

Nitrogen × sulfur interaction on fertiliser-use efficiency in bread wheat genotypes from the Argentine Pampas

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Abstract. Wheat crop response to sulfur (S) depends on nitrogen (N) level, genotype and environmental conditions, demonstrating strong genotype × environment × nutrients interactions. The agronomic-use efficiency of both nutrients has not been evaluated in a wide range of modern genotypes differing in their cycle length and baking quality. The aim of this study was to analyse the effect of N and S fertilisation on yield components and use efficiency of both nutrients in 24 modern, high-yielding bread wheat genotypes (including long and short crop cycles) grown in contrasting environments in the Humid Pampa of Argentina. Two experiments were conducted under contrasting seasonal conditions on a Mollisol in Azul, Buenos Aires. Significant effects of N (range 15–200 kg N ha⁻¹) on grain yield were observed in all genotypes. By contrast, responses to S (30–100 kg S ha⁻¹) were found only at high N level in low soil-fertility environments, differing between long and short cycles. Genotype × fertilisation interaction was significant in the environment with higher soil fertility. Sulfur addition improved N-recovery efficiency (0.15 v. 0.32) and agronomic efficiency of the available N (84 v. 93 g g⁻¹) in the poor-fertility environment, characterised by their N and S deficiency and moderate level of organic matter. Grain N-recovery efficiency was largely explained by increases in grain number, whereas S recovery was also associated with increases in grain nutrient concentration. We conclude that genotype and environment strongly alter fertiliser-use efficiency, providing valuable information for ranking genotypes and optimising site-specific management of wheat crops in the Humid Pampa of Argentina. Grain S percentage may be useful as a physiological marker for selection of bread wheat genotypes with high apparent S recovery.

Additional keywords: fertiliser, genetic variability, nutrient uptake.

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Introduction

Sulfur (S) is an essential nutrient for plant growth and some of its characteristics are similar to those of nitrogen (N), owing to their complexity and dynamics in soil and plant. It is well known that S deficiency reduces wheat yield (Withers *et al.* 1995; Zhao *et al.* 1999; Salvagiotti and Miralles 2008). These symptoms have increased because of the decline in industrial emissions of S, the low use of S fertilisers, the greater S removal from the soil through the application of high N doses, and the reduction of organic matter in agricultural soils with increased cropping intensity (Naeem and Mac Ritchie 2003; Ercoli *et al.* 2011).

Wheat yield is known to respond to N and S fertilisation, associated with an increase in aboveground biomass without changes in harvest index (Salvagiotti and Miralles 2008). However, results in the literature are contradictory regarding

the main components of wheat yield responses to nutrient availability, i.e. numbers of spikes per area and grains per spike (Fischer 1993; Abbate *et al.* 1995). This could be explained by issues related to site fertility and/or different genotypes used in each particular experiment, and application of different strategies to build grain number and, consequently, grain yield. For S in particular, it has been reported that response to fertilisation is the result of an increase in grain number per area but not in grain weight, associated with a larger number of spikes per area unit, without changes in the number of grains per spike (Salvagiotti and Miralles 2008). Because these results correspond only to a particular genotype and location, it is not yet possible to conclude that this is a general response to S.

Nitrogen-use efficiency indicates how effectively crops transform available N into grain yield, recovery efficiency

(ratio between N uptake and N applied) and internal efficiency (ratio between yield or biomass and N uptake) being its main components (Salvagiotti *et al.* 2009). It is widely known that N-use efficiency decreases as the rate of nitrogen fertiliser increases (Sinclair and de Wit 1975; Timsina *et al.* 2001) because other factors become limiting, such as availability of other nutrients or higher N fertiliser losses. Fertilisation with S enhances the effect of N and intervenes in soil processes that improve N-use efficiency by the crop. This improvement has been shown to be due to greater N recovery, without changes in internal efficiency (Salvagiotti and Miralles 2008; Salvagiotti *et al.* 2009). Therefore, the evident interdependence of N and S justifies the joint study of the effect of these nutrients. Particularly for N, genotypic differences in recovery efficiency and agronomic efficiency were found (Guarda *et al.* 2004; Balint *et al.* 2008).

Most of the wheat harvested in Argentina, and globally, is grown under rainfed conditions. The effect of water availability on yield and N- and S-use efficiency is variable between years (Kharel *et al.* 2011). The temperature experienced by the crop also modifies the effect of the addition of mineral nutrients. For example, under controlled conditions, changes in grain weight vary due to temperature regime during the grain-filling period (Altenbach *et al.* 2003; Dupont *et al.* 2006). Thus, the environment strongly modulates bread-wheat yield responses to N and S fertilisation. In turn, it has been reported that the increase in grain number per area explains most of the variation in N fertiliser recovery among years (Lerner *et al.* 2013). However, little is known about the components associated with S fertiliser recovery.

Although several studies have examined the effects of N and S fertilisation on wheat yield, insufficient information is available about their interaction with genotype and environment, as well as the main components associated with yield responses and fertiliser-use efficiency. The aim of this study was to analyse the effects of N and S fertilisation on yield components and use efficiency of both nutrients in 24 modern, high-yielding bread-wheat genotypes contrasting in cycle length, grown in the Humid Pampa of Argentina. This is necessary to improve understanding of the behaviour of these specific varieties under different fertiliser management regimes. This study is also an attempt to elucidate the components associated with the crop's ability to recover the applied fertiliser. This will provide information for selection of genotypes adapted to contrasting conditions of S availability.

Materials and methods

Field experiments

Two experiments were conducted in Azul, Province of Buenos Aires, Argentina. Soil samples were collected from depths of 0–20 and 20–40 cm at sowing of each experiment. During 2005–06, experiment 1 (E1) was performed at the Experimental Farm of the Faculty of Agronomy, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA) (36°83'S, 59°88'W). The trial was conducted under conventional tillage with wheat as the previous crop and in soil with the following features: high level of organic matter, 5.9% (Walkley–Black, 0–20 cm); neutral pH, 6.9 (1 : 2.5 soil : water, 0–20 cm); low

phosphorus (P) availability, 8.6 $\mu\text{g g}^{-1}$ (Bray and Kurtz I, 0–20 cm) (Bray and Kurtz 1945); moderate availability of nitrate-N, 20 kg N ha^{-1} at 0–20 cm and 22.8 kg N ha^{-1} at 20–40 cm (by reflectometry); and moderate availability of sulfate-S, 13.4 mg S kg^{-1} soil at 0–20 cm and 17.4 mg S kg^{-1} soil at 20–40 cm (by turbidimetry).

Twenty modern, high-yielding bread wheat genotypes were tested, including long (LC) and short (SC) crop-cycle duration, and three groups of bread quality (QG1, best bread quality; QG2, intermediate bread quality; and QG3, lowest quality) defined by the National Institute of Seeds (INASE). Genotypes used were: ACA 304 (304: LC, QG1), BioInta 2001 (BI2: LC, QG1), BioInta 3000 (BI3: LC, QG2), Cooperación Liqueñ (LIQ: LC, QG1), Klein Jabalí (JAB: LC, QG1), Relmó INIA Torcaza (TOR: LC, QG1), ACA 601 (601: SC, QG1), Buck Mejorpan (MEJ: SC, QG1), Klein Proteo (PRO: SC, QG1), Relmó INIA Condor (CON: SC, QG1), Buck Chacarero (CHC: LC, QG2), Klein Capricornio (CAP: LC, QG2), ACA 801 (801: SC, QG2), BioInta 1000 (BI1: SC, QG2), Klein Castor (CAS: SC, QG2), Klein Flecha (FLE: SC, QG2), Klein Tauro (TAU: SC, QG2), Relmó INIA Churrinche (CHU: SC, QG2), Buck Aguará (AGU: LC, QG3), Klein Gavilán (GAV: LC, QG3). The sowing dates were 23 June and 20 July with densities of 300 and 400 plants m^{-2} for LC and SC, respectively. Crop phenology was recorded according to Zadoks *et al.* (1974).

During 2012–13, experiment 2 (E2) was performed on a farm under agricultural rotation (36°84'S, 59°88'W). The trial was conducted under no-tillage with soybean as previous crop, in a soil with the following features: moderate level of organic matter, 4.1%; moderately acidic pH, 5.7; low P availability, 6.4 $\mu\text{g g}^{-1}$; low nitrate-N availability (kg N ha^{-1}): LC_{0–20} 6.1, LC_{20–40} 9, SC_{0–20} 8.8, SC_{20–40} 5.9; and low sulfate-S availability (mg S kg^{-1} soil): LC_{0–20} 8.3, LC_{20–40} 8.4, SC_{0–20} 13.2, SC_{20–40} 14.2. Ten modern bread wheat genotypes were tested: ACA 304, ACA 601 (LC), BioInta 3000 (QG2), Buck SY 100 (100: LC, QG2), Buck AGP 127 (127: LC, QG3), Klein Proteo, ACA 801, Buck AGP Fast (FAS: SC, QG3), Klein Chajá (CHJ: SC, QG3), Klein Gavilán (SC). The sowing dates were July 12 and September 12 with 350 and 400 plants m^{-2} densities for LC and SC, respectively. Delay in sowing dates occurred due to heavy rains during the season, especially in the pre-seeding stage for SC.

Fertilisation treatments were performed as a factorial arrangement: N0S0 (unfertilised), N1S0 (N), N0S1 (S) and N1S1 (S + N). Doses of N fertiliser (urea) were calculated by a balance model based on the soil analysis and achievable target yields (Díaz-Zorita *et al.* 2002) to reach 150 kg N ha^{-1} in E1 and 210 kg N ha^{-1} in E2. Doses of S fertiliser (K_2SO_4) were 40 and 25 kg S ha^{-1} for E1 and E2, respectively. Treatments topdressings applied in split doses: 35% at emergence (Z1.0) (Zadoks *et al.* 1974) and 65% at tillering (Z2.2), except in E1, where S was applied at tillering only. In addition, basal P fertilisation with triple superphosphate (4–8 kg P ha^{-1}) was incorporated into the seed line at sowing. Basal potassium (K) fertilisation was not applied because K is not deficient in the Argentine Pampas Mollisols (Lavado and Taboada 2009). Also, the organic matter content of the soils of both experiments was high enough to provide micronutrients. Weeds were chemically controlled (5 g ha^{-1} of metsulfuron-methyl + 100 $\text{cm}^3 \text{ha}^{-1}$ of dicamba) at

4-unfolded leaves (Z1.4), and diseases were chemically controlled ($750 \text{ cm}^3 \text{ ha}^{-1}$ of epoxiconazole+kresoxim methyl) at ear emergence (Z5.0). Pest control was not required.

Measurements

Anthesis (Z6.5) occurred at 134 ± 2 days after sowing (DAS) for LC and at 111 ± 4 DAS for SC in E1, and at 118 ± 4 DAS for LC and at 74 ± 4 DAS for SC in E2. Daily records of mean, minimum and maximum temperatures, and rainfall were taken from the Central Station of Azul (Faculty of Agronomy), located near the experiments, published in the Agrometeorological Bulletin by the Regional Agrometeorology Center, FA-UNCPBA (www1.faa.unicen.edu.ar/centro/centroreg.php). Environmental conditions differed between experiments. In E2, extreme events of precipitation and temperature were recorded in August (215 mm) and December (179 mm) and in July (4.3°C). Average temperatures were similar to the historical average in E1, whereas they were slightly higher after August in E2 (Fig. 1).

At maturity (Z9.9), plants along the five central rows were manually harvested, mechanically threshed, and oven-dried at 60°C. Grain yield (GY), grain number per m^2 (GN) and 1000-grain weight (TGW) were determined. In E2, the subcomponents spike number per m^2 (SN) and grain number per spike (GS), aboveground biomass (AB) and harvest index (HI) were determined. GY was expressed with 13% humidity. Grain protein content (%) was analysed in whole grains with near infrared transmittance (AgriCheck; Bruins Instruments, Salem, NH, USA), and N content in grain (N%) was calculated (factor 5.75). The S content (S%) was determined by atomic absorption spectrophotometry in wholemeal flour samples according to Dunk *et al.* (1969). Use efficiencies of both nutrients were calculated—N agronomic efficiency (NAE), apparent N recovery (ANR) and apparent S recovery (ASR)—by using the following formulae (Eqns 1–3) adapted from Guarda *et al.* (2004):

$$\text{NAE} = \frac{[\text{grain yield (g m}^{-2}\text{)}]}{\text{N soil} + \text{N fertiliser (g m}^{-2}\text{)}} \quad (1)$$

$$\text{ANR} = \frac{[\text{grain N uptake at N1} - \text{grain N uptake at N0 (g N m}^{-2}\text{)}]}{\text{N applied at N1 (g N m}^{-2}\text{)}} \quad (2)$$

$$\text{ASR} = \frac{[\text{grain S uptake at S1} - \text{grain S uptake at S0 (g S m}^{-2}\text{)}]}{\text{S applied at S1 (g S m}^{-2}\text{)}} \quad (3)$$

To compare genotypes, the differential NAE between N0 and N1 treatments (ΔNAE) was calculated. The boundaries for the medium-efficiency interval were formulated by subtracting or adding the value of 1 standard error from the median point of the efficiency criterion according to Balint *et al.* (2008). Differentials between fertilised and unfertilised treatments for each nutrient were also calculated as ΔGY , ΔGN , ΔTGW , and $\Delta\text{N\%}$ and $\Delta\text{S\%}$.

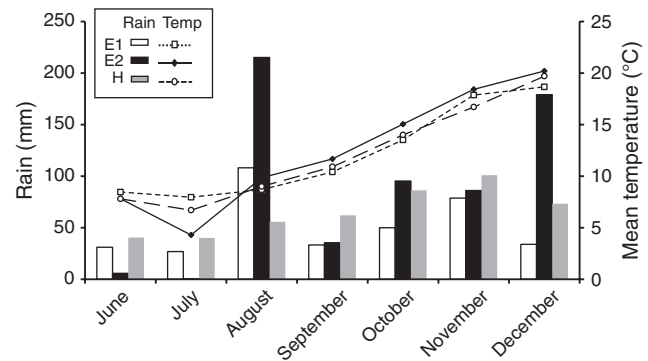


Fig. 1. Seasonal rainfall and mean temperature for both experiments (E1, E2) and historical average (H) of period 1994–2011.

Experimental design and statistical analyses

The experimental design was split-plot in a randomised complete block with three replications, using macroplots of 9.5 m by 5.6 m in E1 and 12.5 m by 5.6 m in E2, assigning the genotypes to the main plots and fertilisation treatments to the subplots. Block \times main plot interaction as error term for the effect assigned to the main plot was used. The effects of cycle length (C), genotype (G), N, S and their interactions were analysed by analysis of variance and Fisher test ($\alpha=0.05$) and the contribution of each source of variation (SV) was expressed as the percentage of sum of squares (SS%). Thus, the SVs were ranked in each case and those variables more influenced by interaction components were determined. Correlation analysis (Pearson), linear regression, principal component analysis (PCA) and cluster analysis (average linkage, Euclidean distance) were also performed. The InfoStat statistical package was used (Di Rienzo *et al.* 2014).

Results

Grain yield response to fertilisation

In E1, the GY for all fertilisation treatments ranged between 217 and 581 g m^{-2} for LC and between 198 and 529 g m^{-2} for SC; and in E2, it ranged between 144 and 592 g m^{-2} for LC and between 155 and 661 g m^{-2} for SC. The effect of S level and its interaction with N strongly depended on the environment (year-site). As expected, N significantly increased GY in E1 (20%); also, effects of G and G \times S interaction were significant in this experiment (Table 1). Thus, only cultivars 304, 601 and 801 responded positively to S addition, and B11 responded negatively. In E2, the effects of S and N \times S, C \times N and C \times N \times S interactions were significant (Table 1). Fertilisation with S increased GY depending on N level, the response being greater for LC (Fig. 2).

Numerical components of grain yield affected by fertilisation

As expected, N fertilisation increased GN in E1 (N0, $7612 \text{ grains m}^{-2}$; N1, $10\,065 \text{ grains m}^{-2}$), explaining >40% of SS%. In addition, the effects of G and G \times S interaction on GN were significant in this experiment (Table 1). Thus, only cultivars 304 and 801 presented a positive response to S addition, whereas JAB

Table 1. Summary of ANOVA for grain yield (GY) and subcomponents number of grains per m² (GN), 1000-grain weight (TGW), number of spikes per m² (SN) and number of grains per spike (GS), including proportion of sum of squares explained (SS%) and significance level of the source of variation considered (C, cycle; G, genotype; N, nitrogen; S, sulfur) for each experiment

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Source	Experiment 1						Experiment 2									
	GY		GN		TGW		GY		GN		TGW		SN		GS	
	SS%	Sign.	SS%	Sign.	SS%	Sign.	SS%	Sign.	SS%	Sign.	SS%	Sign.	SS%	Sign.	SS%	Sign.
C	0.3	n.s.	0.5	n.s.	0.1	n.s.	0.3	n.s.	10.0	***	59.7	***	22.4	***	0.1	n.s.
G	22.8	**	18.8	*	83.2	***	10.3	n.s.	6.4	n.s.	20.9	***	11.9	n.s.	29.1	*
N	42.3	***	45.8	***	1.1	**	43.2	***	40.2	***	0.3	n.s.	19.4	***	18.4	***
S	0.5	n.s.	0.3	n.s.	0.1	n.s.	13.1	***	10.1	***	1.2	*	5.0	**	4.7	**
C × N	0.1	n.s.	0.0	n.s.	0.2	n.s.	1.6	*	0.7	n.s.	0.0	n.s.	2.5	*	0.0	n.s.
C × S	0.0	n.s.	0.1	n.s.	0.0	n.s.	0.2	n.s.	0.1	n.s.	0.0	n.s.	0.0	n.s.	0.3	n.s.
G × N	4.4	n.s.	3.3	n.s.	2.5	*	2.7	n.s.	3.4	n.s.	10.0	***	4.7	n.s.	3.3	n.s.
G × S	6.7	**	6.7	**	1.1	n.s.	0.9	n.s.	1.4	n.s.	1.4	n.s.	4.7	n.s.	4.2	n.s.
N × S	0.0	n.s.	0.0	n.s.	0.6	**	6.8	***	6.4	***	0.0	n.s.	0.7	n.s.	4.8	**
C × N × S	0.0	n.s.	0.0	n.s.	0.1	n.s.	1.5	*	0.4	n.s.	1.1	*	0.8	n.s.	3.3	**
G × N × S	4.4	n.s.	4.6	n.s.	2.0	n.s.	0.5	n.s.	0.7	n.s.	1.7	n.s.	4.8	n.s.	3.4	n.s.

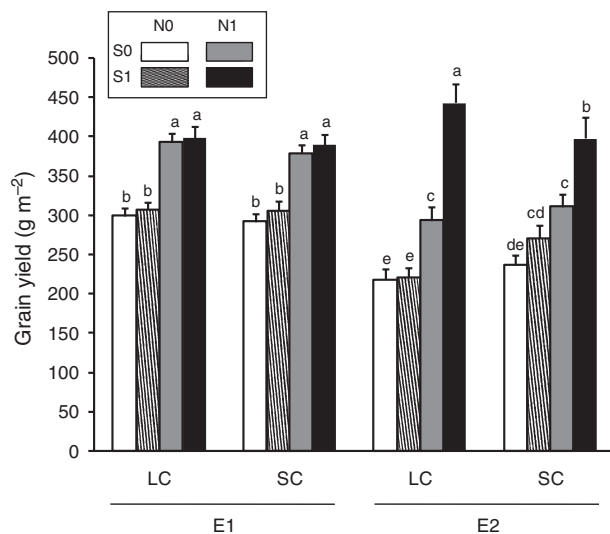


Fig. 2. Average grain yield for the treatments without nitrogen and sulfur (N0S0), without N and with S (N0S1), with N and without S (N1S0) and with N and S (N1S1), for long-cycle (LC) and short-cycle (SC) genotypes in each experiment (E1, E2). Means \pm 1 standard error are shown. Means with the same letter are not significantly different between treatments within each experiment.

was negative. In E2, the effects of S, C and N \times S interaction on GN were significant (Table 1). Hence, S fertilisation increased GN only at high N level, depending on cycle length. For LC, they accounted for variations in GS (LC-N1S0, 18 grains spike⁻¹; LC-N1S1, 24 grains spike⁻¹), whereas for SC, SN was more responsive to S fertilisation (SC-N1S0, 465 spikes m⁻²; SC-N1S1, 539 spikes m⁻²). In turn, the average response to N addition was greater with high S (S0, 1932 grains m⁻²; S1, 4505 grains m⁻²). Furthermore, LC reached, on average, 7373 grains m⁻² and SC 8979 grains m⁻². This difference was mainly due to changes in SN without changes in GS.

In E1, the TGW was affected mainly by G and, to a lesser extent, by N, G \times N and N \times S (Table 1). Thus, 75% of the cultivars showed stability in TGW between different N levels,

whereas with high nutrient level, 20% (AGU, FLE, LIQ and MEJ) responded negatively and only BI3 was positive. In turn, S addition significantly increased TGW only at low N supply, and N addition decreased this component only at high S supply. In E2, TGW was mainly affected by C, G and G \times N interaction and, to a lesser extent, by S and C \times N \times S interaction (Table 1). Similar to E1, 80% of the cultivars presented stability in TGW between different N levels, whereas at high nutrient level, 601 responded negatively and BI3, again, responded positively. Fertilisation with S increased TGW only at high N level for LC and only at low N level for SC. In addition, average TGW was significantly lower for SC than for LC (LC, 34.69 g; SC, 29.42 g), the smaller reductions being for N0S0, 18%; N0S1, 13%; N1S0, 13%; N1S1, 17%.

Changes in GN explained 82% and 87% of GY variation in E1 and E2, respectively (E1, $P < 0.0001$; E2, $P < 0.0001$). In both experiments, the association between GY and TGW was significant (E1, $P = 0.0273$; E2, $P = 0.0356$) but with low correlation (E1, $R^2 = 0.02$; E2, $R^2 = 0.04$). Furthermore, a slight compensation effect between GN and TGW components was observed in E1 ($P < 0.0001$; $R^2 = 0.07$), whereas it was not significant in E2 ($P = 0.0858$).

Aboveground biomass (AB) and harvest index (HI) were also affected by fertilisation treatments. In E2, AB for all fertilisation treatments ranged between 347 and 1331 g m⁻² for LC and between 375 and 1260 g m⁻² for SC. Similar to GY, AB was significantly affected by N and S addition and C \times N, N \times S and C \times N \times S interactions. Therefore, N fertilisation significantly increased AB at both S levels. Also, there was a positive response to S fertilisation at both N levels for SC, and only at high N level for LC. This response was greater for LC due to a lower average AB in the N1S1 treatment for SC.

Harvest index for the genotypes ranged between 0.34 and 0.44. Most cultivars showed stable HI between N levels, whereas at high nutrient level, BI3 and GAV responded positively, in contrast to genotype 127. In turn, HI was stable between N levels for SC and decreased only at the high N level for LC. Addition of N decreased HI only with low S supply (N0S0, N0S1 and N1S1 all 0.41 v. N1S0, 0.38; $P < 0.05$). Furthermore, changes in AB

explained 87% of variation in GY in E2 ($P < 0.0001$), whereas the association between GY and HI was significant ($P = 0.0062$) but with a very low correlation ($R^2 = 0.06$).

Nitrogen and sulfur use efficiency of bread wheat genotypes

Nitrogen fertilisation decreased NAE by 63% and 89%, on average, in E1 and E2, respectively, with genotypic differences in their magnitude (Table 2). In E1, most cultivars exhibited stable NAE between different S levels. At high S level, genotypes 304, 601 and 801 responded positively, whereas the BI1 response was negative. In E2, S fertilisation increased NAE by 11%, on average. Also, genotypic variability in the degree of NAE reduction due to N fertilisation was associated with significant differences in the values of N0 treatments, but not of N1.

Nitrogen fertilisation increased N content in grain (N%) by 28% and 38%, on average, in E1 and E2, respectively, with genotypic differences in magnitude. The N% was higher for SC at both N levels. Furthermore, S addition exhibited a positive effect on N% by 1.85% on average in E1 and, only at high N supply, by 6.56% in E2 (Table 2).

In E1, S content in grain (S%) ranged from 0.115% (BI3) to 0.141% (BI1). In turn, stability in S% between cycles at high N level was observed, and a higher average value for SC at low N

level, the response to N fertilisation being of greater magnitude for LC. With respect to S addition, a positive response was recorded for LC only. On the other hand, in E2, S fertilisation increased S% by 8% and 59%, on average, at low and high N supply, respectively. In addition, average responses to S fertilisation were 39% for LC and 26% for SC. Most cultivars had no response significant to N fertilisation for S%, although FAS, BI3 and 100 responded positively and GAV negatively (Table 2).

Apparent N recovery was stable in most genotypes in E1 between different S levels, although 304 responded positively to S fertilisation and MEJ negatively. In turn, LC showed a greater average ANR than SC (LC, 0.35; SC, 0.30; $P < 0.05$). In E2, S fertilisation increased ANR by 113%, on average (S0, 0.15; S1, 0.32; $P < 0.05$), and the means of the genotypes ranged from 0.31 (BI3) to 0.13 (801) (Table 3).

Apparent S recovery was very low, ranging from 0 to 0.10 in E1 and from 0 to 0.26 in E2. Nitrogen fertilisation significantly increased ASR in most genotypes, with the exception of MEJ, AGU, 801 and FLE (Table 3).

A high correlation of ANR with GY differential between fertilised and unfertilised treatments (Δ GY) was found (Fig. 3a). A similar correlation was observed for GN differential (Δ GN) but not for TGW differential (Δ TGW). In turn, the correlation between ANR and N content in grain differential (Δ N%) was significant but of lesser magnitude

Table 2. Summary of ANOVA for nitrogen agronomic efficiency (NAE), N content in grain (N%) and sulfur content in grain (S%) including proportion of square sum explained (SS%) and significance level of the source of variation considered (C, cycle; G, genotype; N, nitrogen; S, sulfur) for each experiment (E1, E2)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$

Source	Experiment 1						Experiment 2					
	NAE		N%		S%		NAE		N%		S%	
	SS%	Sign.	SS%	Sign.	SS%	Sign.	SS%	Sign.	SS%	Sign.	SS%	Sign.
C	0.0	n.s.	7.9	***	1.0	*	0.3	**	3.1	***	9.1	***
G	3.5	**	13.1	***	28.6	***	1.2	n.s.	9.6	***	18.2	**
N	90.6	***	68.0	***	28.6	***	94.8	***	76.9	***	1.0	**
S	0.1	n.s.	0.3	*	0.5	n.s.	0.5	**	0.9	**	40.9	***
C × N	0.0	n.s.	3.1	***	1.6	*	0.4	**	0.4	*	0.1	n.s.
C × S	0.0	n.s.	0.2	n.s.	1.6	*	0.0	n.s.	0.0	n.s.	0.8	*
G × N	0.8	n.s.	2.7	**	7.0	n.s.	0.6	*	4.4	***	4.5	*
G × S	1.2	**	1.4	n.s.	6.7	n.s.	0.0	n.s.	0.9	n.s.	1.1	n.s.
N × S	0.0	n.s.	0.2	n.s.	0.0	n.s.	0.0	n.s.	1.3	**	22.7	***
C × N × S	0.0	n.s.	0.1	n.s.	0.0	n.s.	0.1	n.s.	0.1	n.s.	0.1	n.s.
G × N × S	0.9	*	0.3	n.s.	6.6	n.s.	0.0	n.s.	0.9	n.s.	1.0	n.s.

Table 3. Summary of ANOVA for apparent nitrogen recovery (ANR) and apparent sulfur recovery (ASR) including proportion of square sum explained (SS%) and significance level of the source of variation considered (C, cycle; G, genotype; N, nitrogen; S, sulfur) for each experiment (E1, E2)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$; SS Type I

Source	ANR				Source	ASR			
	Expt 1		Expt 2			Expt 1		Expt 2	
	SS%	Signif.	SS%	Signif.		SS%	Signif.	SS%	Signif.
C	4.4	**	2.5	n.s.	C	0.9	n.s.	0.7	n.s.
G	34.4	n.s.	19.0	*	G	22.2	**	4.5	n.s.
S	0.6	n.s.	53.2	***	N	44.4	***	79.5	***
C × S	0.2	n.s.	2.5	ns	C × N	0.5	n.s.	2.3	n.s.
G × S	16.9	*	2.5	ns	G × N	11.1	*	2.3	n.s.

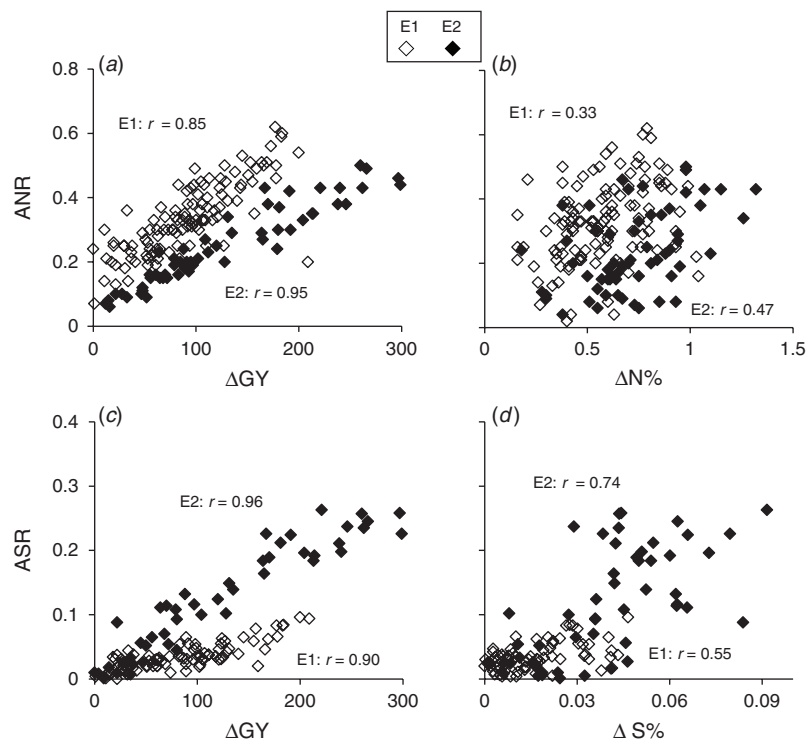


Fig. 3. Correlations between apparent nutrient recovery and differentials of grain yield and nutrient content in grain between fertilised and unfertilised treatments, for both cycle-length genotypes in experiment 1 (E1) and experiment 2 (E2). Correlations between: (a) apparent nitrogen recovery (ANR) and grain yield differential (Δ GY), (b) ANR and differential of N content in grain (Δ N%), (c) apparent sulfur recovery (ASR) and Δ GY, and (d) ASR and differential of S content in grain (Δ S%).

(Fig. 3b). On the other hand, ASR showed high correlation with Δ GY and Δ S% (Fig. 3c, d). In this case, the association of this efficiency with Δ GY was due to changes in Δ GN (E1, $r = 0.88$; E2, $r = 0.92$) but not Δ TGW.

Identifying outstanding genotypes for nutrient efficiency

In each experiment, the effect of N fertilisation on NAE at different S supply was analysed by Δ NAE, and categories of efficient (low Δ NAE), medium (intermediate Δ NAE) and inefficient (high Δ NAE) genotypes were built. Some cultivars retained the same efficiency category between S levels. Thus, CAS, CHU, PRO and TOR in E1, and 127, 304 and 801 in E2, were efficient at both S levels, whereas CON in E1 and CHJ and FAS in E2 were inefficient at both S levels. Of the six genotypes that were repeated between experiments, PRO was efficient only in E1 and 801 was efficient only in E2. In addition, GAV was inefficient or medium in all environments, 304 was efficient in almost all the environments (except in E1 at S1), BI3 was efficient or medium in all environments, and 601 showed instability. Finally, JAB, LIQ and TAU changed from inefficient to efficient category when S fertiliser was applied in E1, and no genotype showed this response in E2 (data not shown).

In each experiment, categories of efficient (high recovery), medium (intermediate recovery) and inefficient (low recovery) genotypes were built for ANR and ASR at different nutrient supply. Data for ASR are shown in Fig. 4. Of the six genotypes

that were repeated between experiments, BI3 was efficient for ANR but medium or efficient for ASR at different nutrient supply in both experiments. By contrast, 304 was efficient for ANR and ASR in almost all the environments (except in E2 at low nutrient level), in a similar way to genotype 601. PRO was efficient for both recovery efficiencies only in E2. On the other hand, GAV was inefficient in most environments, and 801 was more inefficient in E2.

Genotype × environment interaction for grain yield and nutrient recovery

The PCA, which included GY components and fertiliser-use efficiency of the genotypes under high nutrient availability conditions (N1S1) in both experiments indicated that the principal component 1 comprised variables N% (+), S% (+), ASR (+), GN (+), and NAE (–), explaining 39% of observed variability. Principal component 2 comprised GY (+), TGW (+), ANR (+), GN (+) and NAE (+), explaining another 36% of variability. In particular, PRO showed intermediate GY and TGW, and high nutrient content in grain and ASR in both experiments. Main grouping was due to experiment, with higher nutrient content in grain and S recovery in E2. No clear groupings by cycle length and quality group were observed (Fig. 5). In turn, the cluster analysis for the same variables corroborated these groupings (Fig. 6).

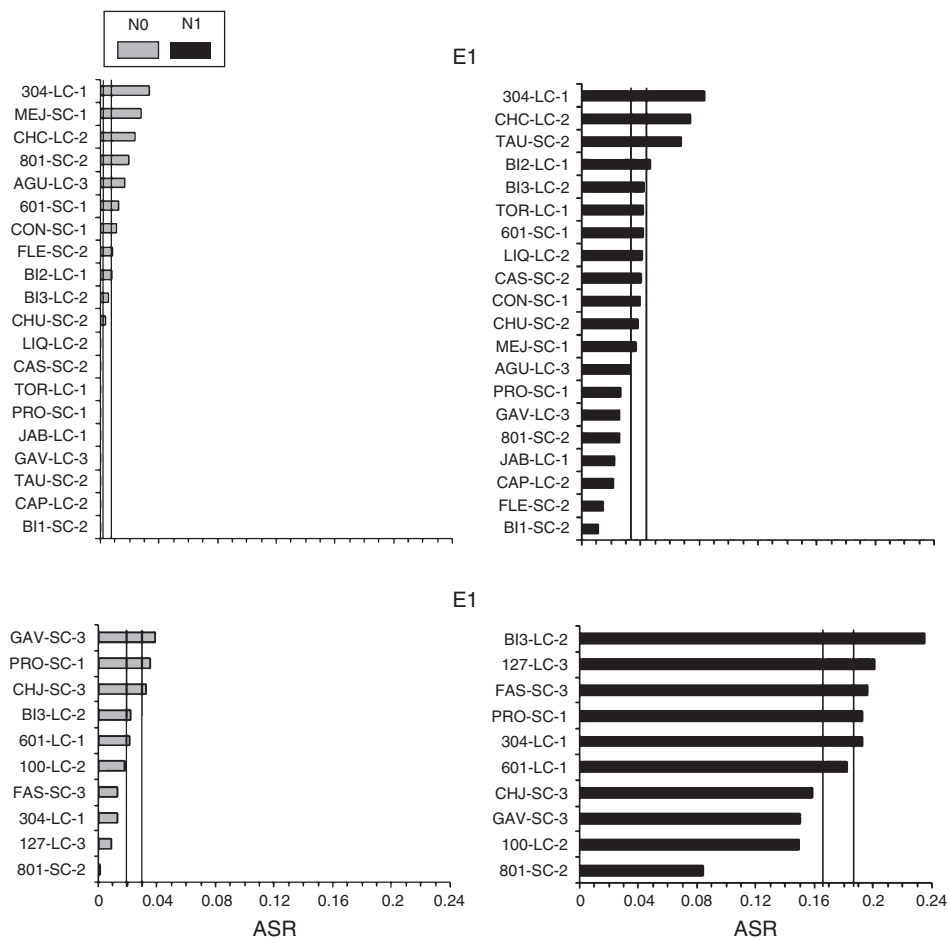


Fig. 4. Apparent sulfur recovery (ASR) for the treatments without N (N0) and with N (N1), for each genotype (see codes in Table 2, number corresponds to quality group) in each experiment (E1, E2). The boundaries for the medium-efficiency interval were formulated by subtracting or adding the value of 1 standard error from the median point of the efficiency criterion.

Discussion

As expected, GY in modern bread wheat genotypes growing in the Argentinean Pampas was limited by N availability in both experiments, whereas S fertilisation response contrasted between E1 and E2 (moderate and low initial soil fertility, respectively). Consequently, the environment in E2 presented higher discriminating power for screening the fertiliser-use efficiency of wheat genotypes. A strong $N \times S$ interaction was observed in E2 for GY, GN and AB, in agreement with Salvagiotti and Miralles (2008), Ercoli *et al.* (2011) and Kumar *et al.* (2012). Interestingly, the higher GN due to S addition at high N level was associated with different components depending on crop cycle, i.e. GS for LC, and SN for SC. A possible explanation may be related to the later sowing date for SC and the rainfall distribution, determining different synchrony between the moment of nutrient availability (supply) and crop development stage (demand) for each cycle length, affecting different processes of GY generation. In addition, heavy rainfall in August could leach the sulfate from the soil in early crop stages, especially for LC. Other authors' findings partially concur with our findings

about the GY components affected by S fertilisation. Salvagiotti and Miralles (2008) found that S fertilisation with ammonium sulfate after sowing increased SN without affecting the GS for a bread wheat cultivar of short cycle, whereas Ercoli *et al.* (2011) observed up to 10% increase of GS without changes in SN due to fertilisation with calcium sulfate before sowing for durum wheat cultivars of intermediate–short cycle.

The TGW was lower for SC genotypes in E2, probably because of the elevated temperature during the grain-filling period (November–December), as observed by González *et al.* (2014) for a broad range of genotypes and temperature-contrasting years in the Rolling Pampas. Interestingly, the average reduction of TGW was of lesser magnitude in N0S1 and N1S0 treatments, showing a possible mitigation effect of fertilisation with only N or with only S, in a similar way to that reported by Altenbach *et al.* (2003). Slight increases of TGW due to S addition were detected in the more restrictive environment E2, characterised by low soil fertility, wet seasonal conditions and short growing season, differing from those found by Salvagiotti and Miralles (2008). Fertilisation effects on TGW are relevant in order to maximise flour yield and milling operations for bread

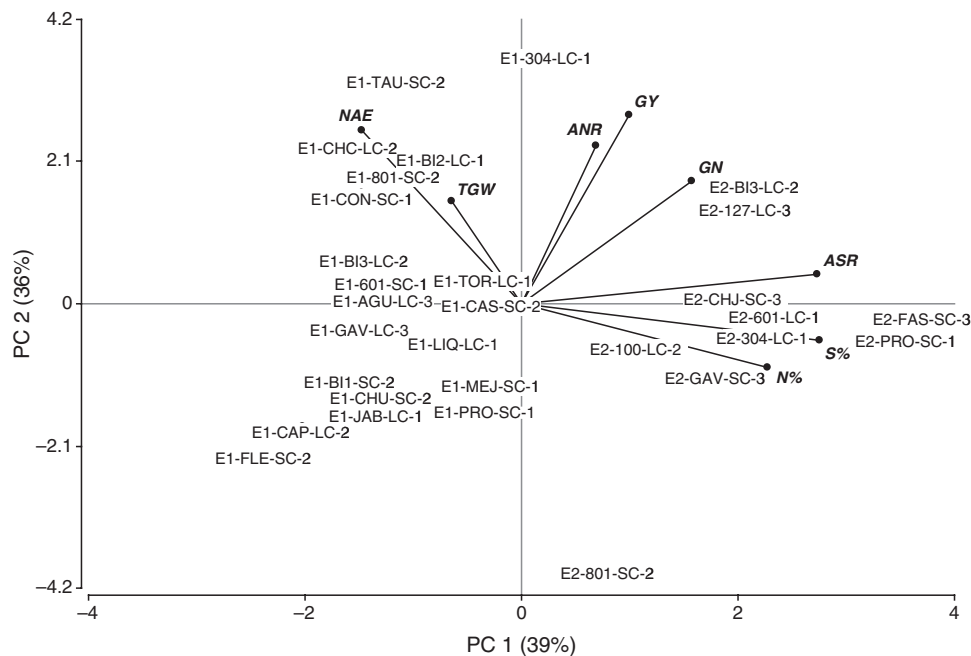


Fig. 5. Biplot of the first and second principal component for grain yield (GY), grain number per m² (GN), 1000-grain weight (TGW), nitrogen content in grain (N%), sulfur content in grain (S%), N agronomic efficiency (NAE), apparent N recovery (ANR) and apparent S recovery (ASR) for treatments with N and S of 24 genotypes (see codes in Table 2, number corresponds to quality group) in both experiments (E1, E2).

wheat. These discrepancies observed in the yield components affected by S fertilisation may also be related to the increase in GY potential of cultivars, which could increase the level of source limitation during grain filling, i.e. the amount of photo-assimilate needed to sustain the growth of all grains (González *et al.* 2014).

Nitrogen agronomic efficiency was primarily affected by N level, according to pioneer work from de Wit (1992) and Timsina *et al.* (2001). In E1, the G × S interaction was significant, grouping genotypes based on their S response in the same manner as for GY. By contrast, S addition in E2 improved NAE by 11%, on average. Similarly, Salvagiotti *et al.* (2009) observed an increase in NUE (kg grain kg⁻¹ N applied) due to greater soil nutrient recovery. Nitrogen content in grain notably increased at high N level and slightly increased at high S level, with a significant N × S interaction in E2. This implies a limitation in the N accumulation in the grain due to S deficiency, as reported by Salvagiotti *et al.* (2009). Genotype × N interaction in both experiments reflects genotypic differences in the capacity of N accumulation in grain, probably associated with the capture of the nutrient, its partition and/or GY potential. In this respect, Sandaña (2016) observed that P uptake was more important than utilisation efficiency in determining P-use efficiency in potato, and detected important genotypic variability in these traits with the potential to be used in breeding and crop-management programs. Furthermore, grain N% was higher for SC at both N levels in both experiments. In E2, this might be partially associated with higher temperature during grain filling, which usually causes a reduction in grain starch content instead of a change in amount of N, thus increasing N concentration (Jenner 1994; Castro *et al.* 2007). However, in E1, post-flowering

temperature did not differ between crop cycles, so other processes may be involved in the N% differences. Mechanisms associated with N-uptake capacity in the pre-flowering period and nutrient translocation to grain should be further studied in the most promising genotypes (Avni *et al.* 2014; Pang *et al.* 2015).

Sulfur fertilisation increased S% in grain in E2, with greater magnitude when N fertilisation was higher. Similarly, Ercoli *et al.* (2011) observed an increase in S% due to S fertilisation in white wheat under water-limited Mediterranean environments. Cycle length also seems to affect S% in grain, being higher for SC, on average. Studies on S accumulation and partitioning in short- and long-cycle bread wheat genotypes grown in the Argentinean Pampas need to be performed.

Apparent N recovery values found in both experiments are similar to those reported by Guarda *et al.* (2004) for winter wheats of different release year, grown in northern Italy. ASR was lower and more variable than ANR, probably associated with the high doses of S fertiliser applied relative to the absolute S crop requirements. ANR showed a strong positive association with ΔGY, explained by variations in GN but not in TGW, and to a lesser extent by ΔN%. Instead, ASR was strongly associated with both ΔGY and with ΔS%. Thus, components associated with the crop ability accounting for the recovery of the applied fertiliser are different between nutrients. In the case of N, it was largely determined by responses in fixed grain number (Abbate *et al.* 1995; Salvagiotti *et al.* 2009), whereas for S, the variations in grain weight and grain S concentration also became important. Similarly, Malhi *et al.* (2007) reported differences in S uptake of different *Brassica* oilseed species/cultivars and observed that the effects of S deficiency and fertilisation were more pronounced in seed than straw. In addition, Girondé *et al.* (2014) reported that

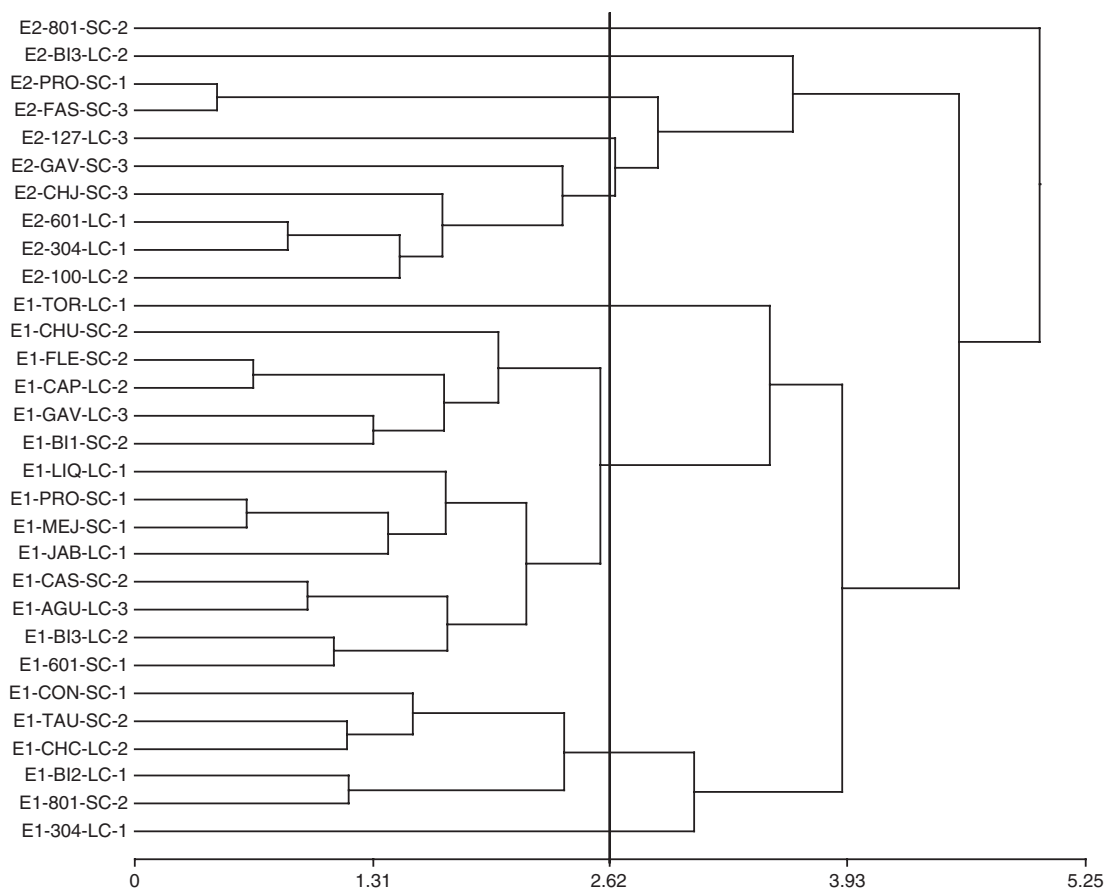


Fig. 6. Cluster analysis for grain yield (GY), grain number per m² (GN), 1000-grain weight (TGW), nitrogen content in grain (N%), sulfur content in grain (S%), N agronomic efficiency (NAE), apparent N recovery (ANR) and apparent S recovery (ASR) for treatments with N and S of 24 genotypes (see codes in Table 2, number corresponds to quality group) in both experiments (E1, E2).

physiological traits such as S remobilisation could be used in breeding programs to select oilseed rape genotypes with high S-use efficiency or those able to limit the impact of mineral S limitation on seed yield and quality. Thus, grain S% can be useful as a physiological marker for selection of bread wheat genotypes with high ASR. In addition, the enhancement of wheat grain nutrients (biofortification) through genetic strategies is a powerful approach for changing the nutritional balance in the human diet at a large scale (Chatzav *et al.* 2010).

Some genotypes exhibited a low Δ NAE, i.e. the decrease of NAE at high N supply was lower than genotypic average, at both S levels. Thus, these genotypes would be adequate to maximise the return of N fertiliser in terms of grain yield under contrasting S-fertility conditions. For example, PRO (SC, QG1) and 801 (SC, QG2) were efficient at both S levels in E1 and E2, respectively. On the other hand, 304 (LC, QG1) and BI3 (LC, QG2) showed good performance of NAE in both environments, being more adaptable. Finally, JAB and LIQ (both LC and QG1) and TAU (SC and QG1) would require balanced N and S fertilisation to maximise NAE in high-fertility environments, whereas GAV (LC and QG3) was inefficient. This information could contribute to optimising the site-specific fertilisation management for bread wheat at Humid Pampa of Argentina.

The ranking of genotypes based on Δ NAE, ANR and ASR differed between environments, and no clear groupings by cycle length and quality group were observed. Other authors found differences between wheat genotypes in N-use efficiency (Le Gouis *et al.* 2000; Guarda *et al.* 2004). The interaction between genotype and nutrient availability for N- and S-use efficiency has also been reported for canola (Balint *et al.* 2008; Balint and Rengel 2009, 2011). The few genotypes (304, BI3, PRO, and 801) with stable N-use efficiency between S levels (Δ NAE) varied in cycle length and quality group, although a trend between good nutrient recovery and bread quality may be observed. A molecular evaluation of baking quality and gluten composition (Lerner *et al.* 2009) for the identified genotypes with high and stable N and S recovery is currently being conducted.

Principal component analysis for GY components and fertiliser-use efficiency showed a close relation between S recovery and nutrient content in grain, with differences in GY between stable cultivars. Thus, PRO (SC, QG1) exhibited high nutrient content and S recovery associated with medium GY and TGW, whereas 304 (LC, QG1) showed the same behaviour but with high GY and TGW in E1. Identifying the molecular mechanisms that explain these differences in S content in grain would be useful to optimise the compromise between GY and

quality in new wheat genotypes, achieving greater S recovery. In this regard, Buchner *et al.* (2010) reported that patterns of expression of the sulfate-transporter gene family are complex when considered for the whole plant during development, and concluded that plasticity of expression is targeted at optimising uptake and allocation for optimal synthesis of storage proteins. In addition, grain tissues showed the most complex expression patterns, and the sulfate levels were low, probably due to rapid assimilation, as described for lupin (Tabe and Droux 2001). Furthermore, it was observed that application of S fertiliser alters gene expression of the sulfate-transporter family, affecting the distribution and accumulation of other nutrients (Shinmachi *et al.* 2010).

Cluster analysis for GY and fertiliser-use efficiency variables also showed a strong grouping by environment (experiment). In turn, the clustering of genotypes was different in each experiment, which did not respond to cycle length and quality group. In this regard, Rotundo *et al.* (2014) reported significant cluster × environment interactions for GY and N-use efficiency parameters in soybean. They concluded that highest yielding cultivars differed in the physiological strategies to attain maximum yield, combining different parameters of biomass partition and N-use efficiency. These results are encouraging for breeding purposes, integrating traits of GY and nutrient-use efficiency.

Conclusions

Our study, which included 24 genotypes grown under different environmental conditions, provides novel information about crop responses to contrasting N and S supply. Effects of S fertilisation on grain number and GY were modified by the environment (initial fertility and temperature) and genotype cycle length. ANR was mostly correlated with increase in yield associated with grain number, whereas S recovery was strongly correlated with increase in grain nutrient concentration. Ranking of genotypes based on fertiliser-use efficiencies differed between contrasting environments. Grain S% may be a useful physiological marker for selection of bread-wheat genotypes with high ASR. Identifying the molecular mechanisms that explain the differences in S content in grain and nutrient recovery would provide information for selection of genotypes adapted to contrasting conditions of S availability.

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