89	8.06	1446	3	Cp
PtAspAT7	Potri.014G143300.1	XP_002321073.3	10931994-10941223	428	47.79	8.35	1287	1	Mt
PtAspAT8	Potri.016G133000.1	PNS99394.1	13652367-13655172	304	33.11	5.66	915	1	Nu, Ex
PtAspAT9	Potri.018G022200.1	XP_002324896.1	1762470-1767522	466	50.92	8.67	1401	4	Cp
PtAspAT10	Potri.018G082500.1	XP_002324997.1	10942388-10947804	407	44.47	7.72	1224	1	Cy

Abbreviations: ORF, open reading frame; aa, amino acid; MW, molecular weight (kDa); pI: isoelectric point; CDS, coding DNA sequence; bp, base pair; Cp, chloroplast; Mt, Mitochondria; Cy, Cytoplasm; Nu, Nucleus; Ex, Extracellular. Note: Parameter of Potri.T079800\* is deduced from both Potri.T079800.1 and Potri.T079800.3.

**Table S3.** Ka/Ks analysis of the *PtAspAT* gene pairs duplication

Gene 1	Gene 2	Ka	Ks	Ka/Ks	Date (MYA)
<i>AspAT1</i>	<i>AspAT6</i>	0.05	0.29	0.17	9.53
<i>AspAT4</i>	<i>AspAT10</i>	0.08	2.28	0.03	1.9
<i>AspAT5</i>	<i>AspAT9</i>	0.02	0.21	0.11	5.99











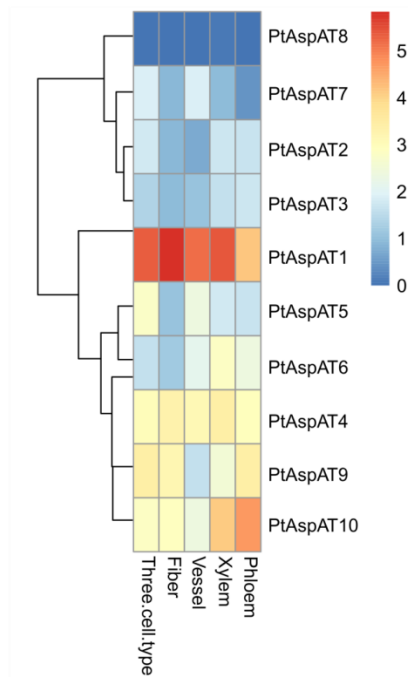




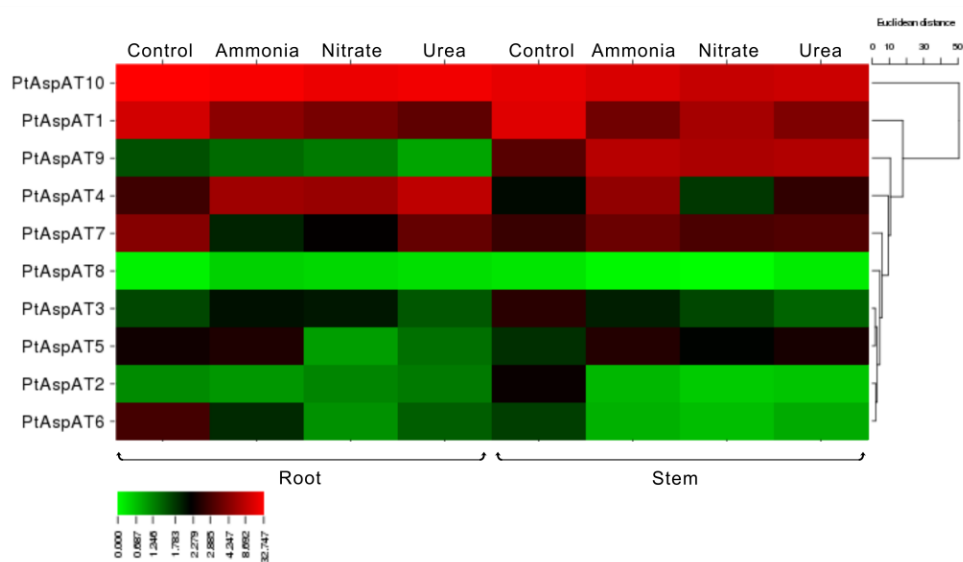
Potri.010G099100	C2H2	PpAspAT8	581	597	+	15.9844	2.22E-06	0.0814	AACGACACGACaaaaa
Potri.010G181000	AP2	PpAspAT8	405	424	+	15.7308	2.32E-06	0.00257	aaagaaaaaanaataaaaaa
Potri.004G056900	Dof	PpAspAT8	483	503	+	14.875	2.43E-06	0.0152	GAATAAAGAGaaaaagaaaaa
Potri.002G114800	AP2	PpAspAT8	1233	1253	-	13.9767	2.59E-06	0.102	GCACACAGTTCCCAAGTATT
Potri.014G074200	MIKC_MADS	PpAspAT8	1577	1597	-	15.5217	2.69E-06	0.00254	CTTTTCTTCTGCTTCTTCCA
Potri.002G151700	MIKC_MADS	PpAspAT8	1577	1597	-	15.5217	2.69E-06	0.00251	CTTTTCTTCTGCTTCTTCCA
Potri.016G112300	MYB	PpAspAT8	93	113	+	15.2278	3.65E-06	0.135	aagaataggataaaaaataca
Potri.014G074100	MIKC_MADS	PpAspAT8	51	69	+	15.1094	3.66E-06	0.0988	tatccaaatagaagaaga
Potri.019G073300	ERF	PpAspAT8	532	546	-	14.7188	3.69E-06	0.0205	ACATGTGTGCGGTGGT
Potri.008G131700	GRAS	PpAspAT8	487	506	+	14.7101	3.93E-06	0.0374	AAGAGaaaaagaaaaa
Potri.004G228800	ARF	PpAspAT8	535	542	+	14.9186	4.42E-06	0.176	ACCGAC
Potri.003G121200	ERF	PpAspAT8	530	544	-	15.3906	4.54E-06	0.18	ATGTGTGCGGTGTAG
Potri.009G055700	MIKC_MADS	PpAspAT8	49	67	-	14.7188	4.79E-06	0.185	TTTTCTTATTTGGGATACT
Potri.016G053200	ERF	PpAspAT8	530	540	+	13.5	5.79E-06	0.0767	CTACCACCGAC
Potri.014G074200	MIKC_MADS	PpAspAT8	480	500	-	14.5652	6.30E-06	0.00466	TTTCTTTTTCTCTTATTCCTC
Potri.002G151700	MIKC_MADS	PpAspAT8	480	500	-	14.5652	6.30E-06	0.00461	TTTCTTTTTCTCTTATTCCTC
Potri.004G065800	TCP	PpAspAT8	617	624	+	15.5192	6.36E-06	0.127	GGCACCA
Potri.010G181000	AP2	PpAspAT8	409	428	+	14.4231	6.60E-06	0.00562	aaaaaataaaaaataata
Potri.001G238400	Dof	PpAspAT8	395	415	-	13.9531	6.82E-06	0.017	TTTTTTTTTCTGATCTTTTTG
Potri.008G131700	GRAS	PpAspAT8	489	508	+	13.9855	7.19E-06	0.039	GAGaaaaagaaaaaaact
Potri.010G181000	AP2	PpAspAT8	486	505	+	14.2692	7.40E-06	0.00562	TAAGAaaaaagaaaaaa
Potri.017G082900	ZF-HD	PpAspAT8	1482	1496	-	13.1618	7.66E-06	0.288	TTTAATFACTAATTA
Potri.002G113300	TALE	PpAspAT8	1579	1598	-	13.48	7.81E-06	0.0501	CCTTTCTCTGCTTCTTC
Potri.008G131700	GRAS	PpAspAT8	481	500	+	13.8116	8.26E-06	0.0393	AGGAATAAGAGaaaaagaa
Potri.014G074200	MIKC_MADS	PpAspAT8	404	424	-	14.2029	8.46E-06	0.0056	TTTTTTTTTATTTTTTCTTG
Potri.002G151700	MIKC_MADS	PpAspAT8	404	424	-	14.2029	8.46E-06	0.00553	TTTTTTTTTATTTTTTCTTG
Potri.010G204800	Dof	PpAspAT8	408	428	-	13.7812	9.02E-06	0.0111	TTTATTTTTTTTATTTTTTTT
Potri.010G101400	BBR-BPC	PpAspAT8	483	506	+	4.82812	9.69E-06	0.0366	GAATAAAGAGaaaaagaaaaa
Potri.014G074200	MIKC_MADS	PpAspAT9	378	398	+	18.3333	7.82E-08	0.000185	tttttttttttttttt
Potri.014G074200	MIKC_MADS	PpAspAT9	379	399	+	18.3333	7.82E-08	0.000185	tttttttttttttttt
Potri.014G074200	MIKC_MADS	PpAspAT9	380	400	+	18.3333	7.82E-08	0.000185	tttttttttttttttt
Potri.014G074200	MIKC_MADS	PpAspAT9	381	401	+	18.3333	7.82E-08	0.000185	tttttttttttttttt
Potri.014G074200	MIKC_MADS	PpAspAT9	382	402	+	18.3333	7.82E-08	0.000185	tttttttttttttttt
Potri.002G151700	MIKC_MADS	PpAspAT9	378	398	+	18.3333	7.82E-08	0.000182	tttttttttttttttt
Potri.002G151700	MIKC_MADS	PpAspAT9	379	399	+	18.3333	7.82E-08	0.000182	tttttttttttttttt
Potri.002G151700	MIKC_MADS	PpAspAT9	380	400	+	18.3333	7.82E-08	0.000182	tttttttttttttttt
Potri.002G151700	MIKC_MADS	PpAspAT9	381	401	+	18.3333	7.82E-08	0.000182	tttttttttttttttt
Potri.002G151700	MIKC_MADS	PpAspAT9	382	402	+	18.3333	7.82E-08	0.000182	tttttttttttttttt
Potri.014G074200	MIKC_MADS	PpAspAT9	383	403	+	17.942	1.48E-07	0.000328	ttttttttttttttttg
Potri.002G151700	MIKC_MADS	PpAspAT9	383	403	+	17.942	1.48E-07	0.000324	ttttttttttttttttg
Potri.010G181000	AP2	PpAspAT9	378	397	-	17.6282	3.71E-07	0.000913	AAAAAAAAAAAAAAAAAAAAA
Potri.010G181000	AP2	PpAspAT9	379	398	-	17.6282	3.71E-07	0.000913	AAAAAAAAAAAAAAAAAAAAA
Potri.010G181000	AP2	PpAspAT9	380	399	-	17.6282	3.71E-07	0.000913	AAAAAAAAAAAAAAAAAAAAA
Potri.010G181000	AP2	PpAspAT9	381	400	-	17.6282	3.71E-07	0.000913	AAAAAAAAAAAAAAAAAAAAA
Potri.010G181000	AP2	PpAspAT9	382	401	-	17.6282	3.71E-07	0.000913	AAAAAAAAAAAAAAAAAAAAA
Potri.010G181000	AP2	PpAspAT9	383	402	-	17.6282	3.71E-07	0.000913	AAAAAAAAAAAAAAAAAAAAA
Potri.002G113300	TALE	PpAspAT9	532	551	-	18.3333	3.73E-07	0.0064	GTCCGCCCCCTCCCGGGT
Potri.002G113300	TALE	PpAspAT9	530	549	-	18.2667	3.92E-07	0.0064	TCCGCCCCCTCCCGGGT
Potri.002G113300	TALE	PpAspAT9	533	552	-	17.9333	4.99E-07	0.0064	TGTCCGCCCCCTCCCGG
Potri.001G188800	HD-ZIP	PpAspAT9	1280	1294	-	16.7385	6.39E-07	0.0119	ATATGTAATGATGAC
Potri.001G238400	Dof	PpAspAT9	54	74	+	15.6406	1.01E-06	0.00474	TTCTCTATTTTACTTTATG
Potri.002G113300	TALE	PpAspAT9	534	553	-	16.3867	1.42E-06	0.0137	TTGTCCGCCCCCTCCCGG
Potri.010G204800	Dof	PpAspAT9	378	398	+	15.3438	2.11E-06	0.00516	tttttttttttttttt
Potri.010G204800	Dof	PpAspAT9	379	399	+	15.3438	2.11E-06	0.00516	tttttttttttttttt
Potri.010G204800	Dof	PpAspAT9	380	400	+	15.3438	2.11E-06	0.00516	tttttttttttttttt
Potri.010G204800	Dof	PpAspAT9	381	401	+	15.3438	2.11E-06	0.00516	tttttttttttttttt
Potri.010G204800	Dof	PpAspAT9	382	402	+	15.3438	2.11E-06	0.00516	tttttttttttttttt
Potri.014G074200	MIKC_MADS	PpAspAT9	384	404	+	15.7391	2.18E-06	0.00216	tttttttttttttttga
Potri.002G151700	MIKC_MADS	PpAspAT9	384	404	+	15.7391	2.18E-06	0.00214	tttttttttttttttga
Potri.010G181000	AP2	PpAspAT9	384	403	-	15.7179	2.34E-06	0.00257	CAAAAAAAAAAAAAAAAAAAAA
Potri.005G139700	bHLH	PpAspAT9	406	414	-	15.1702	3.19E-06	0.127	CCCACGTGA
Potri.001G015900	EIL	PpAspAT9	350	370	+	15.4051	3.21E-06	0.127	TTGTCTAGGTAAGGTCAAT
Potri.015G023200	ERF	PpAspAT9	534	554	-	12.8824	3.46E-06	0.0196	CTTGTCGCCCCCTCCCGG
Potri.001G188800	HD-ZIP	PpAspAT9	1727	1741	-	15.2769	3.52E-06	0.0437	ATATGTAATGATGGC
Potri.010G137600	bHLH	PpAspAT9	407	421	+	15.4375	3.59E-06	0.143	caegtgggtgtcgg
Potri.007G036400	Dof	PpAspAT9	53	73	+	14.1875	3.75E-06	0.014	TTTCTCTATTTTACTTTAT
Potri.014G074200	MIKC_MADS	PpAspAT9	961	981	-	15.058	4.12E-06	0.0038	TTTTATTTTTTCTTTGCTT
Potri.002G151700	MIKC_MADS	PpAspAT9	961	981	-	15.058	4.12E-06	0.00375	TTTTATTTTTTCTTTGCTT
Potri.005G207200	bHLH	PpAspAT9	406	413	+	15.9559	4.42E-06	0.176	tcacgtgg
Potri.015G106900	LFY	PpAspAT9	452	470	+	13.2059	6.00E-06	0.119	caeggccaagtgaatcc
Potri.015G106900	LFY	PpAspAT9	452	470	-	13.2059	6.00E-06	0.119	GGATGTCACGTGGCCGTG
Potri.T044100	TCP	PpAspAT9	162	169	-	15.7212	6.36E-06	0.0843	GACCCCA
Potri.014G066500	bHLH	PpAspAT9	406	413	-	14.8191	6.57E-06	0.262	CCACGTGA
Potri.006G084200	Dof	PpAspAT9	60	74	+	13.7344	7.29E-06	0.068	TATTTTACTTTATG
Potri.009G064700	bHLH	PpAspAT9	1925	1933	-	15.1489	7.36E-06	0.293	GCCACTTGT
Potri.003G207200	bHLH	PpAspAT9	1925	1933	-	15.1489	7.36E-06	0.293	GCCACTTGT
Potri.002G149000	LBD	PpAspAT9	533	551	-	13.9219	7.61E-06	0.301	GTTCGCCCCCTCCCGG
Potri.005G001800	bHLH	PpAspAT9	406	415	-	14.8594	7.82E-06	0.312	CCACGTGA
Potri.010G204800	Dof	PpAspAT9	960	980	-	13.9375	7.88E-06	0.0109	TTTATTTTTTCTTTGCTT
Potri.010G204800	Dof	PpAspAT9	961	981	-	13.9219	7.99E-06	0.0109	ttttttttttttttttg
Potri.002G113300	TALE	PpAspAT9	531	550	-	13.16	9.25E-06	0.0509	TTCCGCCCCCTCCCGGGT

**Table S5.** Conserved motifs of AspATs deduced by MEME analysis

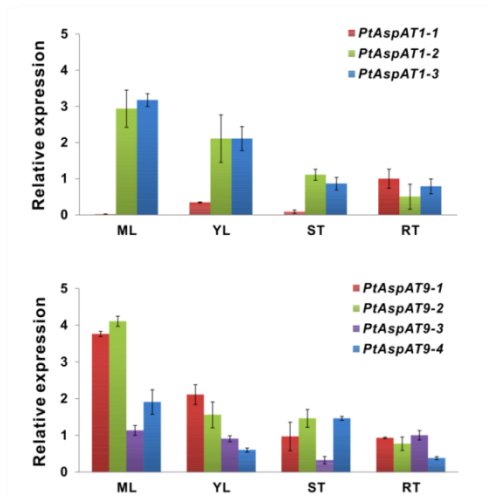
Name	E-value	Sites	Length	MEME best matched sequences	AA Residues	Function of AA residues
Motif 1	1.10E-185	8	50	TYRYYPKTKGLDFQGLLDDIKAAPSGSFVLLHACAHNPTGVDPTPEQWE	N	PLP binding site
Motif 2	3.30E-154	7	50	WSHILKQIGMFTFTGLNPEQVDFMTKEYHIYMTSDGRISMAGLSSKTVPY	Unknown	Unknown
Motif 3	2.20E-114	7	41	RVKSQKLIARPMYSNPPVHGASIVAAAILGDPDLFNEWTV	G I	homodimer interface
Motif 4	5.90E-153	7	50	PFFDVAYQGFASGSLDADAQSVRMFVADGGELLVAQSYAKNMGLYGERVG	Y S AK G[N]ER	PLP binding site; catalytic residue; Homodimer interface
Motif 5	1.80E-151	8	50	IKENRVATVQCLSGTGLRLGAEFLARYYPQSQIYIPSPTWGNHHNIFRD	GTG W	Homodimer interface; PLP binding site
Motif 6	2.30E-145	8	50	PAPEDPILGVTEAFLADPSPKKNLGVGAYRTEEGKPVVNLNVVRKAEQLL	Unknown	Unknown
Motif 7	2.60E-30	7	21	KAMADRIISMQRKQKFDALSAR	Unknown	Unknown
Motif 8	9.80E-32	7	21	PIEGLAAFNKLSAQLLFGADS	Unknown	Unknown
Motif 9	5.30E-20	2	50	HPRLLVLSDEIYEHIYAPATHISFASLPGMWERTLTVNGFSKAFAMTGW	Unknown	specific for I $\beta$ members, Unknown
Motif 10	1.40E-17	2	50	KEENGISYKPDQILVSNQAKQSIYQAILAVCSPGDEVIIPAPFVVSYPEM	Unknown	specific for I $\beta$ members, Unknown
Motif 11	7.90E-16	10	11	LNILCSADVA	Unknown	Unknown
Motif 12	1.40E-13	2	50	VPVIRLAAGEPDFDTPVIAEAGINAIREGFTRYTPNAGTQELRVAICQK	Unknown	specific for I $\beta$ members, Unknown
Motif 13	3.50E-13	2	50	FYLFIDFSSYYGAEVEFGKIEDSDALCRYLLDQAQVALVPGVAFGDDSC	Unknown	specific for I $\beta$ members, Unknown
Motif 14	1.30E-09	2	50	QEILKGKARLGSVSTLNFNKEKGNPFIKKSFGRISMVAVNVSRFEGI	Unknown	specific for I $\alpha$ -C AspAT, Unknown
Motif 15	1.30E-08	2	50	HCFYFKPLEATRRSQLSRISVVVKAESRSEEMQLDISLSPRVNAVKPSKT	Unknown	specific for I $\beta$ members, Unknown



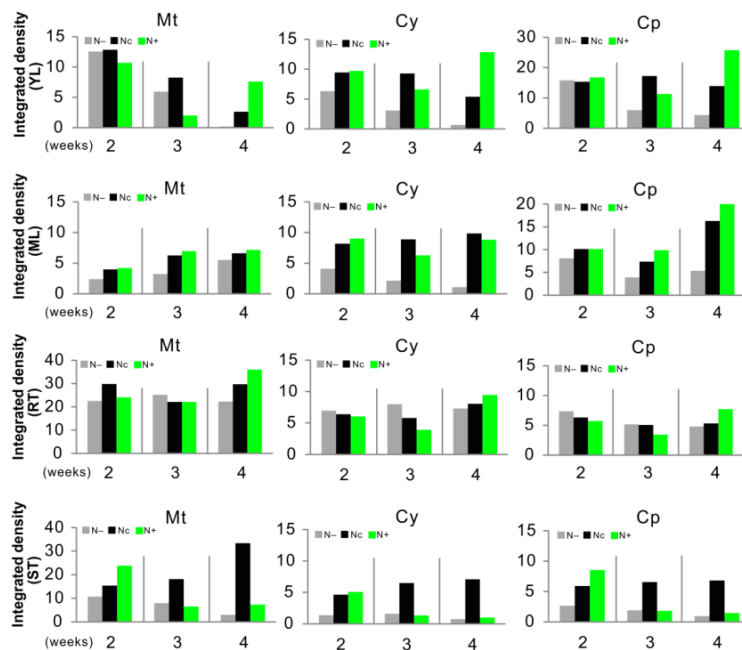
**Figure S1.** Transcriptomic analysis of *PtAspATs* in vascular tissues. Data was collected from GEO Datasets (GSE81077), including phloems, stem differentiating xylems, vessels, fibers, and a mixture of three cell types (fibers, vessels, and ray cells). FPKM (fragments per kilobase per million reads) values of *PtAspAT* genes were transformed by  $\log_2$  and displayed in heat map.



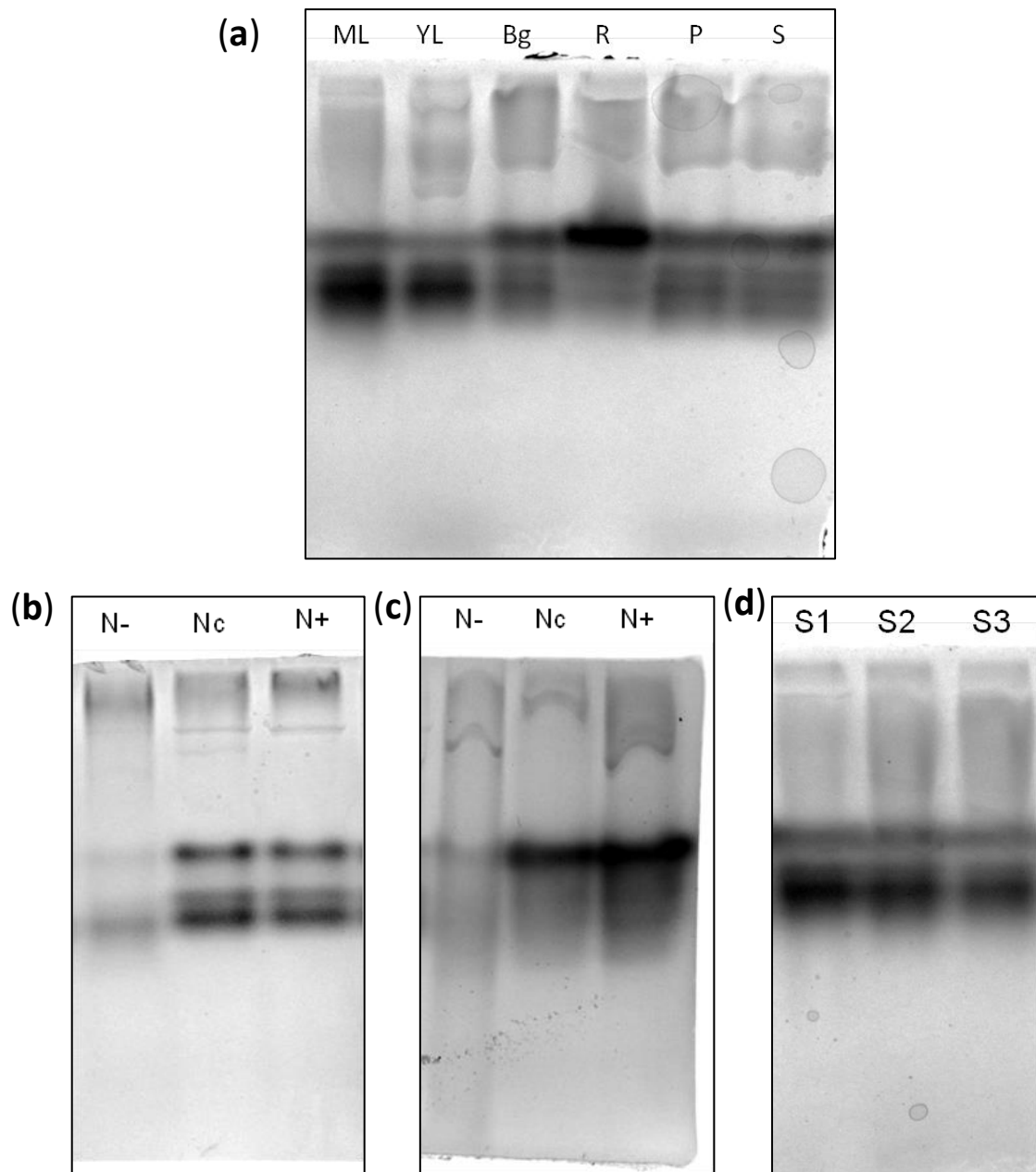
**Figure S2.** Transcriptomic analysis of *PtAspATs* in response to various nitrogen resources. The RNA-seq results were given in FPKM (fragments per kilobase per million reads) expression values.



**Figure S3.** Expression analysis of the transcript variants of *PtAspAT1* and *PtAspAT9* in different tissues. Expression was evaluated by qRT-PCR. Samples were harvested from ML (mature leaves), YL (young leaves), RT (roots) and ST (stems). Data represent mean values  $\pm$ SE of at least three independent biological replicates for qRT-PCR. *PtActin*, *PtUBIC*, and *PtEF1 $\alpha$*  were used as reference genes.



**Figure S4.** Effects on enzyme activities in various tissues of 'Nanlin895'. Enzyme activities were assayed by in-gel analyses under 0 mM (N-), 2 mM (Nc) and 10 mM (N+) nitrogen treatments for 2-4 weeks. Samples were harvested from YL (young leaves), ML (mature leaves), RT (roots), and ST (stems). Mt, Cy, and Cp indicate mitochondria, cytosol and chloroplast, respectively.



**Figure S5.** The entire native PAGE gel images showing AspAT activities that were compatible with Figure 8. (a) Gel activities from different tissues (b) N treated plant leaves (c) N treated plant roots (d) seasonal senescent leaves from poplar plants.