Supplementary Material

Nitrogen application at anthesis increases barley grain protein by enhancing phloem amino acid mobilisation

J. J. Boero[A], *F. H. Gutierrez Boem*[A,B], *P. Prystupa*[A,B], *C. G. Veliz*[B], *M. V. Criado*[A,B], *F. M. Gomez*[A,B], *and C. Caputo*[A,B,*]

[A]Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina.

[B]CONICET – Universidad de Buenos Aires, Instituto de Investigaciones en Biociencias Agrícolas y Ambientales (INBA), Avenida San Martín 4453, C1417DSE, Buenos Aires, Argentina.

[*]Correspondence to: C. Caputo Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina Email: caputo@agro.uba.ar

Supplementary Table S1. Temperature (in °C) and precipitation (in mm) recorded from the weather station nearest to each of the three sites (within 10 km) during the crop cycle.

Site	25 de mayo		Arribeños		Junín	
Month	Temp	Precipitation	Temp	Precipitation	Temp	Precipitation
June	7.9	19	11.8	17	8.8	53
July	8.6	35	11.6	26	9.0	27
Aug	11.2	44	13.1	78	11.8	31
Sept	12.5	40	13.9	65	13.3	47
Oct	15.5	125	16.5	141	16.5	146
Nov	19.0	55	19.9	21	19.4	47
Dec	22.6	97	23.2	67	22.9	101

Supplementary Table S2. Names, accession numbers, and primer sequences of the genes used in the real time qPCR analysis. Primers of the amino acid transporters (AAP) were obtained from Kohl et al. (2012), those for the protease from Parrott et al. (2007; 2010) and those for glutamine synthetase (GS) from Goodall et al. (2013).

Genes	Names	Acc. No.	Forward (5`→3`)	Reverse (5`→3`)
AAP	*HvAAP6*	HE965468	TCGGCAACGTGGTGGGACTT	GCCGCGCTGCCTGATGTA
	HvAAP7	HE965469	CAGGGGGTCATCAGCCAGAAGC	ATGCTCCCCGATCGCTCTAACC
Protease	*HvSAG12*	AK366134	ACGAGGAGCGAGCTATCATT	GACCATTGTACACGCCATTC
GS	*HvGS1_1*	JX878489	GGACCGTCGGTGATGGGG	AAGACGAGAACGAGAAGAGAGACCAGAC
	HvGS1_2	JX878490	CACTTTGGGCAGGCTCTCGTCTC	CAGACTAGACCTTGCAATTGCAAAAGAAAC
	HvGS2	AK360336	AAGCTGGCGCTGAAGGTATGAAGG	GACGGAACCACAGGATCAACAAGAATG
Ref. genes	*HvActin*	AY145451	GTATGGAAACATCGTGCTCAGTGG	CTTGATCTTCATGCTGCTCGGA
	HvTEF	Z50789	AGGTCCACCAACCTTGACTG	CAACAGGCACAGTTCCAATG

Reference

Goodall AJ, Kumar P, Tobin AK (2013) Identification and expression analyses of cytosolic glutamine synthetase genes in barley (*Hordeum vulgare* L.). Plant and Cell Physiology 54, 492–505. https://doi.org/10.1093/pcp/pct006

Supplementary Table S3. Three-way ANOVA for aboveground biomass and spike weight of the control, Na20 and Na40 treatments in relation with the site and sampling time and their interactions. Mean values of each variable are shown in Suppl. Fig. 1 B and D.

	Biomass DW	Spike FW
Site	<0.01	<0.01
Fert.	ns	ns
Time	<0.01	<0.01
Site x Fert.	ns	ns
Site x Time	ns	<0.01
Fert. x Time	ns	ns
Site x Fert. x Time	ns	ns

DW, dry weight; FW, fresh weight; Fert., Fertilization treatment.

Supplementary Figure S1. Aboveground biomass (A) and harvest index (Hi) (C) at harvest of the control, Na20, Na40, Ne and NSe treatments, average aboveground dry weight (DW) (B) and average of spike fresh weight (FW) (C) of control, Na20 and Na40 during grain filling. Data represent the means ± SE. In A and C, n = 4, and in B and D n=12. The inset in Fig A and C shows the *p*-values of the main effects of the two-way ANOVA performed (s, site; F, fertilization; s x F; site x fertilization interaction). Data on B and D are presented as the average value of all treatments for each site, since neither a fertilization effect alone nor any interaction with site or time was observed (Supplementary Table 3). DPA, days post anthesis.