

Supplementary materials to:

Water uptake and hormone modulation responses to nitrogen supply in *Populus simonii* under drought stress

Supplementary materials and methods

Antioxidant enzyme activities

To analyze the activities of antioxidant enzymes in roots or leaves, soluble proteins were extracted as described previously. The activity of SOD was determined according to the method of (Becana et al., 1986). One unit of SOD was defined as the amount of enzyme that caused a 50% decrease in the SOD-inhibited nitrobluetetrazolium reduction at 550 nm. The activities of POD, CAT, and APX were measured as described by (Ekmekci and Terzioglu, 2005) (Kato and Shimizu, 1987) (He et al., 2011).

- Becana, M., P. Aparicio-Tejo, J.J. Irigoyen, and M. Sanchez-Diaz. 1986. Some enzymes of hydrogen peroxide metabolism in leaves and root nodules of *Medicago sativa*. *Plant physiology* 82:1169-1171.
- Ekmekci, Y., and S. Terzioglu. 2005. Effects of oxidative stress induced by paraquat on wild and cultivated wheats. *Pesticide Biochemistry and Physiology* 83:69-81.
- He, J.L., J.J. Qin, L.Y. Long, Y.L. Ma, H. Li, K. Li, X.N. Jiang, T.X. Liu, A. Polle, Z.S. Liang, and Z.B. Luo. 2011. Net cadmium flux and accumulation reveal tissue-specific oxidative stress and detoxification in *Populus x canescens*. *Physiologia plantarum* 143:50-63.
- Kato, M., and S. Shimizu. 1987. Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves; phenolic-dependent peroxidative degradation. *Canadian Journal of Botany* 65:729-735.

Supplementary Table S1. Primers used for qRT-PCR.

Gene model	Gene Name	Closest AGI	Primer-Forward (5'-3')	Primer-Reverse(5'-3')	PCR efficiency (%)
Potri.008G065600	<i>PIP1;2</i>	AT4G00430	GCCATTTGATGAAGTTTGTTC	GCACGCGCTTGCTTATAGGT	104
Potri.009G136600	<i>PIP2;1</i>	AT4G35100	GATCGGATTATGATGGACCTTTCT	AAACTACCCGTGACAATGATACACA	97
Potri.019G030900	<i>SIP1;2</i>	AT3G04090	GTTGGCTATCATGGAGGTGATG	TGCAAGTCCACCTGCAAAGTA	95
Potri.006G121700	<i>TIP1;6</i>	AT2G36830	ACTGGTGGCCTGGAAACCT	CACGATCTCGAGAACGAAAGC	103
Potri.001G186700	<i>TIP2;1</i>	AT3G16240	CCTCTTGTTGGTGGTGGGATT	TCCGGACAAAGGAGTATGATCA	98
Potri.010G044500	<i>TAA1</i>	AT1G70560	TTGGCGAAGGTTGTAGGACT	ATCTCGTGGGTACTTTGGCA	101
Potri.002G254200	<i>YUCCA1</i>	AT4G28720	CAGCAATGTCCCCTATTGGC	GAGACCGGCATTTTCCTTTCC	94
Potri.008G174600	<i>YUCCA3</i>	AT1G04610	GACCTGTCATAGTTGGGGCT	GCGATCATAGGTGCGGTTTT	103
Potri.001G393800	<i>NCED3</i>	AT4G18350	AACTTGCTCGGTCGAAAGAC	ACAGGACACCAGGCTATACCA	94
Potri.009G037300	<i>PP2C</i>	AT1G07430	GACAAGGCGTGCTCTG	CCCCTTAACCTTATCACTCA	102
	<i>Actin2/7</i>		CCCATTGAGCACGGTATTGT	TACGACCACTGGCATAACAGG	97

Supplementary Table S2. Growth parameters of *P.simonii* as affected by experimental drought and nitrogen supply.

N supply (mM)	Drought treatment	Root biomass (mg DW)	Total fine root length (m)	Total fine root surface area (cm ²)	Total root volume(cm ³)	Aerial biomass (mg DW)	Chlorophyll l content (SPAD)	ΔH(cm)
0.01	Control	5.6±0.4a	13.6±0.7a	57.6±2.3a	12.7±0.5a	7.5±0.4a	24.6±1.8a	28.3±1.4a
	Drought	2.3±0.2c	5.8±0.4b	29.5±1.1c	4.2±0.3b	4.6±0.6b	12.7±0.6b	11.7±0.6b
1	Control	4.2±0.2b	11.8±0.6a	40.3±1.4b	11.7±0.6a	7.1±0.4a	25.5±2.3a	25.5±1.8a
	Drought	2.6±0.2c	6.8±0.3b	31.4±1.2c	4.0±0.2b	5.2±0.3b	17.3±1.0b	11.1±0.7b
P-values	N	***	ns	***	ns	ns	ns	ns
	Drought	*	***	***	***	***	***	***
	Drought× N	**	*	***	ns	ns	ns	ns

Data indicate mean± SE (n=6). Different letters in the same column indicate significant difference. P-values of the ANOVAs of drought, nitrogen supply, and their interaction are indicated. *P< 0.05; **P< 0.01; *** P< 0.001; ns, not significant.

Supplementary Figure S1. The typical root state of *P.simonii* as affected by experimental drought and nitrogen supply.



Supplementary Table S3. The concentrations of 17 free amino acids in fine roots and leaves of *P.simonii* as affected by experimental drought and nitrogen supply.

		Asp	Lys	Ile	Met	Thr	Ser	Cys	Gly	Pro	Glu	His
Root	0.01 mM	0.10	0.06	0.01	0.00	0.04	0.06	0.01	0.05	0.04	0.17	0.02
		0.08	0.06	0.03	0.01	0.05	0.04	0.01	0.05	0.03	0.15	0.01
		0.11	0.04	0.03	0.01	0.06	0.06	0.01	0.07	0.04	0.17	0.01
	0.01 mM +PEG	0.10	0.13	0.05	0.01	0.06	0.03	0.01	0.07	0.12	0.07	0.03
		0.10	0.15	0.05	0.01	0.06	0.04	0.02	0.05	0.11	0.08	0.02
		0.11	0.12	0.04	0.02	0.05	0.04	0.01	0.04	0.11	0.05	0.02
	1 mM	0.16	0.10	0.02	0.01	0.05	0.09	0.02	0.08	0.09	0.23	0.04
		0.21	0.07	0.02	0.01	0.03	0.08	0.03	0.07	0.08	0.25	0.03
		0.22	0.11	0.05	0.02	0.05	0.11	0.03	0.08	0.09	0.23	0.04
	1 mM +PEG	0.30	0.28	0.11	0.01	0.16	0.10	0.02	0.08	0.18	0.25	0.06
		0.27	0.25	0.13	0.01	0.14	0.13	0.03	0.09	0.18	0.26	0.04
		0.31	0.25	0.11	0.01	0.17	0.14	0.02	0.12	0.19	0.26	0.06
Leaves	0.01 mM	0.32	0.22	0.16	0.02	0.32	0.16	0.02	0.01	0.20	0.40	0.07
		0.32	0.23	0.18	0.03	0.34	0.15	0.02	0.02	0.22	0.38	0.07
		0.30	0.25	0.18	0.03	0.32	0.12	0.03	0.02	0.19	0.38	0.09
	0.01 mM +PEG	0.39	0.23	0.16	0.04	0.34	0.16	0.04	0.20	0.17	0.40	0.08
		0.37	0.23	0.21	0.02	0.32	0.21	0.03	0.23	0.15	0.41	0.09
		0.37	0.25	0.15	0.02	0.31	0.17	0.04	0.21	0.17	0.37	0.08
	1 mM	0.42	0.20	0.16	0.12	0.20	0.16	0.03	0.02	0.16	0.12	0.03
		0.38	0.18	0.22	0.15	0.25	0.16	0.03	0.03	0.17	0.11	0.05

	0.38	0.21	0.17	0.16	0.23	0.15	0.04	0.05	0.13	0.08	0.05
1 mM +PEG	0.51	0.43	0.34	0.09	0.25	0.44	0.04	0.32	0.22	0.62	0.11
	0.63	0.48	0.39	0.09	0.31	0.48	0.03	0.35	0.28	0.65	0.12
	0.55	0.47	0.37	0.10	0.33	0.49	0.03	0.33	0.27	0.64	0.12

Supplementary Table S3. Continued

		Arg	Tyr	Phe	Ala	Val	Leu
Root	0.01 mM	0.05	0.02	0.06	0.06	0.03	0.05
		0.04	0.02	0.05	0.06	0.04	0.05
		0.04	0.02	0.05	0.08	0.02	0.06
	0.01 mM +PEG	0.05	0.03	0.06	0.06	0.03	0.06
		0.03	0.03	0.07	0.07	0.04	0.08
		0.04	0.02	0.06	0.07	0.03	0.06
	1 mM	0.10	0.03	0.08	0.10	0.03	0.07
		0.10	0.02	0.08	0.10	0.04	0.07
		0.12	0.03	0.09	0.08	0.03	0.11
	1 mM +PEG	0.13	0.03	0.09	0.12	0.13	0.10
		0.13	0.03	0.08	0.11	0.09	0.16
		0.12	0.04	0.08	0.11	0.07	0.12
Leaves	0.01 mM	0.18	0.12	0.21	0.20	0.14	0.13
		0.17	0.10	0.22	0.23	0.16	0.17
		0.15	0.09	0.24	0.19	0.17	0.16

0.01 mM +PEG	0.18	0.09	0.20	0.18	0.13	0.13
	0.14	0.08	0.18	0.21	0.12	0.14
	0.19	0.08	0.20	0.19	0.18	0.18
1 mM	0.02	0.19	0.42	0.23	0.03	0.35
	0.03	0.24	0.41	0.26	0.03	0.42
	0.02	0.27	0.43	0.24	0.07	0.38
1 mM +PEG	0.29	0.15	0.30	0.31	0.27	0.46
	0.28	0.14	0.27	0.30	0.28	0.42
	0.25	0.15	0.32	0.32	0.30	0.49

Supplementary Table S4. PCA of morphological and physiological responses of *P. simonii* as affected

by experimental drought and nitrogen supply. In addition to the two-PC plots in Figure 9, the three dimensional PC plots were also presented here.

Variables	PC1	PC2	PC3	PC4	PC5	PC6
Root biomass	-0.045	0.016	0.044	0.096	0.122	-0.014
Leaf biomass	-0.038	0.045	0.133	0.121	0.087	0.052
Height increment	-0.046	0.029	0.006	0.030	-0.047	0.021
Total root length	-0.047	0.022	0.029	-0.001	0.047	-0.029
Total root surface area	-0.040	0.033	0.078	0.095	0.155	-0.094
Total root volume	-0.039	0.007	-0.016	0.142	-0.034	0.059
Net photosynthetic rate (A)	-0.047	0.033	0.048	0.000	0.015	-0.026
Stomatal conductance (g_s)	-0.049	-0.008	-0.003	-0.033	-0.034	0.012
Transpiration rate (E)	-0.047	0.032	-0.032	-0.004	-0.025	-0.015
Chlorophyll content	-0.040	-0.012	0.077	-0.162	-0.042	0.069
RWC	-0.045	-0.013	0.000	0.025	-0.017	-0.003
Water use efficiency(WUE)	0.020	0.104	-0.069	0.012	-0.020	-0.242
Electrolyte leakage	0.013	0.042	0.140	-0.224	0.062	0.163
Root C concentrations	-0.043	-0.016	-0.017	0.062	-0.089	0.133
Root $\Delta^{13}C$	0.011	-0.048	-0.073	0.018	0.471	-0.165
Leaf C concentrations	0.012	0.002	0.043	-0.267	0.342	-0.210
Leaf $\Delta^{13}C$	0.003	-0.033	0.109	-0.219	-0.167	-0.394
Root N concentrations	-0.016	-0.107	-0.075	0.098	-0.072	-0.002
Root $\Delta^{15}N$	0.044	0.055	-0.003	-0.010	0.006	-0.073
Leaf N concentrations	-0.016	-0.105	-0.121	-0.125	-0.062	-0.092
Leaf $\Delta^{15}N$	0.043	0.034	0.093	0.079	0.080	0.083
Root O ₂ - concentrations	-0.001	-0.030	-0.232	0.163	0.221	0.161
Leaf O ₂ - concentrations	0.034	0.058	-0.137	0.090	-0.132	-0.080
Root MDA	0.041	-0.002	-0.101	0.052	0.036	0.173
Leaf MDA	0.038	0.015	-0.134	-0.073	-0.034	-0.002
Root SOD	-0.016	0.017	-0.049	-0.303	0.142	0.445
Leaf SOD	0.039	-0.034	0.161	0.038	0.015	0.040
Root POD	0.028	-0.077	0.132	0.104	0.116	0.108
Leaf POD	0.033	-0.081	0.082	0.009	0.068	0.147
Root APX	0.019	-0.074	0.134	-0.009	-0.221	0.221
Leaf APX	0.037	0.035	-0.022	0.091	0.003	0.037
Root CAT	0.022	-0.093	0.065	0.105	0.088	-0.152
Leaf CAT	0.021	-0.063	0.182	0.132	-0.052	-0.128
Root soluble sugar	0.048	-0.009	-0.013	-0.010	-0.013	0.100
Leaf soluble sugar	0.047	-0.032	-0.016	-0.027	-0.027	0.052

Root soluble phenolics	0.043	0.035	0.040	0.028	0.027	-0.027
Leaf soluble phenolics	0.025	0.096	-0.003	-0.044	-0.066	0.079
Root IAA	0.010	0.122	0.084	0.088	0.046	0.001
Leaf IAA	-0.013	0.111	0.137	0.080	0.071	0.097
Root ABA	0.045	0.023	0.087	0.013	0.018	0.040
Leaf ABA	0.035	0.082	-0.092	-0.031	-0.096	-0.058
Proportion of variation (%)	49.27	18.24	6.75	4.98	3.81	3.08

