



Synergistic Impact of Co-applied Micronized Sulfur and Nitrogen on Agronomical Traits of a Modern Spring Wheat Cultivars Grown in Alkaline Soil

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Wheat (*Triticum aestivum* L.) uptakes only 40 to 60% of the soil available nitrogen. Sulfur deficiencies depress both nitrate uptake and nitrate reductase activity resulting in low nitrogen use efficiency. Soil and foliar supplied sulfur in combinations with foliar nitrogen were used on three of the modern and commercially grown wheat cultivars. The experiment was conducted in two consecutive growing seasons; 2017 and 2018 in a high pH soil, 8.55. Our results indicated that 714 kg ha⁻¹ of pre-planting sulfur (SS) and spraying twice with 6.66% urea and 2.2% micronized sulfur (S₁FS₁N₁) during stages 13 & 41 on Zadok's scale, increased grain yield, total protein content, straw yield and plant height by 31.58, 26.09, 18.37 and 7.9% respectively. The results indicated a significant and positive impact sulfur-nitrogen combinations when applied on top of pre-planting applied sulfur. However, foliar sprayed sulfur had a more substantial effect on all traits, compared to the pre-planting sulfur or the foliar sprayed N, suggesting interference effect of the alkaline soil with the amount of sulfur recovered from the soil. When N and S foliar were applied simultaneously, a more substantial increase in grain yield, plant height, straw yield and total protein content was observed, suggesting a synergistic effect between these two elements. We attributed the positive effect of S₁FS₁N₁ on improving photosynthates translocation from the sources to sinks. In addition to improving nitrogen use efficiency while reducing the plant content of NO⁻³ by optimizing the S/N

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ratio and reducing sulfur deficiency. Based on our results, we concluded that the foliar application of micronized S has the potential to improve the overall performance of wheat plants. Thus, we recommend enriching nitrogen and phosphate fertilizers with sulfur for alkaline soils.

Keywords: Synergistic impact; nitrogen and sulfur interaction; *Triticum aestivum*; nutrient uptake; photosynthates translocation.

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important cereal crop for human food, as it is considered the primary source of starch and energy for 35% of the world's population [1]. Additionally, wheat also provides substantial amounts of protein, B vitamins, dietary fibers, and phytochemicals [2]. Therefore, wheat is grown across a wide range of environments and geographic regions to secure sufficient grain for the growing human population. As wheat is grown over a wide range of environments, it is frequently exposed to adverse growing conditions such as drought, heat, salinity, and high or low soil pH.

Soil pH (scale from 0 to 14) less than seven is considered acidic, and pH more than seven is alkaline. While both acidic and alkaline pH affects soil nutrients availability, nitrogen immobilization and Losses are greater in alkaline soils compare to the acidic ones [3]. Furthermore, acidic soils tend to retain sulfur (SO_4) better than the alkaline soils [4,5]. Consequently, the requirements of plants grown in alkaline soils from sulfur and nitrogen are higher than these required for the acidic soils [6].

Nitrogen is an essential component for amino acids, which are the building units of protein. Thus, nitrogen is considered indispensable fertilizer to increase grain protein content. Several experiments showed highly significant effects of nitrogen on protein content, grain yield, and yield components on wheat and other crops [7–11]. Appropriate application of nitrogen fertilizers could optimize crop use. Even though soil application of nitrogen is the predominant method, there are other situations where a foliar application may be considered. One of these situations is the interest in reducing the total nitrogen inputs and the amount of nitrogen runoff during crop production [12]. Another case is when the grower urgently needs to apply nitrogen late in the crop life cycle, and there may not be sufficient time for the plant to absorb the soil nitrogen by the roots, and transfer it from the roots to the growing points where it is needed.

Under flood irrigation, foliar application of nitrogen is found to be more efficient as it reduces nitrogen runoff [13].

Sulfur is another necessary element for plant growth and production. Sulfur is involved in amino acids, proteins, vitamins, and many secondary metabolites syntheses [14,15]. Moreover, several studies have shown that sulfur increased wheat grain yield, gluten, cysteine, and methionine [16]. Nonetheless, sulfur levels in the soil were found to be declining during the last decade [17]. The reasons for sulfur declining are; using new, highly purified fertilizers with less sulfur [17,18]. For example, the ordinary superphosphate (0-20-0) contains 11-12% sulfur, while newer triple superphosphate (0-46-0) contains less than 3% sulfur [19]. Using high productive cultivars which extract more sulfur from the soil compared to the low yielding old cultivars, might lead to a sulfur decline in the soil [20]. Correcting sulfur deficiency depends on the soil conditions, several products are available to correct for sulfur deficiency, i.e., ammonium sulfate, ammonium thiosulfate, elemental sulfur, and calcium sulfate [20].

Moreover, the time of supplying nutrients to the plants is one of the factors that affect nutrient use efficiency because the rates of plant's nutrient uptake differ at various growth stages [21]. The maximum nitrogen and sulfur uptake by wheat plants were recorded during the flowering stage [22,23]. Total grain protein content in wheat significantly increased by applying 10 kg ha^{-1} of nitrogen fertilizer at the pre-heading wheat stage [24]. Maximum amounts of nitrogen, sulfur, and potassium uptake were recorded at the beginning of the wheat flowering stage to medium milk stages [25]. Foliar applied nitrogen and sulfur found to have synergistic effects on increasing their assimilation in grain and can improve bread baking qualities [26]. Nitrogen absorption found to be negatively affected by sulfur deficiency and affects the accumulation of the total protein [27]. The interaction between sulfur and nitrogen nutrients directly related to the alteration of physiological and biochemical responses of crops [14].

Inorganic fertilizers such as nitrogen are being used excessively worldwide to increase crop production and to provide sufficient food for the growing population [18,28], whereas a measurable reduction of sulfur in the soil is observed [6,29,30]. Thus, sulfur is gradually becoming a yield-limiting factor for several crops, including wheat [31]. Insufficient sulfur supply reduces crop productivity, diminishes crop quality, impairs the nitrogen utilization efficiency, and thus increased the undesired nitrogen losses to the environment [32]. Furthermore, the soil used in the present investigation is alkaline (pH 8.5 ± 0.5). Plants that were grown in this soil develop symptoms of sulfur deficiency [33]. Sulfur deficiency symptoms is characterized by the yellowing first on the younger or uppermost leaves, while N deficiency symptoms is characterized by yellowing in older leaves [33]. However, both deficiencies can appear as stunted plants with general yellowing of leaves thus, several growers often are mistaken sulfur deficiency for nitrogen deficiency [34]. This confusion between sulfur and nitrogen is partly because historically, S deficiency was most common on irrigated, sandy soils that are low in organic matter and subject to leaching [35].

Additionally, sulfur deficiency symptoms are intensified as high nitrogen fertilization levels are being applied [14]. Consequently, plants uptake an excessive amount of nitrogen greater than its real requirements and suffer from sulfur deficiency [36]. Therefore, plants store that excessive amount of nitrogen in the form of nitrates, nitrites, and nitrosamines [33]. That excessive amount of nitrogen might have no negative effect on the plant [36]. However, plant products with such high levels of nitrogen forms had severe health and environmental ramification [36]. Furthermore, It was estimated that one Kg deficiency of S in the soil decreases N uptake by 10 kg [33,37].

Therefore, the objective of the current study was to assess the effect of soil sulfur application and foliar co-applied sulfur and nitrogen on high yielding and commercially grown wheat cultivars grown in alkaline soil.

2. MATERIALS AND METHODS

2.1 Plant Materials and Treatments

In this study, three wheat cultivars, i.e., "Giza168", "Gimmiza9", and "Sids13" were planted in two successive growing seasons (2016/2017 and 2017/2018; hereafter referred to

by their harvest seasons, 2017 and 2018) in Elkhazan location (31°05'35.1"N, (30°45'19.4" N, 30°29'04.8" E), Behera governorate, Egypt. Within each growing season, a pre-planting calcium sulfate (CaSO_4) was added during the soil preparation in two levels, i.e., 0 kg ha^{-1} (SS_0) and 714 kg ha^{-1} (SS_1). Furthermore, two levels of sulfur, i.e., 0 kg ha^{-1} (S_0) and 10.8 kg ha^{-1} (S_1) of micronized water-soluble elemental sulfur (Micronit, 80%, Agrochim, Egypt), were combined with two levels of nitrogen; 0 kg ha^{-1} (N_0) and 30 kg ha^{-1} (N_1) of urea, to form four levels of sulfur-nitrogen foliar spray (FSN), i.e., FS_0N_0 , FS_1N_0 , FS_0N_1 , and FS_1N_1 . Each level of the sulfur-nitrogen factor was sprayed twice during two different growth stages, in which the first spray was during stage13 (three leaves emerged), while the second spray was during stage 41 (Flag leaf sheath extending). Stage 13 and Stage 41 were defined according to the Zadoks growth scale [38]. At a rate of $450 \text{ Liter hectare}^{-1}$ (l ha^{-1}), an electric sprayer with one upfront fine droplet nozzle was used to provide accurate plot coverage and deposition. Furthermore, spraying was conducted during dry and sunny weather conditions. The first rainfall after the foliar application was three weeks and two weeks later, during the first and second growing seasons, respectively.

2.2 Experimental Design

A split-split plot, with main plots arranged as a randomized complete block design (RCBD) and three replicates, was used [39]. In which Factor (A) or the main plots were randomly assigned to the levels of the pre-planting added sulfur (SS_0 and SS_1). Factor (B) or the subplots were randomly assigned to the sulfur-nitrogen combinations (FSN), i.e., FS_0N_0 , FS_1N_0 , FS_0N_1 , and FS_1N_1 . Factor (C) or the sub subplots were randomly assigned to cultivars, i.e., Giza168, Gimmiza9, and Sids13. Each cultivar was planted in an experimental unit (plot) of 2.5 meters long and four rows wide with 20 cm between rows.

2.3 Experimental Conditions

Across the two growing seasons, the preceding crop was maize (*Zea mays* L.), in which residuals were incorporated into the soil. Soil samples (0–30 cm depth) were collected each season during the first half of November directly before planting and analyzed according to Klute et al. [42]. The main soil physical and chemical properties are presented in Table 1. Within each trial, standard

agronomic practices, including recommended nitrogen, phosphorus, and potassium applications, were followed. Experiments were conducted under weed-free conditions, and in both growing seasons, fungicide recommendations in the growing region were followed. Plots of each genotype were sown on November 12, 2016, and November 16, 2017, during the first and second growing seasons, respectively.

2.4 Phenotypic Measurements

Plant height (PH, cm) was measured on a random sample of five plants in each plot as the distance from the soil surface to the tip of the spike (awns excluded) at harvest time. After full

maturity, plants in each plot were cut at 5 cm above soil surface and left to dry in the middle of the plot. After three days, plants from each plot were threshed separately using locally made single plot thresher, in which seeds and straw were collected, paged, numbered, then dried and weighed as tons ha⁻¹. Grain protein content (% GPC, g protein 100 g⁻¹ grain) was estimated using near-infrared spectroscopy (NIR) with a Perten DA7250 diode array NIR (Springfield, IL). The measurements of GPC were done in the Near Infrared region 950 – 1650 nm, and readings were processed in NetPlus software, which includes validation calculation modules, such as calculations of bias, slope, and standard errors of prediction against the reference methods.

Table 1. Main physical and chemical properties for the soil in the used location

Soil properties	Season	
	2017	2018
Physical properties		
Sand (%)	24.5	27.4
Silt (%)	21.2	22.3
Clay (%)	54.3	50.3
Organic matter (%)*	0.55	0.55
EC (dsm ⁻¹)	1.4	1.4
Ph	8.6	8.5
Saturation paste exchangeable cations (mg L⁻¹)		
Na ⁺	19	20
Ca ⁺²	90	89
Mg ⁺²	0.14	0.13
K ⁺	0.79	0.82
Chloride		
Cl ⁻	24	23

Ece: Electrical conductivity of saturated soil paste extract. *Organic matter: estimated by multiplying the organic carbon content by 1.724

2.5 Data Analysis

Analysis of variance was carried out using SAS 9.4 (SAS v9.4; SAS Institute Inc., Cary, NC, USA), by fitting the following linear model [39]:

$$Y_{ijlm} = \mu + B_i + S_j + BS_{ij} + F_l + SF_{jl} + BSF_{ijl} + G_m + GS_{mj} + GF_{ml} + GSF_{mjl} + \varepsilon_{ijlm}$$

Where Y_{ijlm} is the response measured on the $ijlm$ plot, μ is the overall mean, B_i is the effect of the replicate. S_j is the effect of j^{th} soil sulfur added before planting, BS_{ij} is a random error effect for factor A (Error a), F_l is the effect of the l^{th} level of the foliar application, SF_{jl} is the interaction between j^{th} sulfur level and l^{th} foliar application, BSF_{ijl} is a random error for the foliar applications and the interaction between soil sulfur and the foliar application (Error b). G_m is the effect of m^{th} cultivar, GS_{mj} is the interaction effect between m^{th} cultivar and j^{th} sulfur, GF_{ml} is the interaction effect among m^{th} cultivar and l^{th} foliar application. GSF_{mjl} is the interaction effect for m^{th} cultivar, j^{th} soil sulfur, and l^{th} foliar application, and ε_{ijlm} is the experimental error (Error c).

Means were compared using the least significant difference (LSD) (at P -value < 0.05), according to Gomez and Gomez [40]. Homogeneity of variance across seasons was tested following Bartlett's Test [41]. Combined analyses of variance were performed among seasons with a homogeneous variance, as outlined by Cochran and Cox [42].

3. RESULTS

3.1 Analysis of Variance

The analysis of variance for grain yield, total protein content, straw yield, and plant height are presented in Table 2. The results from the analysis of variance indicated a highly significant effect for the pre-planting soil sulfur (SS) on grain yield, protein content, and straw yield. However, a significant impact of SS was detected for plant height. Furthermore, foliar spray with sulfur and nitrogen (FSN) had a highly significant effect on the four studied traits. In contrast, wheat cultivars (C) had a highly statistically significant effect on plant height only.

Moreover, SS \times FSN interaction had highly significant effects on grain yield and protein content, while it had no significant effects on straw yield and plant height. The interaction between C \times SS had a highly significant impact on straw yield and plant height. Furthermore, C \times FSN interaction had a significant impact on plant height, but it had no statistically significant effects on grain yield, total protein content, and straw yield. Additionally, C \times FSN \times SS had a

significant and highly significant impact on straw yield and plant height, respectively (Table 2). It is also worth noting that the major sources of variance for the studied variables are coming from SS and FSN, and their interactions. At the same time, cultivars and C \times SS, C \times FSN, and C \times SS \times FSN had limited impact on the studied traits.

3.1.1 The effect of the pre-planting sulfur

Comparing the control (Neither soil nor foliar treatments; SS₀FSN₀) with soil sulfur application during the soil preparation indicated an increase in grain yield by 2.63%. Furthermore, soil sulfur also increased the total protein content by 18.84% (Table 3). Nevertheless, straw yield and plant height were increased by 8.16% and 0.72%, respectively.

3.1.2 The effect of foliar applied sulfur, nitrogen, and sulfur-nitrogen

Averages across cultivars were used to estimate the impact of foliar-applied sulfur, nitrogen, and sulfur-nitrogen foliar spray in comparison with the control. Foliar applied nitrogen increased grain yield by 2.11%, total protein content by 5.58%, straw yield by 7.76%, and plant height by 1.7%. Furthermore, spraying with sulfur alone increased grain yield increased by 13.9%, total protein content by 20.2%, straw yield by 7.3%, and plant height by 3.0%. Furthermore, sulfur-nitrogen foliar application increased grain yield, total protein content, straw yield, and plant height by 29.7, 27.1, 24.5, and 6.0%, respectively (Table 3).

Table 2. Analysis of variance for the effects of pre-planting sulfur (SS), sulfur-nitrogen foliar spray (FSN), cultivars (C), and their interactions on grain yield, total protein content (Protein), straw yield and plant height

Source of variance	df	Mean squares			
		Grain yield	Protein	Straw yield	Plant Height
Season	1	2.34	6.7	0.6	1039.69
Block (Season)	4	0.13	0.3	0.2	127.03
SS	1	8.3**	265.1**	9.7**	78.8*
Main plot error	5	0.13	0.3	0.3	11.78
FSN	3	12.7**	89.5**	10.36**	332.7**
SS \times FSN	3	1.4**	14.2**	0.7 ^{ns}	35.07 ^{ns}
Subplot error	30	0.23	1.4	0.5	31.37
C	2	0.22 ^{ns}	0.1 ^{ns}	0.2 ^{ns}	74.16**
C \times SS	2	0.16 ^{ns}	0.5 ^{ns}	0.73**	79.35**
C \times FSN	6	0.34 ^{ns}	0.6 ^{ns}	0.2 ^{ns}	32.1*
C \times SS \times FSN	6	0.22 ^{ns}	1.0 ^{ns}	0.3*	79.8**
Error	80	0.18	0.8	0.1	12.95

ns, not significant at 0.05 probability level. *, ** Significant and highly significant at 0.05 and 0.01 probability levels, respectively

3.1.3 The interaction between pre-planting add sulfur and the sulfur-nitrogen foliar spray

Comparing the control with 714 kg ha⁻¹ of sulfur applied to the soil pre-planting, topped with spraying twice with 6.66% urea (SS₁FS₀N₁) during stage 13 followed by another spray with 6.66% urea during stage 41 increased all traits studied (Table 3). In which grain yield, total protein content, straw yield, and plant height were increased by 28.95, 33.62, 22.04, and 5.06%, respectively (Table 3 & Fig. 1). Furthermore, adding 714 kg ha⁻¹ of sulfur pre-planting and spraying twice with 2.22% micronized sulfur (SS₁FS₁N₀) during stage 13 & 41 increased grain yield, total protein content, straw yield and plant height by 15.53, 35.14, 15.31 and 2.25%, respectively (Table 3 & Fig. 1). Furthermore, using 714 kg ha⁻¹ of sulfur pre-planting and spring twice with 6.66% urea and 2.22% micronized sulfur (SS₁FS₁N₁) during stage 13 & 41, increased grain yield, total protein content, straw yield, and plant height by 31.58, 26.09, 18.37 and 7.9%, respectively (Table 3 & Fig. 1). The interaction between SS × FSN had a highly significant impact on Grain yield and total protein content.

Data presented in Fig. 2 indicated that, under SS₀, FSN levels had a similar impact pattern on grain yield and the total protein content, i.e., FS₀N₀ < FS₀N₁ < FS₁N₀ < FS₁N₁. Furthermore, SS₁FS₁N₁ and SS₁FS₁N₀ outperformed the other treatments for grain yield and total protein content, respectively. Even though SS₁FS₁N₀ and SS₁FS₀N₁ were not statistically different, FS₁N₀ outperformed the other FSN levels in terms of the absolute average of the total protein content (Fig. 2).

Furthermore, the significant interaction between the pre-planting applied sulfur (SS) and the studied cultivars indicated different responses for the cultivars to SS for straw yield and plant height (Fig. 3). As such, under SS₀, Giza168 produced the highest straw yield (5.58 ton ha⁻¹) followed by Sids13 (5.32 ton ha⁻¹), then Gimmiza9 (5.24 ton ha⁻¹). Moreover, data depreciated in Fig. 3 indicated that Gimmiza9 produced the highest straw yield (5.8 ton ha⁻¹) under SS₁, while Giza168 and Sids13 produced similar Straw yield. Furthermore, the tallest cultivar under SS₀ was Giza168 followed by Sids13 and Gimmiza9. Additionally, under SS₁, the tallest cultivar was Gimmiza9, followed by Giza168 and Sids13. Sulfur-nitrogen foliar

applications had significant interaction with cultivars on plant height. The tallest cultivar under control (FS₀N₀) was Gimmiza9 (112.46 cm), followed by Giza168 (112.02 cm) then Sids13 (110.92 cm). While, when foliar nitrogen applied, the tallest cultivar was Giza168 (117.86 cm), then Sids13 (114.94 cm) followed by Gimmiza9 (112.69 cm). Foliar spray with sulfur increased Gimmiza9 plant height from 112.46 cm to 116.01 cm. However, spraying sulfur only resulted in reduced plant height in Giza168 and Sids13 compared to N sprayed. A significant increase in plant height was observed when foliar spraying with S and N combination (FS₁N₁) compared to control (FS₀N₀) in which plant height for Gimmiza9, Giza168, and Sids13, were increased by 5.75, 8.69, and 7.56 cm, respectively.

4. DISCUSSION

Alkaline soil accounts for more than 30 % of the world's soils, which poses problems to plant nutrient availability. Furthermore, alkaline soils are the dominant soil class in Egypt, in which the soil pH ranges from 7.9 to 9.3 [43,44]. Sulfur element is a cheap and readily available source of soil acidulates, which reduces pH for the alkaline soils to improve nutrient availability and, consequently, plant growth. For example, plants uptake sulfur from the soil in SO₄⁻² under pH range between 4 to 7 [33]. Additionally, during the last decade, the Egyptian chemical fertilizer industry has experienced tremendous improvement, in which they have been producing concentrated fertilizers containing little or no sulfur. For example, the triple superphosphate has replaced single superphosphate in several regions; triple superphosphate contains three folds less S, compared to single superphosphate. As a result, less S is being added to the soil [29]. Moreover, sulfur additions to the crop from atmospheric deposition were decreased during the last decade worldwide [45,46].

Several studies were conducted to identify the optimum sulfur rate that can be added to the soil to decrease pH and remedy sulfur deficiency in wheat, i.e., 714 kg ha⁻¹ [47,48]. The optimum foliar-applied nitrogen and sulfur rates, and forms, i.e., 6.66% from urea and 2.4% from micronized sulfur [49]. The optimum growth stage to successful foliar spray with N and S was also considered [50–52]. However, no reports were published to date to address the impact of pre-planting sulfur, foliar-applied micronized sulfur, and foliar co-applied

micronized sulfur with nitrogen, simultaneously on the performance of spring wheat grown in alkaline soil. Therefore, in the current experiment, we are building upon previously reported optimum rates for pre-planting added sulfur (714 kg ha⁻¹), foliar sprayed micronized

sulfur (2.4% micronized S), and N (6.66% urea) during two growth stages; 13 & 41, simultaneously, to elucidate the synergistic relationship between sulfur and nitrogen on essential physio-agronomical traits in spring wheat.

Table 3. The overall effect (%) of the pre-planting sulfur (SS), foliar sprayed sulfur-nitrogen (FSN), on grain yield (ton ha⁻¹), total protein content (%), straw yield (ton ha⁻¹) and plant height (cm), compared to the control (Neither pre-planting added sulfur nor any foliar spray)

Soil application	Foliar application	The effect in percentage (%)			
		Grain yield	Protein	Straw yield	Plant Height
SS ₀	FS ₀ N ₁	2.11	5.58	7.76	1.70
SS ₀	FS ₁ N ₀	13.9	20.2	7.3	3.0
SS ₀	FS ₁ N ₁	29.7	27.1	24.5	6.0
SS ₁	FS ₀ N ₀	2.63	18.84	8.16	0.72
SS ₁	FS ₀ N ₁	28.95	33.62	22.04	5.06
SS ₁	FS ₁ N ₀	15.53	35.14	15.31	2.25
SS ₁	FS ₁ N ₁	31.58	26.09	18.37	7.90



Fig. 1. The effect of pre-planting sulfur (SS) and foliar sprayed sulfur-nitrogen (FSN) across cultivars on grain yield (ton ha⁻¹), total protein content (%), straw yield (ton ha⁻¹) and plant height (cm)

Table 4. The effect of pre-planting sulfur (SS), foliar sprayed sulfur-nitrogen (FSN), and cultivars (C) on grain yield (ton ha⁻¹), total protein content (%), straw yield (ton ha⁻¹) and Plant height (cm)

FSN	Cultivars	Grain yield			Protein			Straw yield			Plant Height		
		SS ₀	SS ₁	Mean	SS ₀	SS ₁	Mean	SS ₀	SS ₁	Mean	SS ₀	SS ₁	Mean
FS ₀ N ₀	Gimmiza9	3.7	4.2	3.9	14.0	16.0	15.0	4.8	5.6	5.2	109.8	115.2	112.5
	Giza168	3.8	3.7	3.7	13.6	16.8	15.2	4.8	5.2	5.0	112.7	111.3	112.0
	Sids13	3.9	3.8	3.8	13.8	16.5	15.1	5.0	5.1	5.0	111.6	110.2	110.9
Mean of FS₀N₀		3.8	3.9		13.8	16.4		4.9	5.3		111.4	112.2	
FS ₀ N ₁	Gimmiza9	4.0	4.9	4.4	14.3	18.6	16.4	5.2	5.8	5.5	113.2	112.2	112.7
	Giza168	3.9	5.0	4.4	14.6	18.0	16.3	5.5	6.2	5.8	115.0	120.7	117.85
	Sids13	3.7	4.8	4.2	14.9	18.8	16.8	5.2	6.0	5.6	111.6	118.3	114.95
Mean of FS₀N₁		3.9	4.9		14.6	18.4		5.3	6.0		113.3	117	
FS ₁ N ₀	Gimmiza9	4.2	4.4	4.3	17.0	18.4	17.7	5.2	5.8	5.5	115.5	116.5	116.0
	Giza168	4.4	4.2	4.3	16.5	19.1	17.8	5.6	5.4	5.5	118.9	111.7	115.3
	Sids13	4.4	4.6	4.5	16.3	18.5	17.4	5.0	5.7	5.3	110.0	113.6	111.8
Mean of FS₁N₀		4.3	4.4		16.6	18.7		5.3	5.6		114.8	113.9	
FS ₁ N ₁	Gimmiza9	5.2	5.1	5.15	17.5	17.5	17.5	5.9	6	6.0	113.4	123.0	118.2
	Giza168	5.1	5.1	5.1	17.7	17.1	17.4	6.4	5.8	6.1	121.8	119.6	120.7
	Sids13	4.5	4.9	4.7	17.4	17.7	17.5	6.0	5.8	5.9	118.8	118.2	118.5
Mean of FS₁N₁		4.9	5.0		17.5	17.4		6.1	5.8		118.0	120.2	
Mean of SS		4.2	4.5		15.6	17.7		5.4	5.7		114.36	115.86	
LSD for SS		0.15			0.24			0.21			1.5		
LSD for FSN		0.23			0.57			0.33			2.6		
LSD for SS × FSN		0.23			0.81			0.47			3.81		
LSD for C × SS		0.24			0.49			0.18			2.06		
LSD for C × FSN		0.34			0.7			0.26			2.92		
LSD for C × SS × FSN		0.48			0.99			0.36			4.13		

LSD: is the least significant difference at 0.05 probability level

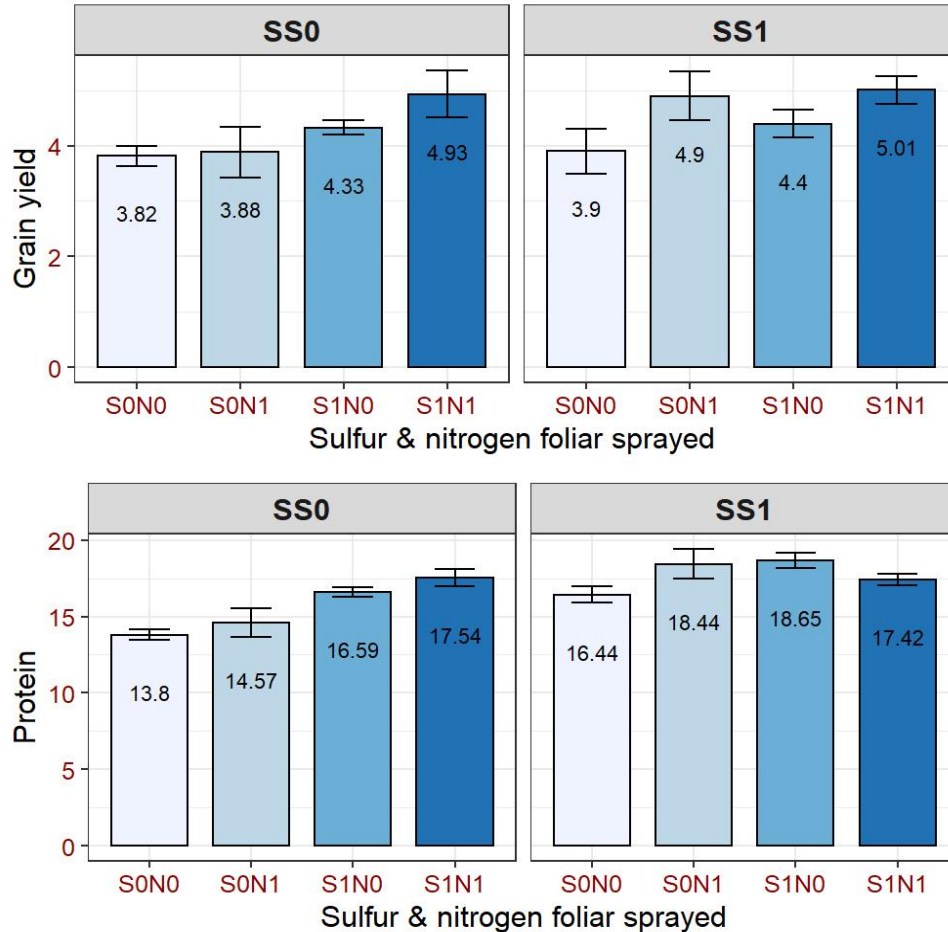


Fig. 2. The interaction between pre-planting sulfur (SS) and sulfur-nitrogen foliar spray on the grain yield (ton ha⁻¹), and protein %

Our results indicated a highly significant impact for the pre-planting sulfur on grain yield, protein content, straw yield, and plant height. The results showed that applying 714 kg ha⁻¹ of sulfur to the soil pre-planting increased all traits studied. Several researchers found that sulfur deficiency reduced grain yield and quality for several field crops, in which grain yield reduction of about 5% per year has been reported because of sulfur deficiency [53]. Significant positive effects of sulfur on grain yield and its components were also reported [54]. Our results agree with those reported [55], in which they reported a positive influence of sulfur on grain yield, yield component, straw yield, and plant height. Furthermore, the addition of sulfur to the soil showed a positive and significant effect on plant height, where supplying 30 kg sulfur ha⁻¹ resulted in taller plants at the harvest stage of wheat [56].

Moreover, pre-planting added sulfur positively affected grain yield in comparison to the control [57]. The reported positive effects of the added soil sulfur on grain production, from our study and previous reports, could be attributed to the increased Ca, K, and decreased Na in the soil because of sulfur addition [49]. Therefore, the overall positive effect of the added soil sulfur could be attributed to its acidic impact on the soil, particularly as soil alkalinity increases, which improves the availability of nutrients and increased chlorophyll content in leaves [23,58]. Additionally, soil added sulfur resulted in higher translocation of photosynthates and accounted for a more significant number of productive tillers during the reproductive stage [49]. Additionally, our results indicated a tremendous and unexpected impact on the foliar application of the micronized sulfur on the studied traits. In which foliar sprayed S increased grain yield by 13.9%,

total protein content by 20.2%, straw yield by 7.3% and plant height by 3%. Furthermore, foliar application of the micronized sulfur resulted in better grain yield compared to the soil added sulfur. As pre-planting sulfur application during the soil preparation increased grain yield by 2.63%, total protein content by 18.84%, straw yield by 8.16% and plant height by 0.72%, respectively. Sulfur oxidation can happen in the soil or on the plant leaves [59]. Recently, it was reported that stomata could absorb SO_2 and transfer it into HSO_3^- and SO_3^{2-} in the water film of the substomatal chamber. Then they will be reduced in the sulfur reduction pathway to S^{2-} . Similarly, the micronized sulfur can be received and absorbed by the plant aerial parts. The

reactivity of the micronized sulfur is a result of its small particle size, around $41 \mu\text{m}$ [60]. Due to that small particle size present in the micronized sulfur, it can be applied through the foliar route [60]. Furthermore, micronized sulfur small particle size facilitates its ability to be a source of sulfur for rapid assimilation by plants to serve as biostimulants [61] and works as a tolerance-inducing factor against biotic and abiotic stresses [62–64]. Unlike urea, micronized elemental S withstands washdown by rainfall due to its small particle size and its preferential adhesion to the inner and outer leaf surfaces. Furthermore, recently, 6 to 12% of the micronized S applied by foliar spray found to be recovered in the wheat grain [49].

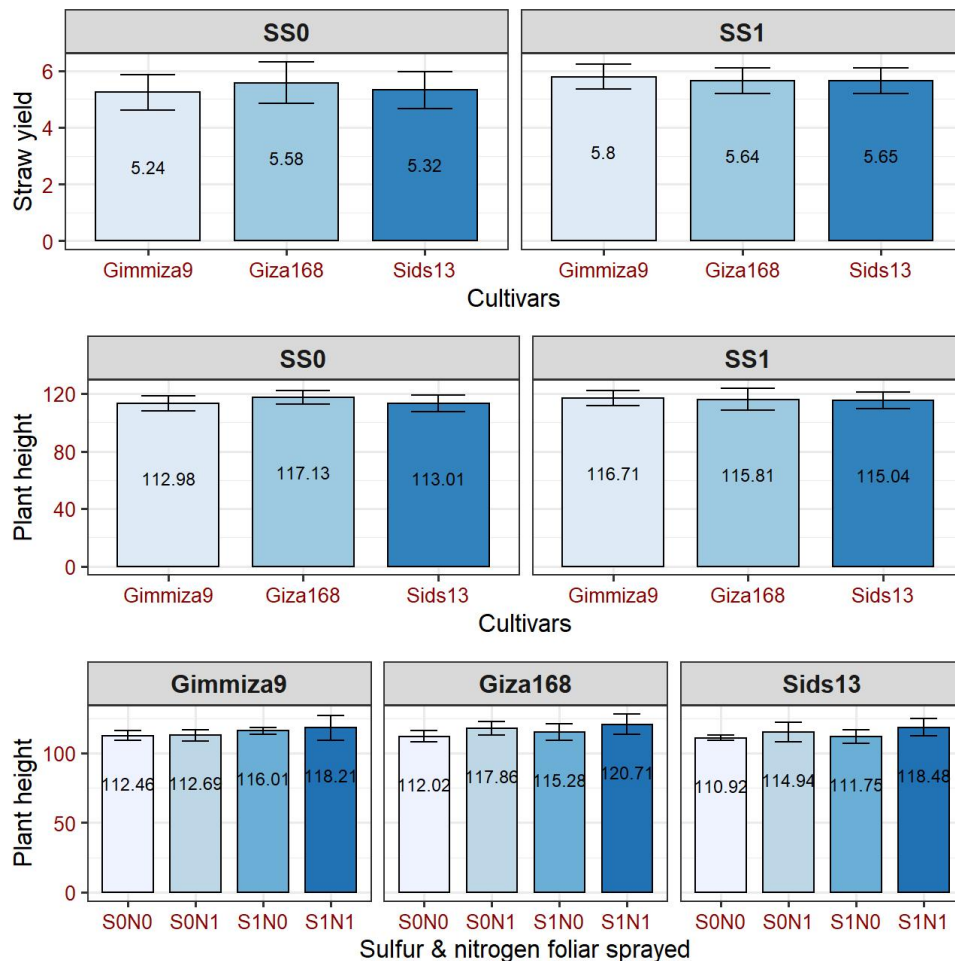


Fig. 3. The effect of the interaction between cultivars (C) and pre-planting added sulfur (no Soil sulfur; SS0 and added soil sulfur; SS1) on plant height and straw yield. The interaction between cultivars (C) and sulfur- nitrogen foliar sprayed (FSN) on plant height (cm) is presented in the lower part of the plot

The rapid assimilation and being a tolerance inducing factor might explain the superiority of sulfur foliar application compared to the pre-planting added S form. Moreover, in the current experiment, we applied the foliar micronized sulfur twice; the first was during stage 13 (three leaves emerged), while the second spray was during stage 41 (Flag leaf sheath extending). When three leaves emerged is the time when wheat plants are actively tillering [65]. That means higher translocation of photosynthates from the main stem to tillers or to generate more tillers is required. Therefore, the foliar application at that growth stage might have rapidly provided sulfur in a critical time window at which plants needed it the most to translocate photosynthates. Similarly, the foliar application of the micronized sulfur during stage 41, is also applied when the plants are in critical need to translocate stem stored photosynthates to initiate spikes and, consequently, the seed setting process.

Even though adding the pre-planting sulfur during soil preparation as gypsum, has several merits, sulfur can be lost due to leaching [66]. It is giving the fact that the dominant irrigation method in Egypt is flood irrigation, which found to have a tremendous impact on increasing soil nutrients leachates [67]. Thus, a most likely large proportion of the soil added sulfur was already leached during the seedling stage because of the first two flood irrigations. At the same time, the amount left in the soil will be affected by the soil properties that affect sulfur transformation to a plant-available form. It is well known that the sulfur element is not readily available for plants to use. Thus, it must be transformed in the soil into sulfate form. The transformation of elemental sulfur to sulfate depends on the activity of the soil microorganism, soil temperature and moisture, and the degree of sulfur crumbling [27]. If the soil environment is not suitable for the sulfur transformation process, then the projected impact of the added sulfur will also be minimized.

Foliar absorption of N and S elements was proven [49]. During the tillering and the reproductive stages applying N from urea found to improve photosynthesis, whereas at a later stage N from urea is progressively incorporated into spikes [49]. Our results also demonstrated a highly significant impact of nitrogen-sulfur foliar spray on all traits. In which using 714 kg ha⁻¹ of sulfur pre-planting and spring twice with 6.66% urea and 2.4% micronized sulfur (SS₁FS₁N₁) during stage 13 & 41, increased grain yield, total protein content, straw yield and plant height by

31.58, 26.09, 18.37 and 7.9%, respectively. The observed impact of using pre-planting sulfur while spraying with micronized S and Urea could be attributed to the critical role of S/N ratio and positive interaction between N and S on the grain yield attributed traits. That positive interaction between N and S was reflected in a higher N use efficiency in several crops such as wheat [49]. Furthermore, the balance between nitrogen and sulfur plays an essential role in dry matter accumulation in plants. The balance is represented by the S/N ratio, which is relatively constant across plant species, i.e., 0.025 for legumes and 0.032 for grasses [60]. Thus, the sulfur required by a giving plant is dependent on its available nitrogen fertilizers.

Consequently, if the available sulfur is not adequate, nitrogen applied will not be efficiently used [68,69]. Moreover, several studies had shown an increase in nitrogen use efficiency when S fertilizer was provided [68]. Sulfur deficiency causes profound changes in N metabolism with reduced protein synthesis and accumulation of soluble organic and inorganic nitrogenous compounds [14]. Furthermore, a favorable effect of sulfur and nitrogen on plant height, dry matter accumulation, photosynthesis activity, and chlorophyll synthesis was observed [70].

Our results demonstrated no significant differences among cultivars in response to S or N treatments for grain yield, protein content, and straw yield. Generally, the reasons for the insignificant difference among genotypes for a given trait might be attributed to using a small number of genotypes with insufficient genetic variability. Moreover, the studied cultivars are modern high yield potential wheat cultivars. Thus higher input conditions might have a favorable impact regardless of the genetic makeup of the modern cultivar [71]. In the current study, the treatments accounted for more than 80% of the variation across all variables, while cultivars combined with their two- and three-way interactions accounted for less than 20% of the variance. The three cultivars used in the current study (Sids13, Gimmiza9, and Giza168) are among the modern wheat cultivars that are widely cultivated in Egypt. Sids13 pedigree is as following: KAUZ"S" //TSI//TSI/SNB"S"ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP-0SD. Moreover, Gimmiza9 pedigree: Ald"S"/Huac "S" / / CMH74A.630 / 5x CGM4583-5GM-1GM-0GM. While Giza 168 is a result of a three-way cross between "MIL" and "BUC" genotypes,

then their offspring were crossed with “Seri CM93046-8M-0Y-0M-2Y-0B”. The pedigree information suggested that the cultivars are genetically different. Early flowering, short, reduced root system, and high input requirements are the key features of the modern high-performance widely adopted wheat cultivars. The modern cultivars were found to be more sensitive to the soil properties and water stress [71].

Across the three cultivars, our results indicated that pre-planting and foliar fertilization with sulfur had increased total protein content compared to only N foliar or S soil application. Several reports have shown a similar impact for adequate sulfur and nitrogen fertilizers in which they were found to improve the gluten index by 22.7 to 64.4% compare to N-fertilizer alone [71]. The first indication is that sufficient sulfur has increased sulfur metabolism, which produces more cysteine. Cysteine is mainly responsible for protein aggregation [72]. It became evident from several studies that fertilization with S affected the protein contents of the major amino acids, such as cysteine, threonine, methionine, and lysine in wheat [72].

During the current experiment sulfur, foliar application found to have a more positive influence on grain yield, plant height, straw yield, and total protein content compared to foliar application of N, suggesting that N availability was less wheat performance restricting factor compare to S. Pre-planting added sulfur had a lower impact on the traits studied suggesting that alkaline soil may have interfered with the amount of sulfur recovered from the soil. When N and S foliar fertilizers were applied simultaneously, a more robust increase in grain yield, plant height, straw yield, and total protein content was observed, suggesting a synergistic effect between these fertilizers. This synergistic effect could be attributed to their positive effect on improving photosynthates translocation from sources to sinks [73]. Furthermore, improved nitrogen use efficiency while reducing the plant content of NO^{-3} by optimizing the S/N ratio and reducing sulfur deficiency [35].

5. CONCLUSION

Sulfur deficiencies depress both nitrate uptake and nitrate reductase activity resulting in low nitrogen use efficiency in plants. Our results demonstrated the importance of the simultaneous application of S and N to optimize

the yield potential of the modern cultivars. Overall, from our study and previous studies fertilizing with sulfur was found to have a more significant positive impact on protein accumulation and composition compared to nitrogen when nitrogen deficiency is not a production restricting factor. Furthermore, 714 kg ha^{-1} of pre-planting sulfur and spraying twice with 6.66% urea and 2.4% micronized sulfur ($\text{SS}_1\text{FS}_1\text{N}_1$) during stage13 & 41, increased grain yield, total protein content, straw yield, and plant height by 31.58, 26.09, 18.37, and 7.9%, respectively. The results indicated a significant and positive impact of the soil sulfur application and foliar added sulfur-nitrogen combination. However, foliar added sulfur had a more substantial effect on all traits, compared to the added soil sulfur. Similarly, Foliar-applied nitrogen and sulfur were found to have synergistic effects on increasing grain yield, total protein content, straw yield, and plant height. Overall, sulfur alone or sulfur combined with nitrogen improved all the studied traits. We attributed the positive effect observed of $\text{SS}_1\text{FS}_1\text{N}_1$ on improving photosynthates translocation from the sources to sinks. And to improve nitrogen use efficiency while reducing the plant content of NO^{-3} by optimizing the S/N ratio and reducing sulfur deficiency. Based on our results, we concluded that the foliar application of micronized S has the potential to improve the overall performance of wheat plants. Thus, we recommend enriching nitrogen and phosphate fertilizers with sulfur for alkaline soils.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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