

Supplementary Material

Genetic diversity for root plasticity and nitrogen uptake in wheat seedlings

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Table S1. Oligonucleotide primer sequences used for quantitative real time RT-PCR analysis

Primer name	Forward primer	Reverse primer
TaNPF6.3	CAY GGG AGC AAC GAC GGC TG	ATG CGT TTC TCC TTG TAC ACG TAG
TaNRT2.1	GCT GCT CWT AGT TGT GAG TGY AAC	TGA AGG ACT TGG CCT TGT GCT C
TaNRT2.2	GCA ACG TCA TCC TTG CCA CRG	AAA CAT ACT TGC GGA TTT CAG GCG
TaCyclophilin	CAA GCC GCT GCA CTA CAA GG	AGG GGA CGG TGC AGA TGA A
TaEFA α	CAG ATT GGC AAC GGC TAC G	CGG ACA GCA AAA CGA CCA AG
TaActin	GAC AAT GGA ACC GGA ATG GTC	GTG TGA TGC CAG ATT TTC TCC AT

Table S2. Wheat seedling root growth in response to external N supply - Figure 2 data presented in table format

Root dry weight, RDW; Total root length, RL; Total root tip number, RTN; Specific root length, SRL; Total root volume (RV) and Root surface area, RSA of 17 day old wheat seedlings. Plants were grown in potting soil treated with urea (+; 56 mg N kg⁻¹ soil) or without urea (-; 15 mg N kg⁻¹ soil).

Gladius-07	+		-	
	Mean	s.e.m.	Mean	s.e.m.
RDW	24.5	3.6	32.2	3.3
RL	457.6	58.1	720.4	66.7
RTN	363.7	41.5	552.3	30.4
SRL	18.8	0.5	22.4	0.3
RV	0.6	0.1	0.7	0.1
RSA	52.7	7.1	71.2	7.8

Kukri-99	+		-	
	Mean	s.e.m.	Mean	s.e.m.
RDW	26.79	4.05	35.04	3.33
RL	532.48	82.53	830.13	90.46
RTN	438.33	55.93	539.33	70.24
SRL	19.97	1.08	23.63	0.36
RV	0.53	0.08	0.76	0.08
RSA	51.90	8.02	77.86	7.77

RAC875	+		-	
	Mean	s.e.m.	Mean	s.e.m.
RDW	28.62	5.31	33.98	5.11
RL	627.17	112.38	808.92	127.91
RTN	502.00	91.24	585.00	38.00
SRL	21.99	0.27	23.74	0.88
RV	0.64	0.19	0.84	0.04
RSA	61.02	13.50	80.25	8.75

Inia-66	+		-	
	Mean	s.e.m.	Mean	s.e.m.
RDW	19.14	3.62	27.47	4.78
RL	427.29	74.59	835.72	142.11
RTN	323.00	54.08	562.67	65.36
SRL	22.51	0.62	30.44	0.69
RV	0.45	0.09	0.57	0.10
RSA	42.64	8.11	68.27	11.77

Jupateco-73	+		-	
	Mean	s.e.m.	Mean	s.e.m.
RDW	24.91	2.96	32.20	4.64
RL	509.95	73.81	755.24	164.63
RTN	371.33	49.19	476.67	99.15
SRL	20.33	0.66	22.93	1.96
RV	0.57	0.04	0.61	0.15
RSA	53.22	5.52	67.61	15.61

Ciano-79	+		-	
	Mean	s.e.m.	Mean	s.e.m.
RDW	21.36	2.99	24.03	3.42
RL	457.87	53.07	636.28	82.20
RTN	374.67	62.74	396.67	45.72
SRL	21.61	0.67	26.58	0.51
RV	0.48	0.04	0.50	0.06
RSA	45.82	4.46	55.67	7.00

Opata-85	+		-	
	Mean	s.e.m.	Mean	s.e.m.
RDW	20.38	6.53	19.68	2.56
RL	261.95	85.70	593.90	48.25
RTN	243.67	65.03	452.33	21.50
SRL	17.37	7.28	30.55	1.52
RV	0.22	0.07	0.37	0.05
RSA	23.86	7.34	46.62	5.47

Table S3. Variation of growth related traits in wheat seedlings. Figure 4 data presented in table format

The percentage of explained variation attributed to the genotype (G), treatment (T, +N; 56 mg N kg⁻¹ soil or -N; residual 15 mg N kg⁻¹ soil) and genotype by treatment interactions (GT) for each of the traits was derived from an ANOVA analysis using the mean averaged across all time points (7, 9, 13 and 17 days) of seedling development. Values displayed are the mean sum of squares for each variation (G, T or GT) as a percentage of the total mean sum of squares (G + T + GT). N content (mg N plant⁻¹), N concentration (mg N g DW⁻¹), C content (mg C plant⁻¹) and C concentration (mg C g DW⁻¹), total plant biomass (Plant DW, g), shoot biomass (Shoot DW, g); root biomass (Root DW, g); ratio of root to shoot dry weight (R:S); total root length (RL, cm), total RSA (cm²), total root volume (RV, cm³), total root tip number (RTN), rate of root tip development (Rate RT, root tip number h⁻¹) and specific root length (SRL, cm mg⁻¹)

Trait	Genotype	Treatment	GT
N content	16%	74%	10%
N concentration	4%	90%	6%
C content	39%	37%	24%
C concentration	26%	25%	49%
Plant DW	95%	2%	2%
Root DW	30%	66%	4%
Shoot DW	71%	25%	4%
R:S	3%	93%	4%
RL	15%	83%	2%
RSA	27%	71%	2%
RV	55%	43%	3%
RTN	29%	67%	4%
Rate RT	74%	18%	8%
SRL	24%	60%	16%

Table S4. Nitrate influx and N translocation of wheat genotypes with contrasting N responsiveness - Figure 6 data presented in table format

Unidirectional NO₃⁻ influx rates of 14-day-old (14 DAS) and 20-day-old (DAS) Kukri-99 and Inia-66 seedlings grown at either 5 mM K¹⁴NO₃⁻ (HN) or 0.2 mM K¹⁴NO₃⁻ (LN). ¹⁵N content of roots and shoots was measured after exposing roots to external N at 0.1 mM or 1 mM KNO₃ with 10% ¹⁵N label over a 10 min incubation period. High-affinity nitrate uptake capacity (HAT) and low-affinity nitrate uptake capacity (LAT) are shown. Low-affinity nitrate uptake capacity was calculated by subtracting nitrate influx at 0.1 mM from the nitrate influx at 1 mM K¹⁵NO₃⁻. The % ¹⁵N translocated to the shoot was calculated by the equation: (¹⁵N shoot/(¹⁵N root + ¹⁵N shoot))*100

Inia-66	14 DAS				20 DAS			
	HN		LN		HN		LN	
	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.
HAT	47.61	2.42	35.97	2.25	53.09	2.08	65.24	1.68
LAT	9.69	2.63	8.72	2.63	10.81	2.63	5.43	2.63
% ¹⁵N translocated	39.33	1.91	33.00	1.90	43.20	1.32	38.17	1.30

Kukri-99	14 DAS				20 DAS			
	HN		LN		HN		LN	
	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.
HAT	47.80	2.38	43.29	1.84	57.52	1.13	65.00	0.98
LAT	9.69	2.63	5.78	2.63	5.78	2.63	9.54	2.63
% ¹⁵N translocated	41.83	2.93	37.00	1.82	40.20	1.32	42.67	1.15

*HAT and % ¹⁵N translocated means ± s.e.m., n = 6. LAT means ± s.e.d, n = 24, see text for equation of LATS s.e.d.

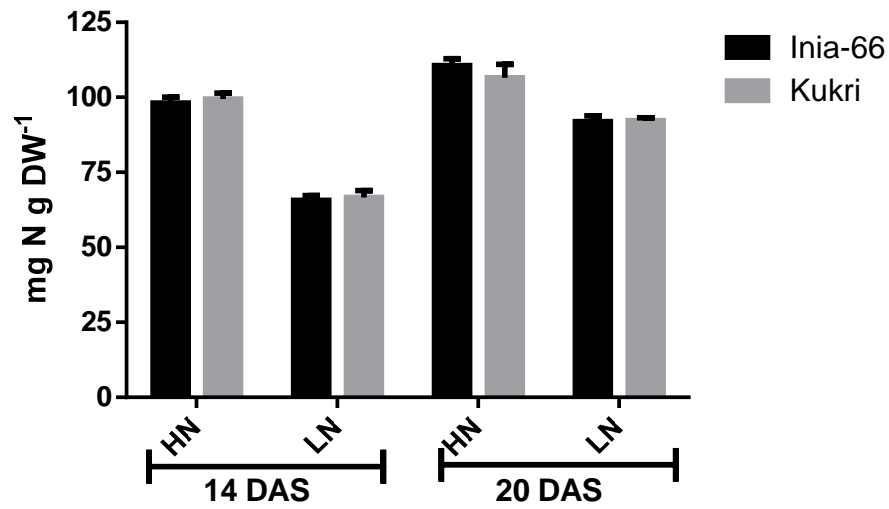


Fig. S1. Nitrogen concentration of wheat seedlings grown in a hydroponics growth system. 14-day-old (14 DAS) and 20-day-old (DAS) Kukri-99 and Inia-66 seedlings were grown at either 5 mM KNO₃ (HN) or 0.2 mM KNO₃ (LN). Values are means \pm s.e.m. and $n = 12$.