

Supplementary materials

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Table S1. Two-way ANOVA of Ct' value of each gene expression at different time points after low-nitrogen stress in shoots of the two barley cultivars

Source	Gene	SS	df	MS	F	Sig.
Cultivar	<i>HvNRT3.1</i>	0.216	1	0.216	0.379	0.551
	<i>HvNRT3.3</i>	0.038	1	0.038	0.040	0.846
	<i>HvNIA2</i>	0.678	1	0.678	1.217	0.294
	<i>HvNiR1</i>	1.144	1	1.144	1.384	0.259
	<i>HvGS1_1</i>	1.252E-05	1	1.252E-05	0.000	0.992
	<i>HvGS2</i>	1.786	1	1.786	5.373	0.034
	<i>HvGLU2</i>	0.818	1	0.818	0.459	0.508
	<i>HvASN1</i>	0.330	1	0.330	1.214	0.303
Time Point	<i>HvNRT3.1</i>	4.884	3	1.628	2.852	0.086
	<i>HvNRT3.3</i>	2.543	3	0.848	0.893	0.478
	<i>HvNIA2</i>	2.591	3	0.864	1.549	0.257
	<i>HvNiR1</i>	6.916	3	2.305	2.789	0.079
	<i>HvGS1_1</i>	5.197	3	1.732	16.334	0.001
	<i>HvGS2</i>	1.185	3	0.395	1.188	0.346
	<i>HvGLU2</i>	3.451	3	1.150	0.646	0.597
	<i>HvASN1</i>	7.391	3	2.464	9.070	0.006
Cultivar × Time Point	<i>HvNRT3.1</i>	1.499	3	0.500	0.875	0.483
	<i>HvNRT3.3</i>	0.216	2	0.108	0.114	0.894
	<i>HvNIA2</i>	0.849	3	0.283	0.507	0.685
	<i>HvNiR1</i>	1.363	3	0.454	0.549	0.657
	<i>HvGS1_1</i>	1.248	3	0.416	3.922	0.048
	<i>HvGS2</i>	2.286	3	0.762	2.293	0.117
	<i>HvGLU2</i>	13.271	3	4.424	2.483	0.098
	<i>HvASN1</i>	0.012	1	0.012	0.043	0.841
Total	<i>HvNRT3.1</i>	338.511	19			
	<i>HvNRT3.3</i>	285.484	17			
	<i>HvNIA2</i>	274.413	19			
	<i>HvNiR1</i>	45.332	22			
	<i>HvGS1_1</i>	330.869	17			
	<i>HvGS2</i>	367.074	24			
	<i>HvGLU2</i>	264.552	24			
	<i>HvASN1</i>	197.599	14			

Table S2. Two-way ANOVA of Ct' value of each gene expression at different time points after low-nitrogen stress in roots of the two barley cultivars

Source	Gene	SS	df	MS	F	Sig.
Cultivar	<i>HvNRT2.2</i>	15.224	1	15.224	35.243	0.000
	<i>HvNRT2.3</i>	0.107	1	0.107	0.258	0.619
	<i>HvNRT3.1</i>	2.086	1	2.086	13.396	0.002
	<i>HvNRT3.3</i>	3.366	1	3.366	26.148	0.000
	<i>HvNIA2</i>	1.152	1	1.152	2.392	0.143
	<i>HvNiR1</i>	4.625	1	4.625	14.021	0.002
	<i>HvGS1_1</i>	2.241	1	2.241	4.824	0.045
	<i>HvGS2</i>	1.480	1	1.480	13.763	0.002
	<i>HvGLU2</i>	6.497	1	6.497	26.644	0.000
	<i>HvASN1</i>	4.484	1	4.484	3.096	0.109
Time Point	<i>HvNRT2.2</i>	84.236	3	28.079	65.001	0.000
	<i>HvNRT2.3</i>	67.433	3	22.478	54.418	0.000
	<i>HvNRT3.1</i>	6.630	3	2.210	14.190	0.000
	<i>HvNRT3.3</i>	4.575	3	1.525	11.847	0.000
	<i>HvNIA2</i>	36.998	3	12.333	25.603	0.000
	<i>HvNiR1</i>	14.831	3	4.944	14.988	0.000
	<i>HvGS1_1</i>	11.715	3	3.905	8.408	0.002
	<i>HvGS2</i>	23.116	3	7.705	71.633	0.000
	<i>HvGLU2</i>	4.255	3	1.418	5.817	0.008
	<i>HvASN1</i>	5.916	3	1.972	1.362	0.310
Cultivar × Time Point	<i>HvNRT2.2</i>	0.531	3	0.177	0.410	0.748
	<i>HvNRT2.3</i>	2.369	3	0.790	1.912	0.174
	<i>HvNRT3.1</i>	1.229	3	0.410	2.629	0.086
	<i>HvNRT3.3</i>	2.243	3	0.748	5.808	0.007
	<i>HvNIA2</i>	3.246	3	1.082	2.246	0.125
	<i>HvNiR1</i>	0.282	3	0.094	0.285	0.836
	<i>HvGS1_1</i>	0.641	3	0.214	0.460	0.715
	<i>HvGS2</i>	0.272	3	0.091	0.844	0.491
	<i>HvGLU2</i>	0.421	3	0.140	0.576	0.640
	<i>HvASN1</i>	3.971	3	1.324	0.914	0.469
Total	<i>HvNRT2.2</i>	411.789	23			
	<i>HvNRT2.3</i>	351.726	22			
	<i>HvNRT3.1</i>	82.016	24			
	<i>HvNRT3.3</i>	70.878	24			
	<i>HvNIA2</i>	466.910	23			
	<i>HvNiR1</i>	61.300	23			
	<i>HvGS1_1</i>	636.533	22			
	<i>HvGS2</i>	68.875	23			
	<i>HvGLU2</i>	511.137	23			
	<i>HvASN1</i>	656.328	18			

Table S3. Two-way ANOVA of Ct' value of each gene expression at different time points after low-nitrogen stress between shoots and roots of BI-04

Source	Gene	SS	df	MS	F	Sig.
Tissue	<i>HvNRT3.1</i>	175.118	1	175.118	1034.642	0.000
	<i>HvNRT3.3</i>	141.376	1	141.376	291.690	0.000
	<i>HvNIA2</i>	1.734	1	1.734	5.858	0.030
	<i>HvNiR1</i>	35.283	1	35.283	93.812	0.000
	<i>HvGS1_1</i>	1.975	1	1.975	6.151	0.029
	<i>HvGS2</i>	162.114	1	162.114	1759.644	0.000
	<i>HvGLU2</i>	32.016	1	32.016	134.911	0.000
	<i>HvASN1</i>	26.347	1	26.347	30.617	0.000
Time Point	<i>HvNRT3.1</i>	4.370	3	1.457	8.607	0.002
	<i>HvNRT3.3</i>	1.701	3	0.567	1.170	0.359
	<i>HvNIA2</i>	7.689	3	2.563	8.658	0.002
	<i>HvNiR1</i>	13.085	3	4.362	11.597	0.000
	<i>HvGS1_1</i>	6.404	3	2.135	6.648	0.007
	<i>HvGS2</i>	10.938	3	3.646	39.573	0.000
	<i>HvGLU2</i>	6.933	3	2.311	9.738	0.001
	<i>HvASN1</i>	4.284	3	1.428	1.659	0.233
Tissue × Time Point	<i>HvNRT3.1</i>	0.812	3	0.271	1.599	0.234
	<i>HvNRT3.3</i>	0.840	3	0.280	0.578	0.640
	<i>HvNIA2</i>	2.812	3	0.937	3.167	0.058
	<i>HvNiR1</i>	0.216	3	0.072	0.191	0.901
	<i>HvGS1_1</i>	0.520	3	0.173	0.540	0.664
	<i>HvGS2</i>	4.588	3	1.529	16.599	0.000
	<i>HvGLU2</i>	1.569	3	0.523	2.204	0.127
	<i>HvASN1</i>	8.815	3	2.938	3.414	0.057
Total	<i>HvNRT3.1</i>	210.110	22			
	<i>HvNRT3.3</i>	177.900	21			
	<i>HvNIA2</i>	405.440	22			
	<i>HvNiR1</i>	61.898	23			
	<i>HvGS1_1</i>	461.833	20			
	<i>HvGS2</i>	202.271	24			
	<i>HvGLU2</i>	425.956	24			
	<i>HvASN1</i>	541.360	19			

Table S4. Two-way ANOVA of Ct' value of each gene expression at different time points after low-nitrogen stress between shoots and roots of BI-45

Source	Gene	SS	df	MS	F	Sig.
Tissue	<i>HvNRT3.1</i>	188.150	1	188.150	382.090	0.000
	<i>HvNRT3.3</i>	162.322	1	162.322	402.346	0.000
	<i>HvNIA2</i>	1.218	1	1.218	1.586	0.232
	<i>HvNiR1</i>	22.584	1	22.584	29.060	0.000
	<i>HvGS1_1</i>	7.624	1	7.624	23.275	0.001
	<i>HvGS2</i>	154.557	1	154.557	424.735	0.000
	<i>HvGLU2</i>	4.255	1	4.255	2.250	0.154
	<i>HvASN1</i>	8.528	1	8.528	8.303	0.024
Time Point	<i>HvNRT3.1</i>	8.222	3	2.741	5.566	0.011
	<i>HvNRT3.3</i>	7.107	3	2.369	5.872	0.009
	<i>HvNIA2</i>	9.236	3	3.079	4.010	0.034
	<i>HvNiR1</i>	9.035	3	3.012	3.875	0.033
	<i>HvGS1_1</i>	10.090	3	3.363	10.268	0.002
	<i>HvGS2</i>	6.822	3	2.274	6.249	0.006
	<i>HvGLU2</i>	2.742	3	0.914	0.483	0.699
	<i>HvASN1</i>	3.819	3	1.273	1.239	0.365
Tissue × Time Point	<i>HvNRT3.1</i>	0.327	3	0.109	0.221	0.880
	<i>HvNRT3.3</i>	0.045	2	0.023	0.056	0.946
	<i>HvNIA2</i>	17.133	3	5.711	7.438	0.004
	<i>HvNiR1</i>	1.668	3	0.556	0.715	0.559
	<i>HvGS1_1</i>	0.729	3	0.243	0.742	0.549
	<i>HvGS2</i>	4.722	3	1.574	4.325	0.022
	<i>HvGLU2</i>	9.881	3	3.294	1.742	0.201
	<i>HvASN1</i>	0.062	1	0.062	0.060	0.813
Total	<i>HvNRT3.1</i>	210.417	21			
	<i>HvNRT3.3</i>	178.461	20			
	<i>HvNIA2</i>	335.883	20			
	<i>HvNiR1</i>	44.733	22			
	<i>HvGS1_1</i>	505.568	19			
	<i>HvGS2</i>	233.678	23			
	<i>HvGLU2</i>	349.733	23			
	<i>HvASN1</i>	312.567	13			

Table S5. Ct' value of gene expression at different time points after low-nitrogen stress and the significant difference analysis in shoots and roots of the two barley cultivars

Cultivar	tissue	gene	Time			
			0h	1h	24h	48h
BI-04	shoots	<i>HvNRT3.1</i>	2.5242	3.2238	4.0189	3.7157
		<i>HvNRT3.3</i>	2.5150	2.7862	1.8412	4.2263
		<i>HvNIA2</i>	3.9454	4.4565	3.6706	3.4719
		<i>HvNiR1</i>	0.3571a	0.3204ab	-1.5360c	-1.4461bc
		<i>HvGS1_1</i>	5.1318a	4.2449b	3.8322b	4.2746b
		<i>HvGS2</i>	-2.8683a	-3.8355b	-3.9644b	-3.6570b
		<i>HvGLU2</i>	3.9071a	2.3470b	2.3832b	2.6935b
		<i>HvASN1</i>	3.5239ab	2.9935a	4.8000bc	5.0842c
	roots	<i>HvNRT2.2</i>	6.7723a	5.9227a	3.2888b	1.8972b
		<i>HvNRT2.3</i>	4.7880a	5.7318b	1.8953c	2.2047c
		<i>HvNRT3.1</i>	-1.0198a	-1.0565a	-1.7425b	-1.8126b
		<i>HvNRT3.3</i>	-1.0148	-0.9863	-1.3063	-1.4467
		<i>HvNIA2</i>	5.7547a	4.9258a	3.5062b	3.7656b
		<i>HvNiR1</i>	2.7222a	2.0690ab	1.2638bc	0.8654c
		<i>HvGS1_1</i>	6.2142a	4.7159ab	4.6027b	4.5166b
		<i>HvGS2</i>	2.9248a	2.2228b	1.1785c	0.1406d
		<i>HvGLU2</i>	5.5853a	4.4208b	4.7993ab	5.7652a
		<i>HvASN1</i>	5.2107	7.0003	6.6396	7.1538
BI-45	shoots	<i>HvNRT3.1</i>	2.9189	4.3348	3.6058	3.5165
		<i>HvNRT3.3</i>	ND	4.3781	3.5497	3.9017
		<i>HvNIA2</i>	4.0654	4.1348	3.5576	3.5701
		<i>HvNiR1</i>	-0.8519	-0.8786	-1.3155	-1.8846
		<i>HvGS1_1</i>	5.4732a	4.2707b	4.3229ab	3.4097b
		<i>HvGS2</i>	-4.1740	-3.9364	-3.7611	-4.6361
		<i>HvGLU2</i>	2.3878	4.1788	4.2006	2.0410
		<i>HvASN1</i>	3.0834	2.6925	ND	ND
	roots	<i>HvNRT2.2</i>	5.0655a	4.2191a	1.2984b	0.7303b
		<i>HvNRT2.3</i>	6.1106a	5.3524a	1.5773b	2.1455b
		<i>HvNRT3.1</i>	-0.9174a	-1.8583ab	-2.8721c	-2.3423bc
		<i>HvNRT3.3</i>	-0.8259a	-1.8644b	-2.8286c	-2.2312bc
		<i>HvNIA2</i>	6.5658a	4.2340b	2.5597b	2.7860b
		<i>HvNiR1</i>	2.1342a	1.2808ab	0.1203bc	-0.2347c
		<i>HvGS1_1</i>	6.9357a	5.9080ab	4.9494b	4.8490b
		<i>HvGS2</i>	2.3697a	1.5854a	0.4742b	-0.0106b
		<i>HvGLU2</i>	4.5295	3.7097	3.7679	4.2733
		<i>HvASN1</i>	5.2287	4.5014	6.1026	6.1170

Different letters represent significant differences of the related gene expression among different time points at 0.05 level in shoots and root respectively of BI-04 and Bi-45. ND indicates that expression was not detectable.

Table S6. Ct' value of gene expression at different time points and significant difference analysis between shoots and roots of the two barley cultivars

Cultivar	Gene	Tissue	Time			
			0h	1h	24h	48h
BI-04	<i>HvNRT3.1</i>	shoots	2.5242a	3.2238a	4.0189a	3.7157a
		roots	-1.0198b	-1.0565b	-1.7425b	-1.8126b
	<i>HvNRT3.3</i>	shoots	2.5150a	2.7862a	1.8412a	4.2263a
		roots	-1.0148b	-0.9863b	-1.3063b	-1.4467b
	<i>HvNIA2</i>	shoots	3.9454	4.4565	3.6706	3.4719
		roots	5.7547	4.9258	3.5062	3.7656
	<i>HvNiR1</i>	shoots	0.3571a	0.3204a	-1.5360a	-1.4461a
		roots	2.7222b	2.0690b	1.2638b	0.8654b
	<i>HvGS1_1</i>	shoots	5.1318	4.2449	3.8322	4.2746
		roots	6.2142	4.7159	4.6027	4.5166
	<i>HvGS2</i>	shoots	-2.8683a	-3.8355a	-3.9644a	-3.6570a
		roots	2.9248b	2.2228b	1.1785b	0.1406b
	<i>HvGLU2</i>	shoots	3.9071a	2.3470a	2.3832a	2.6935a
		roots	5.5853b	4.4208b	4.7993b	5.7652b
	<i>HvASN1</i>	shoots	3.5239	2.9935a	4.8000	5.0842
		roots	5.2107	7.0003b	6.6396	7.1538
BI-45	<i>HvNRT3.1</i>	shoots	2.9189a	4.3348a	3.6058a	3.5165a
		roots	-0.9174b	-1.8583b	-2.8721b	-2.3423b
	<i>HvNRT3.3</i>	shoots	ND	4.3781a	3.5497a	3.9017a
		roots	-0.8259	-1.8644b	-2.8286b	-2.2312b
	<i>HvNIA2</i>	shoots	4.0654a	4.1348	3.5576	3.5701
		roots	6.5658b	4.2340	2.5597	2.7860
	<i>HvNiR1</i>	shoots	-0.8519a	-0.8786	-1.3155	-1.8846a
		roots	2.1342b	1.2808	0.1203	-0.2347b
	<i>HvGS1_1</i>	shoots	5.4732a	4.2707	4.3229	3.4097
		roots	6.9357b	5.9080	4.9494	4.8490
	<i>HvGS2</i>	shoots	-4.1740a	-3.9364a	-3.7611a	-4.6361a
		roots	2.3697b	1.5854b	0.4742b	-0.0106b
	<i>HvGLU2</i>	shoots	2.3878a	4.1788	4.2006	2.0410a
		roots	4.5295b	3.7097	3.7679	4.2733b
	<i>HvASN1</i>	shoots	3.0834	2.6925	ND	ND
		roots	5.2287	4.5014	6.1026	6.1170

Different letters represent significant differences of the related gene expression between shoots and roots at 0.05 level in BI-04 and BI-45 respectively. ND indicates that the expression was not detectable.