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Supplementary Materials

Nitrogen–sulfur fertilisation effects on gluten composition and industrial quality in Argentinean bread wheat cultivars differing in apparent sulfur recovery

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Supplementary material

Table S1. Results of the soil analysis in each experiment (E1, E2) for the variables: pH (1:2.5 soil:water), organic matter (Walkley-Black), phosphorus (Bray-Kurtz I), Nitrate N (Reflectometry), and Sulfate S (Turbidimetry).

Soil samples were collected at sowing from depths of 0-20 and 20-40 cm as appropriate. In experiment 2, separate analysis of N and S were carried out for the long (LC) and short (SC) cycle genotypes due to the delay in the sowing date of the latter.

Soil depth (cm)	pH		Organic matter (%)		Phosphorus (μg g^{-1})		Nitrate N (kg N ha^{-1})			Sulfate S (mg S kg^{-1} soil)		
	E1	E2	E1	E2	E1	E2	E1	E2		E1	E2	
								LC	SC		LC	SC
0-20	6.9	5.7	5.9	4.1	8.6	6.4	20	6.1	8.8	13.4	8.3	13.2
20-40	-	-	-	-	-	-	22.8	9	5.9	17.4	8.4	14.2

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Table S2. Grain yield (13% humidity, g m^{-2}) mean values for the treatments without N and S (N0S0), without N and with S (N0S1), with N and without S (N1S0) and with N and S (N1S1), for long cycle (LC) and short cycle (SC) genotypes in each experiment (E1, E2). Different letters indicate significant differences for the CxNxS interaction within each experiment.

Grain yield (g m^{-2})									
Experiment 1									
	Long Cycle					Short Cycle			
Genotype	N0S0	N0S1	N1S0	N1S1	Genotype	N0S0	N0S1	N1S0	N1S1
304	280	375	397	489	601	262	329	353	392
BI2	309	316	408	448	801	303	397	387	449
BI3	281	310	383	394	BI1	352	297	411	367
AGU	295	349	397	380	MEJ	234	317	404	339
CHC	304	351	454	469	CAS	260	262	325	381
LIQ	306	290	386	365	FLE	268	291	355	317
CAP	292	259	382	334	PRO	249	247	341	340
GAV	344	301	365	383	TAU	333	266	432	510
JAB	309	256	370	338	CHU	268	285	356	346
TOR	272	265	388	387	CON	387	374	429	461
Overall mean	299 b	307 b	393 a	399 a		292 b	307 b	379 a	390 a

Experiment 2									
	Long Cycle					Short Cycle			
Genotype	N0S0	N0S1	N1S0	N1S1	Genotype	N0S0	N0S1	N1S0	N1S1
304	179	171	281	414	801	206	216	245	290
601	253	255	338	435	FAS	245	281	337	428
BI3	223	232	328	488	CHJ	284	324	345	451
127	199	204	269	477	GAV	226	265	298	401
100	236	248	251	404	PRO	221	267	330	419
Overall mean	218 e	222 e	293 c	443 a		236 de	271 cd	311 c	398 b

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Table S3. Summary of ANOVA for the ratio between contents of N and S in grain, protein content (Pro%), sedimentation test (SDSS), dough strength (W), tenacity (P) and extensibility (L), including the proportion of the sum of squares explained (%) and the significance level of the source of variation considered (C: Cycle, G: Genotype, N: Nitrogen, S: Sulfur) for each experiment (E1, E2).

	N/S				Pro%				SDSS				W				P				L			
SV	E1		E2		E1		E2		E1		E2		E1		E2		E1		E2		E1		E2	
	SS%	Sig	SS%	Sig	SS%	Sig	SS%	Sig	SS%	Sig	SS%	Sig	SS%	Sig	SS%	Sig	%SS	Sig	SS%	Sig	SS%	Sig	SS%	Sig
C	6.7	***	2.5	**	7.9	***	2.9	***	5	***	0.4	ns	12.3	***	1.7	**	6.6	***	0.2	ns	7	***	2.6	***
G	32.5	***	5	*	13.2	***	9.6	***	58.8	***	54.5	***	48.3	***	46.8	***	66	***	33.1	***	35.6	***	31.5	***
N	29.1	***	36.9	***	68.2	***	76.9	***	5.1	***	0.5	*	24.3	***	24.8	***	0.1	ns	17.1	***	22.5	***	3.5	***
S	0	ns	29.5	***	0.3	*	0.8	**	0	ns	9.2	***	0	ns	0.6	*	0.7	**	24.6	***	1.7	**	28.6	***
C*N	1	*	0.4	ns	3.1	***	0.4	*	0.8	**	0.1	ns	0	ns	0.7	*	0.6	**	2.7	**	3.3	***	0.2	ns
C*S	0.2	ns	1.1	*	0.2	ns	0	ns	0.3	*	0.9	**	0	ns	4.1	**	0.2	ns	0	ns	1.8	**	0.5	ns
G*N	12	***	1.8	ns	2.8	**	4.5	***	20.2	***	4.8	**	6.3	***	3.5	**	7.5	***	3.8	*	6.6	**	2	ns
G*S	7.1	**	2.7	ns	1.5	ns	0.8	ns	3.2	***	2.2	*	3.6	***	3.5	**	6.6	***	1.2	ns	6	**	1.8	ns
N*S	0.3	ns	15.4	***	0.2	ns	1.2	**	0.9	**	18.5	***	0.1	ns	2.6	***	0.1	ns	10.9	***	0.1	ns	20.9	***
C*N*S	0.1	ns	0.1	ns	0.1	ns	0.1	ns	0.1	ns	0.9	*	0.2	*	3.2	***	0.5	*	0.1	ns	0.1	ns	0.3	ns
G*N*S	5.3	ns	1.4	ns	0.5	ns	0.8	ns	3.6	***	3.6	**	1.4	ns	1.9	ns	2.6	*	2	ns	3.9	ns	2.2	ns

*, ** and *** correspond to $p < 0.05$, $p < 0.01$ and $p < 0.0001$; respectively. C: Cycle, G: Genotype, N: Nitrogen, S: Sulfur. Type I SS.

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Table S4. Protein content (Pro%, %) mean values for the treatments without N and S (N0S0), without N and with S (N0S1), with N and without S (N1S0) and with N and S (N1S1), for long cycle (LC) and short cycle (SC) genotypes in each experiment (E1, E2).

Protein content (Pro%) (%)									
Experiment 1									
	Long Cycle					Short Cycle			
Genotype	N0S0	N0S1	N1S0	N1S1	Genotype	N0S0	N0S1	N1S0	N1S1
304	9.87	9.99	13.37	14.42	601	12.15	12.76	14.73	14.78
BI2	9.63	10.79	12.7	13.81	801	11.1	10.96	14.37	13.53
BI3	9.07	9.74	13.3	13.44	BI1	10.62	11.39	13.43	13.37
AGU	9.12	10.19	14	14.43	MEJ	12.36	12.66	15.9	15.85
CHC	10.85	10.13	13.51	12.47	CAS	11.21	11.51	14.45	14.56
LIQ	10.72	11.56	14.85	15.96	FLE	10.94	11.62	13.37	13.05
CAP	10.67	10.97	14	13.29	PRO	13.21	13.16	16.63	16.42
GAV	10.01	9.9	13.83	13.82	TAU	11.73	11.4	13.1	12.73
JAB	10.24	10.14	13.8	14.72	CHU	11.98	12.42	14.29	13.72
TOR	10.08	10.38	13.48	14.04	CON	11.2	11.69	13.54	13.78

Experiment 2									
	Long Cycle					Short Cycle			
Genotype	N0S0	N0S1	N1S0	N1S1	Genotype	N0S0	N0S1	N1S0	N1S1
304	12.2	13.83	15.26	16.06	801	11.76	11.66	14.96	15.45
601	11.57	10.66	15.13	16.04	FAS	11.04	10.85	15.46	17.72
BI3	10.02	9.8	14.72	15.53	CHJ	11.6	11.1	14.22	15.02
127	9.69	9.81	14.15	14.35	GAV	12.25	11.72	14.56	15.01
100	9.72	9.47	13.96	15.69	PRO	12.35	12.29	16.25	17.96

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Table S5. Summary of ANOVA for the ratio between contents of gliadins and glutenins (GLI/GLU), the ratio between contents of high molecular weight and low molecular weight glutenin subunits (HMW/LMW) and the ratio between ω-gliadins and α-β-γ-gliadins (ω-gli/α-β-γ-gli), including proportion of the sum of squares explained (%) and the significance level of the source of variation considered (C: Cycle, G: Genotype, N: Nitrogen, S: Sulfur) for each experiment (E1, E2).

	GLI/GLU				HMW/LMW				ω-gli/α-β-γ-gli			
	E1		E2		E1		E2		E1		E2	
SV	%SS	Sig	%SS	Sig	%SS	Sig	%SS	Sig	%SS	Sig	%SS	Sig
C	19.1	***	2.7	**	0.8	**	18.4	***	1	**	4.9	***
G	64.3	***	24.7	**	83.5	***	60.4	***	70.2	***	23.7	***
N	2.8	***	12.7	***	1.5	***	5.6	***	16.1	***	46.1	***
S	0	ns	19.3	***	0	ns	3.1	***	0	ns	2.7	***
C*N	0.1	ns	2.3	**	1.2	***	0	ns	0	ns	1.7	**
C*S	0.1	ns	2.1	*	0.7	**	0.8	**	0.3	ns	1	**
G*N	4	**	3	ns	2.1	*	2	**	4.4	**	6.5	***
G*S	5.2	**	4.7	ns	5.1	***	2.3	**	2.5	ns	4.6	**
N*S	0.1	ns	19.1	***	0.1	ns	1.3	**	0.5	*	1.4	**
C*N*S	0.3	ns	0	ns	0.4	*	0.5	*	0.2	ns	0.2	ns
G*N*S	1.8	ns	1.1	ns	1.8	*	1.1	ns	1.7	ns	4.2	**

*. ** and *** correspond to $p < 0.05$. $p < 0.01$ and $p < 0.0001$; respectively. C: Cycle. G: Genotype. N: Nitrogen. S: Sulfur. Type I SS.

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Table S6. Preliminary (a) and final (b) eigenvectors corresponding to each variable included in the biplot of Figure 3

a) Variables	e1	e2
N/S	0.35	0.12
Pro%	0.26	0.24
SDSS	0.41	0.10
W	0.43	0.05
P	0.20	0.01
L	0.38	0.15
GLI/GLU	-0.17	0.06
HMW/LMW	0.09	0.51
GLU-A1/HMW	-0.03	0.02
GLU-B1x/HMW	0.05	-0.17
GLU-B1y/HMW	0.08	0.14
GLU-D1x/HMW	-0.24	0.08
GLU-D1y/HMW	0.14	-4.3E-03
GLU-A3/LMW	-0.27	0.29
GLU-B3/LMW	0.22	-0.49
GLU-D3/LMW	-0.08	0.48
ω-gli/α-β-γ-gli	0.14	-0.15

b) Variables	e1	e2
N/S	0.39	-0.06
Pro%	0.28	-0.24
SDSS	0.44	-0.08
W	0.42	-0.03
L	0.43	-0.12
HMW/LMW	0.13	-0.51
GLU-D1x/HMW	-0.23	-0.06
GLU-A3/LMW	-0.30	-0.33
GLU-B3/LMW	0.22	0.53
GLU-D3/LMW	-0.06	-0.50

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Table S7. Preliminary (a) and final (b) eigenvectors corresponding to each variable included in the biplot of Figure 4

a) Variables	e1	e2
N/S	0.23	0.13
Pro%	0.14	-0.16
SDSS	0.25	0.37
W	0.38	0.23
P	0.29	0.33
L	0.04	-0.28
GLI/GLU	-0.20	0.05
HMW/LMW	-0.26	0.22
GLU-A1/HMW	1.2E-03	-0.18
GLU-B1x/HMW	0.12	0.16
GLU-B1y/HMW	-0.04	-0.04
GLU-D1x/HMW	-0.24	-0.24
GLU-D1y/HMW	0.17	0.36
GLU-A3/LMW	-0.30	0.26
GLU-B3/LMW	0.39	-0.31
GLU-D3/LMW	-0.33	0.25
ω-gli/α-β-γ-gli	0.26	-0.24

b) Variables	e1	e2
N/S	0.27	0.08
SDSS	0.28	0.35
W	0.38	0.25
P	0.29	0.38
L	0.03	-0.31
GLI/GLU	-0.23	0.02
HMW/LMW	-0.26	0.18
GLU-D1x/HMW	-0.22	-0.24
GLU-D1y/HMW	0.18	0.38
GLU-A3/LMW	-0.31	0.29
GLU-B3/LMW	0.40	-0.35
GLU-D3/LMW	-0.34	0.28
ω-gli/α-β-γ-gli	0.24	-0.22

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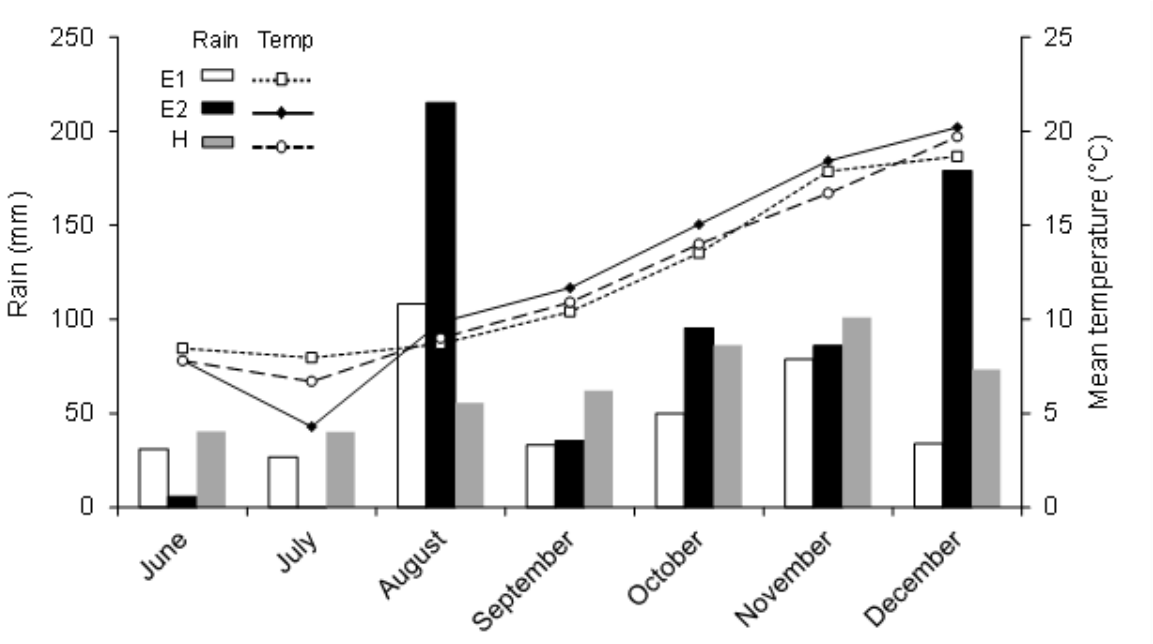

Figure S1. Seasonal rainfall (mm) and mean temperature (°C) for experiment 1 (E1), experiment (E2), and the historical average from 1994 to 2011 (H). Taken from Arata *et al.* (2017).