

## **Sulfur affects root growth and improves nitrogen recovery and internal efficiency in wheat**

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# **Sulfur affects root growth and improves nitrogen recovery and internal efficiency in wheat**

Wheat plants were cultivated in pots with the objective of evaluate the effect of two sulfur rates (+S and -S) on i) shoot growth, S and nitrogen uptake and nitrogen use efficiency (NUE) and ii) root growth and architecture and its relations with sulfur and nitrogen uptake. Plant samplings were at Z39, Z51 and Z92 stages.

Shoot mass and NUE were greater in +S treatment at the three stages. -S plants increased root growth at Z39 (14% more length and 16% more tips) in comparison with +S, but the opposite occurred at Z51 (31% less area and 42% less mass). Sulfur uptake per unit root mass, area and length were greater in +S treatment at Z39 and Z51. A similar pattern was determined for nitrogen uptake at Z39, but the opposite occurred at Z51. This indicates that nitrogen uptake is mainly controlled by shoot growth and not by root growth.

Keywords: nitrogen use efficiency; root architecture; sulphur uptake; nitrogen uptake

## **1 Introduction**

Nitrogen (N) is the most limiting nutrient in many of the world's agricultural systems. During the past four decades, worldwide agricultural food production has doubled, leading to a sevenfold increase in the use of N fertilizers (Hirel et al., 2007). Based on estimates of world population growth, it is evident that agricultural intensification will continue to increase, together with the consumption of N fertilizers (Alexandratos and Bruinsma, 2012).

Despite the benefits of N fertilization on crop production, such as yield and quality increases, this nutrient may have detrimental effects on the environment. For example, the eutrophication of freshwater as a result of N leaching (Giles, 2005) or the

emission of N oxides and ammonia into the atmosphere (Stulen et al., 1998). Therefore, improving N use efficiency (NUE) is desirable to increase crop yield, reduce production costs and maintain environmental quality.

NUE indicates how effectively crops extract this nutrient from the soil and produce shoot mass or grain. It can be divided into two components: (i) the recovery efficiency (NRE), defined as the amount of N absorbed by the crop per unit of N applied, and (ii) the internal efficiency (NIE), which represents the biomass or grain produced per unit of N absorbed. NUE is affected by the N rate (Barraclough et al., 2010) and several variables such as crop genotype (Giambalvo et al., 2010), soil water content (Campbell et al., 1993; Timsina et al., 2001) and the availability of other nutrients, such as sulfur (S) (Aulakh and Malhi, 2004; Salvagiotti et al., 2009).

S has been recognized as an essential element for plant growth and as an important limiting factor for crop production (Havlin et al., 2005). Generally, low organic matter content in soils, soil erosion and high nutrient removal by crops may determine S shortages (Scherer, 2001). Although these deficiencies were not common in the past, nowadays they have become widespread (Scherer, 2001).

Several studies have analyzed the interaction between N and S with regard to crop production. Most of them only considered the effect of this interaction on shoot mass or grain yield (Randall et al., 1981; Reneau et al., 1986; Salvagiotti and Miralles, 2008) without evaluating the effect on NUE. An exception to this is the work of Salvagiotti et al. (2009) who determined that S addition increased wheat biomass and grain yield, and reported a positive interaction between N and S, which was reflected in a greater NUE. This increase in NUE was attributable to greater NRE without alterations in the NIE. However, the mechanisms by which S affects the NUE and its components remain unclear. Salvagiotti et al. (2009) hypothesized that the addition of S

promotes a larger soil exploration by the roots, allowing for greater N uptake (Nu). This assumption was based on the extrapolation of the response of barley (Atkinson, 1990) and wheat (Katterer et al., 1993; Mandal et al., 2003) root growth to N additions. These authors also discussed that for a specific root:shoot ratio, the observed increase in shoot mass caused by S fertilization could also generate an increase in root growth.

Even if the previous hypothesis were accepted, the root growth pattern under different nutrient availability is nutrient-specific. For example, deficiencies of N (Hirai et al., 2004; Scheible et al., 2004) and phosphorus (Cakmak et al., 1994; Sánchez-Calderón et al., 2006) result in increases in root:shoot ratio, which is in part opposite to the hypothesis of Salvagiotti et al. (2009). In contrast, potassium (Cakmak et al., 1994; Zhao et al., 2001) and magnesium-deficient plants (Fischer et al., 1998; Hermans and Verbruggen, 2005) rarely increase their root biomass under deficient conditions. The information regarding the effect of different S rates on cereal root growth is scarce.

It is accepted that root growth and distribution is an important trait controlling plant NRE under suboptimal N availability. But under ample N supply, NRE mainly depends on shoot growth rate (Devienne-Barret et al., 2000). This indicates that in the latter condition, S deficiency alleviation may improve NRE mainly by a greater plant growth rate rather than by increasing root growth as Salvagiotti et al. (2009) proposed.

The aim of this study is to analyze the effects of two S fertilizer rates on (i) shoot growth, S uptake (Su) and Nu and NUE and its components, and (ii) root growth and architecture and its relations with Su and Nu.

## **2 Materials and methods**

### **2.1 Growth conditions**

The experiment was carried out at the Balcarce INTA Research Station, located in the

south east of Buenos Aires Province, Argentina (37° 45' S, 58°18' W). Seeds of *Triticum aestivum* L., cv. Baguette 9 were germinated on moistened filter paper in darkness at 21 °C and transferred to pots 24 hours later. Six seeds were sown in each pot. After they emerged, plants were thinned to three per pot. This density was approximately 380 plants m<sup>-2</sup>, recommended for field crops. Wheat plants were cultivated in plastic pots of 10 cm diameter and 60 cm deep placed in a greenhouse, from August 10<sup>th</sup> to December 20<sup>th</sup>, when wheat is usually grown in the region. Pots were placed on a table inside the greenhouse, and their contours were covered with white plastic to prevent the sun's rays from directly reaching their surface.

The substrate consisted of a mixture of perlite, vermiculite and soil in a 3:3:1 volume proportion. Soil was collected from the surface layer (0 to 20 cm) of a local Typic Argiudoll with the following characteristics: clay-loam texture, organic matter 45.6 g kg<sup>-1</sup>; pH 5.5; available phosphorus (Bray and Kurtz I) 25.5 mg kg<sup>-1</sup>; N-NO<sub>3</sub><sup>-</sup> 5.0 mg kg<sup>-1</sup>; y S-SO<sub>4</sub><sup>-2</sup> 4.9 mg kg<sup>-1</sup>. Soil was sieved (5 mm) and recognizable crop residues and roots were eliminated.

## 2.2 Treatments

Two different rates of S were evaluated (+S and -S), in a completely randomized design with four replications. Nutrients were supplied by a modified Hoagland nutrient solution (Taiz and Zeiger, 2010) containing the following ions (g L<sup>-1</sup>): 1.18 Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O; 0.51 KNO<sub>3</sub>; 0.49 MgSO<sub>4</sub>·7H<sub>2</sub>O; 0.14 KH<sub>2</sub>PO<sub>4</sub>; 0.07 FeEDTA; 0.0134 H<sub>3</sub>BO<sub>3</sub>; 0.0091 MnCl<sub>2</sub>; 0.0006 ZnCl<sub>2</sub>; 0.0003 CuCl<sub>2</sub>; 0.0001 Na<sub>2</sub>MoO<sub>4</sub>. The solution was diluted in distilled water in a ratio of 1:10, and was applied to the pots two or three times a week in order to maintain a substrate moisture content close to field capacity.

The two different S levels were generated as follows: for +S, the nutrient solution was used with the original S concentration and for -S, the S concentration from

the Hoagland solution was reduced to 3%. The removal Mg in  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  was compensated by the addition of  $0.1847 \text{ g L}^{-1}$  of  $\text{Cl}_2\text{Mg}$ . Table 1 indicates the amounts of S and N applied to each treatment at each phenological stage.

[Table 1 near here]

### 2.3 *Shoot and root biomass measurements*

Plants were collected 57 days after planting (DAP), at flag leaf visible stage (Z39, Zadoks et al., 1974); 83 DAP, at inflorescence emergence (Z51); and 127 DAP, at physiological maturity stage (Z92). Shoot mass was determined at the three stages. However, root mass was measured only at Z39 and Z51, because at Z92 plant biomass to pot volume ratio was greater than the  $2 \text{ g L}^{-1}$  threshold proposed by Poorter et al. (2012) to not negatively affect root growth.

Leaf area was measured at Z39 and Z51 using an area meter (LI-COR 3100, Licor Inc, Lincoln, Nebraska, USA). At Z92, grains were separated from the spike manually. Shoot, root and grain samples were oven dried at  $60 \text{ }^\circ\text{C}$  until they reached constant weight and then were weighed.

### 2.4 *Root morphological measurements and calculations.*

Root morphological parameters were determined using an EPSON Expression 1000XL scanner. Individual root systems were spread out on a clear tray with water and placed on a flat-bed scanner. Root length, area, mean diameter and number of tips were determined using the WinRHIZO software (Regent instruments Inc, Quebec, Canada). Following the protocol described by Ostonen et al. (2007), the relative root length ( $\text{cm g}^{-1}$ ), the relative root area ( $\text{cm}^2 \text{ g}^{-1}$ ) and the relative number of tips ( $\text{tips g}^{-1}$ ) were calculated. These indicate the root length, surface and number of tips produced per unit of root mass respectively.

## 2.5 *Biomass nitrogen and sulfur content.*

Dried samples were ground and sieved. N and S were quantified using the Dumas method, which consists of the combustion of the sample at 950 °C and 1350 °C, respectively, with subsequent detection by thermal conductivity using a TruSpec CNS analyzer (LECO, 2013). Nu and Su were calculated as the product between biomass and N and S content, respectively.

Nitrogen use efficiency was calculated as:

$$\text{NUE} = \text{NRE} * \text{NIE} \text{ (Eq. 1)}$$

$$\text{NUE} = \text{SM}/\text{Na} = \text{Nu}/\text{Na} * \text{SM}/\text{Nu} \text{ (Eq. 2)}$$

where SM is shoot mass, Na is N applied with the nutrient solution and Nu is N uptake. Considering that NIE could be represented as the inverse of N concentration (N%), NUE could be expressed as:

$$\text{NUE} = \text{SM}/\text{Na} = \text{Nu}/\text{Na} * 1/\text{N\%} \text{ (Eq. 3)}$$

Nitrogen use efficiency for grain production (NUEg) was calculated including a biomass harvest index:

$$\text{NUEg} = \text{GY}/\text{Na} = \text{Nu}/\text{Na} * 1/\text{N\%} * \text{GY}/\text{SM} \text{ (Eq. 4)}$$

where GY is the grain yield. All units were expressed as g pot<sup>-1</sup>.

## 2.6 *Statistical analyses*

Analysis of variance was made using the GLM (General Linear Model) procedure, included in the Statistical Analysis System software (SAS Institute, 1985). Significance levels used were 0.05 and 0.1. When significant treatment differences were found, mean difference test (LSD) was used.

### 3 Results and Discussion

#### 3.1 Shoot growth and grain yield

At Z39, the S fertilized treatment accumulated 20% more shoot mass ( $p < 0.05$ ), and at Z51, 14% more ( $p < 0.1$ ) compared to the S-deficient plants (Table 2). Leaf area was similar between treatments at the first sampling date (Table 2), but it was 15% higher at Z51 with the full S rate ( $p < 0.05$ ). These results are in line with previous experiments which indicate that a shortage in S provision caused less shoot growth and leaf area in wheat (Salvagiotti and Miralles, 2008;) and barley (De Bona et al., 2011). Similarity between treatments in leaf area at Z39 may be explained because leaves were not fully expanded at this stage.

[Table 2 near here]

At physiological maturity (Z92), plants that received full S rate also produced more shoot mass ( $p < 0.05$ ) and more grain yield ( $p < 0.1$ ) (Table 3). Within yield components, grain weight was similar between treatments so the differences were a consequence of the number of grains, which was 16% greater in the +S treatment ( $p < 0.05$ ) (Table 3). This was also a consequence of a higher number of grains per spike (20% more), while there were no differences in the number of spikes per pot (data not shown). These results are in line with other studies that shown that the S starvation primarily affects the number of grains per spike, indicating a negative effect on the initiation of spikelet and/or floret or an increase in the mortality of florets (Archer, 1974). Zhao et al. (1999) suggest that other yield components, such as the number of tillers or grain weight, are less affected by S availability unless the deficiency is severe.

[Table 3 near here]



### 3.2 *Sulfur and nitrogen uptake*

Sulfur concentration (S%) in shoots and Su were greater in the +S treatment at Z39 and Z92 ( $p < 0.05$ ) (Table 4). At Z51 and Z92 (grain) there was no difference in S% between treatments. However, plants that received full S rate had greater Su ( $p < 0.05$ ) (Table 4).

Nitrogen concentration in shoots was higher at Z39 ( $p < 0.1$ ) and Z51 ( $p < 0.05$ ) in the -S treatment (Table 4). However, Nu was similar (Table 4) as a consequence of the greater shoot mass of the +S treatment (Table 2). At Z92, N% in shoots did not differ between treatments, but the greater biomass of the +S treatment caused a greater Nu. There were no differences between treatments in N% or Nu in grain.

[Table 4 near here]

Similar results were reported by Zhao et al. (1996) and Fitzgerald et al. (1999) for S% and Su. They observed that S additions to wheat plants increased Su due to an increase in shoot mass production and higher S%. Several experiments reported increases in S% when S changes from deficient to sufficient conditions (Zhao et al., 1999; Reussi Calvo et al., 2011), and this was commonly associated with increases in cysteine and methionine concentration which improves grain quality in wheat.

The greater shoot N% in S-deficient plants at Z39 and Z51 coincides with the observations of Barney and Bush (1985) for tobacco and Varin et al. (2010) for white clover under S-deficient conditions. De Bona et al. (2011) determined that S-deficient barley plants fertilized with nitrate contained significantly higher levels of N in the form of nitrate, but also an accumulation of asparagine. Otherwise, they reported that S-deficient plants under urea supply did not increase asparagine concentration, but it was compensated for by a greater glutamine concentration. This is also in line with the reports of Carfagna et al. (2011), and could be explained by an increase in proteolysis and a reduction in protein synthesis rates (Watanabe et al., 2010). Coinciding with our

results, Abdallah et al. (2010) did not observe differences in Nu between oilseed rape plants growing under different S rates.

At Z92, N% was similar between treatments (Table 4), so the greater Nu in the +S treatment was mainly caused by the increase in shoot mass (Table 3). Although several experiments give evidence of the direct link between  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$  uptake at root level (Clarkson et al., 1989), the increase of the plant growth rate is proposed as the main cause of the increase of Nu when a S stress is alleviated. Westerman et al. (2001) determined that the enrichment of the atmosphere with  $\text{H}_2\text{S}$  increased shoot mass growth of S-deficient *Brassica oleracea* L. plants and consequently the  $\text{NO}_3^-$  uptake by the roots.

### 3.3 Nitrogen use efficiency

Nitrogen use efficiency was greater in plants that received full S rate at all sampling dates ( $p < 0.05$  for Z39 and Z92, and  $p < 0.1$  for Z51) (Table 5). At Z39 and Z51, NIE was greater in S-fertilized treatments while there was no difference in NRE. In contrast, at Z92, the difference in NUE was attributable to NRE, as no differences were observed in NIE (Table 5).

[Table 5 near here]

These results should be interpreted considering all the components of the NUE. Thus, based on Eq. 2 it could be inferred that NRE is directly related to Nu, and NIE is inversely related with N% in shoot. Thus, at Z39 and Z51, the reduction of NIE in the S-deficient plants was mainly explained by the diminution in shoot mass and the increase in N%. Moreover, NRE was similar between treatments because the greater N% in plants of the -S treatment was compensated by the reduction in shoot mass, resulting in similar values in Nu.

At Z92, NUE was greater in plants that received full S rate as a consequence of a greater NRE (Table 5). Following Eq. 2, NRE was higher in +S treatment as a consequence of greater Nu. This increase was mainly caused by an increase in shoot mass, as it was determined a tendency to less N% (-7.4%) (Table 4). The analysis of the NUEg shows that the NRE was the same as calculated for shoot mass, so the change in the harvest index ( $p < 0.05$ , 0.31 and 0.29 for -S and +S respectively) slightly affected NIE.

Our results partially differed from those reported by Salvagiotti et al. (2009), who performed six experiments in the Argentinean Pampas. They determined that S fertilization increase grain yield, Nu and consequently NRE and NUE in all the experiments. However, NIE did not have a consistent tendency, as in some experiences it was greater for the S-fertilized treatment while it was lower in others. Interpretation of Eq. 2 indicates that any of both components of NUE could be increased by S fertilization, depending on the relative effect of S on shoot growth and Nu.

#### **3.4 Root growth and its relation with nitrogen and sulfur absorption**

Root mass and area were similar between treatments at Z39 but length and number of tips increased ( $p < 0.05$ ) and root diameter lowered ( $p < 0.1$ ) (Table 6) when S was restricted. Meanwhile, at Z51 the treatment that receives the full S rate produced more mass and also greater area and root diameter ( $p < 0.05$ ) than the S-deficient. The length and number of tips did not differ between treatments (Table 6).

[Table 6 near here]

The differences between treatments in root mass should be analyzed considering also the patterns in shoot mass. Therefore, at Z39 the shoot:root ratio was higher for the +S treatment ( $p < 0.05$ ) (ratios 1.44 and 1.77 for -S and +S, respectively). This is in line with several previous reports which conclude that when a nutrient limits plant growth,

the proportion of carbohydrates partitioned from shoots to roots increases (Thornley, 1972). In particular, there is scarcely any evidence about the effect of S availability on shoot:root ratio, and this experiment indicates that in early developmental stages, wheat plants define strategies to optimize S capture similar to those reported for *Medicago sativa* L. (Wang et al., 2003) and *Brassica oleracea*; (Buchner et al., 2004). Hermans et al. (2006) reported that N and phosphorus-deficient plants also reduced the shoot:root ratio compared to non-deficient plants. However, they determined that potassium and magnesium-starved plants did not modify this ratio compared to non-stressed plants. This reduction in shoot:root under N and phosphorus deficiencies were linked to the greater transport of sugars from shoots to root (Hermans et al., 2006). In contrast, at Z51 shoot:root ratio was higher in the treatment with the shortage of S ( $p < 0.05$ ) (ratios 3.48 and 2.88 for -S and +S, respectively). Similarly, Bonifas et al. (2005) reported that maize plants grown under N starvation evidenced less shoot:root ratio prior to the V13 stage (Ritchie and Hanway, 1982). But from V13 to R2 the ratios were similar. Gedroc et al. (1996) and McConnaughay and Coleman (1999) also report a similar pattern for non-cultivated species.

Most of the information regarding shoot and root allometry refers to early development stages of crops. However, when the growth of reproductive structures begins, the shoot:root ratio increases greatly and rapidly (Parsons and Robson, 1981; Barraclough, 1984) as they constitute a priority sink in plants (Gregory and Atwell, 1991). Based on this information, it could be proposed that as spike growth increased, plants that received less S prioritized high shoot growth rates in relation to root. In contrast, plants that received full S rates could reach high shoot rates but also could partition more carbohydrates to roots.

Root length and number of tips were greater in the -S treatment at Z39 ( $p < 0.05$ ) (Table 6). These show that wheat plants not only modified carbon partitioning to roots, but also root architecture in order to increase soil exploration. At Z51, root mass was markedly higher in the +S plants ( $p < 0.05$ ), but length and number of tips were similar between treatments (Table 6). This indicates that the pattern to more soil exploration under S deficiency was maintained at this stage. Similarly, Gilbert and Robson (1984) reported that S addition increased root mass and area, but had no effect on root length in ryegrass plants. In addition, root diameter was lower when S was restricted ( $p < 0.1$  and  $p < 0.05$  at Z39 and Z51, respectively) (Table 6). Similar results were reported for N restriction by Hackett (1972) in wheat and Drew et al. (1973) in barley. All these patterns can be interpreted as a general strategy to improve S acquisition. Hoefgen and Nikiforova (2008) and Amtmann and Armengaud (2009) suggested that these patterns are presumably associated with an increase in auxin levels derived from the glucosinolate catabolism that occurs in S-deficient plants.

Plants under S shortage showed 14% and 34% more root length per unit of mass at Z39 and Z51, respectively ( $p < 0.05$ ) (Figure 1). In addition, the number of tips per unit of root mass was 17% and 33% higher for -S treatment at Z39 and Z51, respectively ( $p < 0.05$ ; Figure 1). The relative area was higher only at Z39 for -S treatment ( $P < 0.1$ ), but no differences were observed at Z51 (Figure 1). These patterns indicate that S-deficient plants displayed mechanisms to increase the efficiency of carbon allocation for soil exploration. Schippers and Olf (2000) reported similar results for the relative root length of six herbaceous species grown in a N-deficient substrate. Trubat et al. (2006) also reported a similar pattern for *Pistacia lentiscus* (L.) under phosphorus deficiency. In addition, in absolute terms, at Z51 a general trend was determined for more soil exploration in plants that receive the full S rate (Table 6). This

is in line with the hypothesis of Salvagiotti et al. (2009) that relates the increases in shoot mass with increases in root mass and soil exploration.

[Figure 1 near here]

Sulfur uptake per unit root mass was greater for the +S treatment at Z39 ( $p < 0.05$ ), and no differences between treatments were determined at Z51 (Figure 2). In addition, plants that received the full S rate absorbed more S per unit of root length at Z39 and Z51 ( $p < 0.05$ ) (Figure 2), and per unit of root area at Z39 ( $p < 0.05$ ) (Figure 2). Nitrogen uptake per unit root mass was similar between treatments at Z39 but was greater for the -S treatment at Z51 ( $p < 0.05$ ) (Figure 2). It was also determined that the treatment that receives the full S rate absorbed more N per unit root area and length at Z39 ( $p < 0.05$ ), but the tendency was the opposite at Z51, when -S treatment had more Nu per unit root area ( $p < 0.05$ ) (Figure 2).

[Figure 2 near here]

Sulfur uptake per unit root area and length was greater in +S treatment at Z39, when plants prioritized shoot growth (Table 2) instead of root growth. At Z51, plants of the +S treatment increased root growth and area compared to the -S maintaining similar values of Su per unit of root mass and area or even higher as occurred with Su per unit of root length (Figure 2). Similar results were reported by Gilbert and Robson (1984), who found that the S application increased Su per unit root mass, length and area in ryegrass and clover. These patterns indicate that Su is mainly driven by shoot growth (S demand) and not by root growth or architecture.

At Z39, Nu per unit root mass, area and length was similar to that described for Su (Figure 2). But at Z51, the plants of the +S treatment had lower Nu per unit of root mass and area than S-deficient plants (Figure 2). This indicates that Nu increased in a lower proportion than root growth or expansion in the +S plants. These results differed

from those reported by Varin et al. (2010), who determined similar Nu per unit of root area in white clover plants growing under different S rates. However, our results should not be considered as negatives because for plants growing under field conditions, a greater root growth in response to S application could be beneficial for increasing Nu if N availability is limited by quantity or location in the soil profile (Wiesler and Horst, 1994).

#### **4 Summary and conclusions**

The results of this experiment indicates that wheat plants grown with the full S rate produced more shoot mass and grain yield than those of the -S treatment. The greater S rate increased NUE through a positive effect on both NIE and NRE. We propose that either of the components of NUE could be increased by S addition, depending on the relative effect of S on shoot growth and Nu.

Meanwhile, plants grown under S starvation developed mechanisms that tended to increase soil exploration which were evident in absolute terms at Z39 (length and number of tips) and relative to root mass at Z39 and Z51. This is one of the main contributions of our investigation, given the scarcity of information regarding the S effect on cereal root growth. However, at Z51 root growth in absolute terms tended to be greater for +S treatment, which could be considered as beneficial for nutrient uptake.

As a general tendency, Su per unit root mass, area and length was greater in plants grown under full S rate. A similar pattern was determined for Nu at Z39, but the opposite occurred at Z51, as S-deficient plants had a greater Nu per unit of root mass, area and length. This indicates that +S plants increased Nu in a lower proportion than root growth or expansion and suggests that Nu is mainly controlled by shoot growth rate and not by the root mass or architecture.

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Table 1. Sulfur (S) and nitrogen (N) addition ( $\text{mg pot}^{-1}$ ) at each phenological stage (Z39, Z51 and Z92; Zadoks *et al.*, 1974) at two S rates (-S and +S).

Table 2. Shoot mass ( $\text{g pot}^{-1}$ ) and leaf area ( $\text{cm}^2 \text{pot}^{-1}$ ) at Z39 and Z51 stages (Zadoks *et al.*, 1974) for the two S rates (-S and +S). SE indicates the standard error of the mean.

Table 3. Shoot mass ( $\text{g pot}^{-1}$ ), grain yield ( $\text{g pot}^{-1}$ ), number of grains ( $\text{grains pot}^{-1}$ ) and grain weight (mg) at Z92 stage (Zadoks *et al.*, 1974) for the two S rates (-S and +S). SE indicates the standard error of the mean.

Table 4. Sulfur concentration (S%), sulfur uptake (Su;  $\text{mg pot}^{-1}$ ), nitrogen concentration (N%) and nitrogen uptake (Nu;  $\text{mg pot}^{-1}$ ) in shoots at Z39, Z51 and Z92 stages and grains at Z92 (Zadoks *et al.*, 1974) for the two S rates (-S and +S). SE indicates the standard error of the mean.

Table 5. Nitrogen use efficiency ( $\text{NUE} = \text{kg shoot mass kg N applied}^{-1}$ ), nitrogen recovery efficiency ( $\text{NRE} = \text{kg de N uptake kg N applied}^{-1}$ ) and nitrogen internal efficiency ( $\text{NIE} = \text{kg shoot mass kg of N uptake}$ ), at Z39, Z51 and Z92 stages (Zadoks *et al.*, 1974) for the two S rates (-S and +S). At Z92, NUE and its components are presented for shoot biomass and grain. SE indicates the standard error of the mean.

Table 6. Root mass ( $\text{g pot}^{-1}$ ); length ( $\text{cm pot}^{-1}$ ); area ( $\text{cm}^2 \text{pot}^{-1}$ ); mean diameter (mm) and number of tips ( $\text{tips pot}^{-1}$ ) at Z39 and Z51 stages (Zadocks *et al.*, 1974) for the two S rates (-S and +S). SE indicates the standard error of the mean.

Figure 1. Relative root length ( $\text{cm g}^{-1}$ ); relative root area ( $\text{cm}^2 \text{g}^{-1}$ ) and relative number of tips ( $\text{tips g}^{-1}$ ) at Z39 and Z51 stages (Zadoks *et al.*, 1974) for the two S rates (-S and +S). Vertical bars indicate the standard error of the mean. For each growth stage, similar letters on top of the columns indicate statistically equal values at  $P < 0.05$  using Fisher-protected LSD. \* Indicates  $p < 0.1$ .

Figure 2. Sulfur uptake per unit root mass (Su:RM,  $\text{mg g}^{-1}$ ), per unit root length (Su:RL;  $\text{mg cm}^{-1} \times 1000$ ) and per unit root area (Su:RA;  $\text{mg cm}^{-2} \times 100$ ), and nitrogen uptake per unit root mass (Nu:RM,  $\text{mg g}^{-1}$ ), per unit root length (Nu:RL;  $\text{mg cm}^{-1} \times 100$ ) and per unit root area (Nu:RA;  $\text{mg cm}^{-2} \times 10$ ) at Z39 and Z51 stages (Zadoks *et al.*, 1974) for the two S rates (-S and +S). For each growth stage, similar letters on top of the columns indicate statistically equal values at  $P < 0.05$  using Fisher-protected LSD. Vertical bars indicate the standard error of the mean.