Sulfur and nitrogen nutrition status in flag leaf and shoot samples collected from wheat growing areas in Çukurova, Central Anatolia and GAP regions of Turkey

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Abstract

Sulfur (S) deficiency in soils and plants has been increased in the recent decade which is reducing crop yield and quality. Unfortunately, no extensive study has been conducted on S nutritional status of plants in Turkey. In this study, soil and plant samples were collected from Çukurova, Central Anatolia and GAP regions where wheat is extensively cultivated. Plant samples either as flag leaf or the whole shoot were collected depending on growth stage of wheat crop at sample collection. Similarly, surface (0–20 cm) and sub-surface (20–40 cm) soil samples were collected from plant sampling sites and a total 963 plant and 1947 soil samples were collected during the study. The S concentration in flag leaf samples varied between 0.18 and 0.67%, 0.11–0.59% and 0.17–0.82% for central Anatolia, Çukurova and GAP regions, respectively. According to S concentration in flag leaf samples, 99% of the plants in Çukurova region were found sufficient in S nutrition. However, 49% of the samples collected from central Anatolia and GAP regions were deficient in S. Critical N:S ratio indicating S nutrition status of plants was lower than the widely accepted critical value of 17. This low N:S ratio was a consequence of deficient N nutrition rather than S nutrition. Moreover, it was observed that plant available SO4-S concentration of soils varied within and among sampled provinces with an average value of 20.6 and 31.6 mg kg⁻¹ for surface and sub-surface samples, respectively. The SO4-S concentration increased with increasing soil depth. The results indicate a significantly positive correlation between S concentration in plant shoot and plant available SO4-S concentration in soils. In conclusion, S-containing fertilizer use in central Anatolia and GAP regions must be considered as an important approach for the prevention of yield and quality losses. Furthermore, rapid and sensitive plant and soil analysis methods are needed, which must also consider the local and site-specific conditions.

1. Introduction

Sulfur (S) is an essential nutrient required for all plants and animals (Sager, 2012). It is involved in the structure of cysteine, methionine, many co-enzymes (such as biotin, co-enzyme A, thiamine, pyrophosphate and lipoic acid), thioredoxins and sulfolipids (Bouranis et al., 2020). In addition, there are many S-containing compounds that are not vital for plants; however, play an important role in the defense mechanism against pathogens, pesticides and weeds. Moreover, these compounds give plants a special taste and fragrance (Capaldi et al., 2015; Hasanuzzaman et al., 2018). Apart from these, S has important effect on the quality of wool product due to its important roles in amino acid synthesis, proteins and some secondary metabolites (Stahl et al., 1949).

Although S has long been known to play an important role in plant growth and development, S-deficiency in plants was mentioned in Europe until a decade ago (Bloem et al., 2015; Haneklaus et al., 2008). Many plants require S in the similar quantity as of phosphorus (P). However, due to the lack of importance given S-fertilization, its deficiency is becoming common. Sulfur-deficiency is common in the UK and many European countries in agricultural lands and meadows (Tisdale et al., 1986; Wilhelm Scherer, 2009). One of the main reasons for this is the decrease in S coming from the atmosphere and applied to soil through fer-

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2. Materials and methods

2.1. Materials

To determine S nutrition levels of wheat production areas in central Anatolia (Konya, Karaman, Aksaray, Niğde and Nevşehir), GAP (Şanlıurfa and its districts) and Çukurova region (Adana and its districts, Adana-Osmaniyeh road) plant and soil samples were collected. Sampling was conducted at the beginning of heading stage. When there were no plants that have completed heading, whole shoot samples were collected at booting stage. Flag leaf and whole shoot samples were evaluated separately and classified as adequate (optimum) and insufficient (incomplete) according to the reference limit values specified in the literature. A total 963 plant samples, including 916 flag leaves and 47 whole shoot were collected. Surface (0–20 cm) and subsurface (20–40 cm) soil samples were also taken. A total 1009 surface (0–20 cm) and 938 subsurface (20–40 cm) soil samples were collected.

2.2. Method

2.2.1. Sulfur analysis

Plant samples were washed with distilled water and dried at 70 °C for 24 h. After drying, grinding process was started. The ground samples were burned in a mixture of H2O2–HNO3 acid in the microwave oven (Milestone) for half an hour according to the wet burning method and filtered through a blue-banded filter paper. The final volume of the filtered samples was completed to 20 ml with distilled water and S analysis was performed in ICP at a wavelength of 182.037 nm (Zhao and McGrath, 1994).

2.2.2. Total nitrogen analysis with Kjeldahl method

Total N analysis was conducted by Kjeldahl distillation method (Bremner, 1960). The basis of this method is to transform the organic N in plant sample burned with H2SO4 into NH4-N. The chemicals used in this method were concentrated H2SO4, 33% NaOH, 4% boric acid-indicator mixture solution and Kjeldahl tablet [(potassium sulfate (K2SO4), copper sulfate (CuSO4·5H2O) and selenium blends tablet)].

The dried and ground 0.2 g plant sample was weighed and placed into the Kjeldahl tubes and placed in incinerator. Afterwards, half Kjeldahl tablet and 5 ml concentrated H2SO4 were added to the samples. Then, the incineration process was started at 385 °C. The burning process continued until approximately 1–2 ml of clear liquid remained in the sample tube. After the burning phase, the distillation process was started with 15 ml of boric acid-indicator solution. Distillation continued until the pink boric acid turned green. In the last stage, the green boric acid-indicator solution was titrated with 0.1 N H2SO4 solution and turned into pink color again. When the color is pink, the H2SO4 consumption was replaced with the formula below and the total %N in the plant was calculated.

\[ \text{N concentration} = \left( \frac{T - B}{N} \right) \times 1.4 \]

Here, T = Acid used in titration (ml), B = Acid used in witness titration (ml), N = normality of the acid and S = sample quantity (g).

2.2.3. Determination of the available S concentration in soils

The method of Blair et al. (1991) modified by Bloem et al. (2002) was used to determine S concentration of soil samples. According to the method modified by Bloem et al. (2002), 10 g of soil passed through a 2 mm sieve and 50 ml of 0.025 M KCl solution was added to it. Afterwards soil-KCl suspension was shaken at 100 rpm in a horizontal shaker for 3 h at room temperature. The suspension was then filtered through blue band filter paper and the resulting filtrate was measured on ICP (Inductively-Coupled Plasma).

3. Results

3.1. Sulfur and nitrogen nutrition status of wheat crop in central Anatolia

A total 287 flag leaf and 10 whole shoot samples were collected from Konya province, which is one of the major crop production center in Turkey. While S concentration in flag leaf samples collected from Konya province varied between 0.18 and 0.67%, the mean was 0.32%. The S range in whole shoot samples was 0.12–0.21% with the mean S accumulation of 0.15% (Table 1).

The sampling sites within Konya province varied in terms of S concentration of flag leaf samples. While the lowest average S concentration in the flag leaf was found in samples collected from Hüyük district (0.23%), the highest average was found in the samples collected from Konya-Merkez (0.36%) and Çumra (0.36%) districts (Table 1). Kadınhanı district had the lowest average S (0.12%) in whole shoot samples, whereas Konya-Merkez, Çumra and...
Cihanbeyli districts had the highest average S (0.17%) in whole shoot samples (Table 1).

The S concentration in flag leaf and whole shoot samples collected from Konya was evaluated for critical S limit values given by (Westfall et al., 1990). According to the aforementioned literature, the S concentration required in the flag leaf of the plant at the beginning of the heading and in the whole plant at harvest should be 0.19%.

There was hardly any flag leaf sample having lower S percentage than the critical S value collected from Konya province. On the other hand, 70% of the samples collected during heading stage were fed well with S, while 30% were undernourished according to the critical limit value for S.

The average S concentration in the flag leaf and whole shoot samples taken from Aksaray province was 0.27% and 0.17%, respectively (Table 2). Compared to Konya, average S in flag leaf samples of Aksaray province was lower than Konya, while whole shoot samples of Aksaray province was higher than the critical S limit value collected from Konya province. Moreover, 99% of the collected flag samples from these provinces were S-sufficient, while only 1% of the samples were classified as S-deficit.

Apart from S, N nutrition status of wheat in the central Anatolia region was also determined. The average N concentration of flag leaf and whole shoot samples from Aksaray province was 2.79% and 1.64%, respectively (Table 1). Similarly, N concentration varied between 1.64 and 6.15% and 1.02–2.65% in flag leaf and whole shoot samples, respectively (Table 1). It has been understood that N nutrition status of wheat plants under farmer

| Location | Sample | n | Mean | Range | Mean | Range | Mean | Range |
|----------|--------|----|------|-------|------|-------|------|-------|
| Akören   | Flag leaf | 4  | 0.24 | 0.24–0.27 | 2.75 | 2.35–3.20 | 12   | 11–12 |
| Akşehir  | Flag leaf | 11 | 0.30 | 0.23–0.42 | 4.26 | 3.00–6.15 | 14   | 10–21 |
| Altınekin| Flag leaf | 26 | 0.35 | 0.23–0.54 | 4.02 | 2.99–4.75 | 12   | 9–15  |
| Beyşehir | Flag leaf | 7  | 0.28 | 0.23–0.37 | 3.32 | 2.74–4.09 | 13   | 10–14 |
| Cihanbeyli| Whole shoot | 2  | 0.17 | 0.14–0.21 | 2.21 | 1.76–2.65 | 13   | 12–13 |
| Çumra    | Flag leaf | 24 | 0.36 | 0.25–0.67 | 3.86 | 1.64–5.54 | 11   | 5–15  |
| Çumra    | Whole shoot | 2  | 0.17 | 0.12–0.21 | 1.89 | 1.48–2.30 | 12   | 11–12 |
| Emirgazi | Flag leaf | 7  | 0.28 | 0.19–0.43 | 3.05 | 2.31–3.69 | 11   | 9–13  |
| Ereğli   | Flag leaf | 6  | 0.33 | 0.26–0.35 | 3.54 | 2.92–3.91 | 11   | 9–12  |
| Huyük    | Flag leaf | 4  | 0.23 | 0.18–0.27 | 2.91 | 2.42–3.23 | 13   | 12–14 |
| İlın     | Flag leaf | 5  | 0.35 | 0.30–0.39 | 4.19 | 3.83–4.42 | 12   | 11–13 |
| Kadınhanı| Flag leaf | 27 | 0.34 | 0.22–0.42 | 3.99 | 2.81–5.12 | 12   | 9–14  |
| Kadınhanı| Whole shoot | 1  | 0.12 | 0.12 | 1.20 | 1.20 | - | 10 |
| Karapınar| Flag leaf | 14 | 0.30 | 0.19–0.44 | 3.47 | 2.76–4.18 | 13   | 8–19  |
| Konya-Merkez| Flag leaf | 44 | 0.36 | 0.21–0.66 | 3.81 | 1.93–4.63 | 11   | 7–14  |
| Konya-Merkez| Whole shoot | 2 | 0.17 | 0.15–0.19 | 1.79 | 1.35–2.22 | 11   | 9–12  |
| Kulu     | Flag leaf | 10 | 0.27 | 0.21–0.32 | 3.48 | 2.82–4.26 | 13   | 11–14 |
| Kulu     | Whole shoot | 1 | - | 0.13 | - | 1.35 | - | 10 |
| Sarayönü | Flag leaf | 20 | 0.29 | 0.19–0.40 | 4.08 | 2.65–4.97 | 14   | 12–16 |
| Sarayönü | Whole shoot | 1 | - | 0.13 | - | 1.69 | - | 13 |
| Seydişehir| Flag leaf | 5  | 0.29 | 0.25–0.32 | 3.61 | 3.30–3.97 | 13   | 11–13 |
| Seydişehir| Whole shoot | 1 | - | 0.15 | - | 1.02 | - | 7 |
| Tuzlukuç | Flag leaf | 8  | 0.30 | 0.25–0.37 | 3.77 | 3.19–4.55 | 13   | 12–14 |
| Yunak    | Flag leaf | 24 | 0.30 | 0.23–0.43 | 3.75 | 1.93–4.69 | 13   | 8–16  |
| Mean     | Flag leaf | 287 | 0.32 | 0.18–0.67 | 3.79 | 1.64–6.15 | 12   | 5–21  |
| Mean     | Whole shoot | 10 | 0.15 | 0.12–0.21 | 1.70 | 1.02–2.65 | 11   | 7–13  |

Cihanbeyli districts had the highest average S (0.17%) in whole shoot samples (Table 1). The S concentration in flag leaf and whole shoot samples collected from Konya was evaluated for critical S limit values given by (Westfall et al., 1990). According to the aforementioned literature, the S concentration required in the flag leaf of the plant at the beginning of the heading and in the whole plant at harvest should be 0.19%.

The average S concentration in the flag leaf and whole shoot samples collected from Aksaray province was 0.27% and 0.17%, respectively (Table 2). Compared to Konya, average S in flag leaf samples of Aksaray province was lower than Konya, while whole shoot samples of both provinces had almost similar S. The S deficiency was higher in flag leaf and whole shoot samples collected from Karaman province compared with Konya province. While S deficiency in the flag leaf was 4% in Karaman, it was 1% in Konya province. For whole shoot, S deficiency reached 80% in Karaman, while 30% in Konya (Table 2). This reveals that more importance should be given S nutrition in wheat production areas of Karaman province.

The S nutrition status in the flag leaf and whole shoot samples collected from Aksaray province was the highest among all provinces included in the study. The average S concentration of the flag leaf samples taken from Aksaray province was 0.36%, 0.31% and 0.33%, respectively (Tables 3–5).

The S concentration in flag leaf samples collected from all three provinces was higher than the critical S limit value. Moreover, 99% of the collected flag samples from these provinces were S-sufficient, while only 1% of the samples were classified as S-deficit. The S nutrition status in the flag leaf and whole shoot samples collected from Aksaray, Niğde and Nevşehir provinces are presented in Tables 3, 4 and 5, respectively.

Apart from S, N nutrition status of wheat in the central Anatolia region was also determined. The average N concentration of flag leaf and whole shoot samples collected from Konya province was 2.79% and 1.70%, respectively (Table 1). Similarly, N concentration varied between 1.64 and 6.15% and 1.02–2.65% in flag leaf and whole shoot samples, respectively (Table 1). It has been understood that N nutrition status of wheat plants under farmer
Critical limit value. While the average N concentration of wheat suffered from other provinces with higher N concentration than the literature in the rest of the sampled provinces. The average N value in flag leaf samples was higher than the critical limit value in Karaman, different from the other sampled provinces. The average N value in the flag leaf in Karaman, differed from the other provinces. The average N concentration in wheat production areas in different locations of Aksaray province.

Average sulfur (S) and nitrogen (N) concentrations and N:S ratio in flag leaf and whole shoot samples collected from wheat production areas in different locations of Nevsehir province.

Table 3

| Location       | Sample     | n  | S concentration (%) | N concentration (%) | N:S ratio |
|----------------|------------|----|---------------------|--------------------|----------|
| Ağızikaranı     | Flag leaf  | 2  | 0.32 (0.28–0.36)    | 4.17 (4.03–4.31)   | 13       |
| Guzelyurt      | Flag leaf  | 8  | 0.35 (0.26–0.42)    | 3.98 (2.91–4.58)   | 11       |
| Aksaray-Merkez | Flag leaf  | 6  | 0.33 (0.24–0.38)    | 3.88 (2.98–4.71)   | 12       |
| Aksaray-Merkez | Whole shoot| 1  | 0.11                | 1.25               | –        |
| Esık            | Flag leaf  | 12 | 0.37 (0.27–0.48)    | 4.39 (3.36–5.86)   | 12       |
| Mean           | Flag leaf  | 28 | 0.36 (0.24–0.48)    | 4.15 (2.91–5.86)   | 12       |
| Mean           | Whole shoot| 1  | 0.11                | 1.25               | –        |

Average sulfur (S) and nitrogen (N) concentrations and N:S ratio in flag leaf and whole shoot samples collected from wheat production areas in different locations of Niğde province.

Table 4

| Location        | Sample     | n  | S concentration (%) | N concentration (%) | N:S ratio |
|-----------------|------------|----|---------------------|--------------------|----------|
| Aksaray-Merkez  | Flag leaf  | 2  | 0.21 (0.12–0.34)    | 2.26 (1.27–3.12)   | 11       |

Average sulfur (S) and nitrogen (N) concentrations and N:S ratio in flag leaf and whole shoot samples collected from wheat production areas in different locations of Nevsehir province.

Table 5

| Location     | Sample     | n  | S concentration (%) | N concentration (%) | N:S ratio |
|--------------|------------|----|---------------------|--------------------|----------|
| Aksaray-Merkez| Flag leaf  | 4  | 0.32 (0.23–0.39)    | 3.59 (2.51–4.83)   | 11       |
| Aksaray-Merkez| Whole shoot| 2  | 0.29 (0.28–0.31)    | 3.40 (3.39–3.42)   | 12       |
| Aksaray-Merkez| Whole shoot| 2  | 0.29 (0.28–0.31)    | 3.40 (3.39–3.42)   | 12       |
| Aksaray-Merkez| Whole shoot| 5  | 0.3 (0.24–0.37)     | 4.03 (3.82–4.31)   | 14       |
| Aksaray-Merkez| Whole shoot| 2  | 0.12 (0.12–0.13)    | 1.99 (1.87–2.11)   | 16       |
| Mean          | Flag leaf  | 16 | 0.33 (0.23–0.44)    | 3.74 (2.51–4.83)   | 12       |
| Mean          | Whole shoot| 4  | 0.21 (0.13–0.31)    | 2.70 (1.87–3.42)   | 14       |

conditions is quite different. These results were compared with critical limit values recommended for N in wheat proposed by Westfall et al. (1990). The N concentration should be 3.5–4.5% and 3.0–4.0% in the flag leaf and at heading stage, respectively. According to these critical values, flag leaf samples collected from Konya were sufficient in N; however, whole shoot samples were N-deficient. This finding for the adequacy or insufficiency of N concentration in the flag leaf and the whole plant was also obtained for the S concentration in the same samples. This result may be due to the difficulty of finding and sampling plants at the same period under farmer conditions and/or the fact that most of the N has been moved to flag leaf.

The N concentration in flag leaf and whole shoot samples collected from Karaman province was 3.35% and 2.23%, respectively (Table 2). Both of these mean values below the critical level accepted for optimum N in wheat. This average N value, which is lower than the critical limit value in the flag leaf in Karman, differed from the other sampled provinces. The average N value in the flag leaf samples was higher than the critical limit value in the literature in the rest of the sampled provinces.

The whole shoot samples collected from Nevshehir province differed from other provinces with higher N concentration than the critical limit value. While the average N concentration of wheat samples at the end of heading period in Nevshehir province was 2.70%, it was 1.25% and 2.26% in samples collected from Aksaray and Niğde, respectively (Tables 3, 4 and 5). The N:S ratio was computed for all the collected plant samples in the study. This ratio is one of the important parameters used to determine S nutritional status of plants. If this ratio is >17, it is accepted that the plants are deficient in S (Zhao et al., 1999a). Average N:S ratio for whole shoot and flag leaf samples was <17 (Tables 1–5). According to these results, N:S ratios of flag leaf and the whole shoot samples indicate that plants are S-sufficient. In contrast, 64% of the whole shoot samples were S-deficient.

It is mentioned above that the average N concentration of whole plant samples in all provinces except Nevshehir was lower than the critical N concentration (3.0–4.0%). Assuming that S nutrition status of plants is accepted as such and N nutritional level of the plant during heading period is within the optimum limits (3.0–4.0%), it has been observed that the N:S ratio can be >17. For example, while the average S and N concentrations in whole shoot samples collected from Konya were 0.15% and 1.70%, respectively, N:S ratio was 11 (Table 1). When N concentration was 3.0%, N:S ratio increased to 20, the same ratio increased to 23 when N concentration was 3.5% and to 27 with 4.0% N concentration.
3.2. Sulfur and nitrogen nutrition status of wheat crop in Çukurova region

A total 362 flag leaf samples were collected from Çukurova region to determine S nutrition status. The S concentration ranged from 0.11% to 0.59% in the collected samples (Table 6). Furthermore, the average S concentration was 0.34% in the collected samples (Table 6). Except for one of the flag leaf samples collected in Adana, all samples had higher than the adequate S concentration. Average N concentration in flag leaf samples collected from Adana province was within the optimum limit. The N concentration in flag leaf samples collected from all locations in Adana except İmamoğlu was higher than sufficient N level (3.5–4.5%) (Table 6).

According to the collected flag leaf samples from Adana, there was no N and S nutrition problem in the region. A similar finding was observed when N:S ratio was considered as all samples had <17 N:S ratio (Table 6). However, these results should not mean that there is no S nutrition problem in wheat growing areas of Adana province. Like central Anatolia region, a significant S-deficiency was recorded in whole shoot samples collected from Adana province.

3.3. Sulfur and nitrogen nutrition status of wheat crop in GAP region

A total 180 flag leaf and 22 whole shoot samples were collected from GAP region to determine S nutrition status. Şanlıurfa province observed the greatest change in S nutrition in the studied samples. The average S concentration in the samples collected from Akçakale district in Şanlıurfa had quite high S and the average S concentration in flag leaf samples was 0.40. This value is the highest location average among all provinces. Hilvan district had the lowest S average (0.29%) among the studied locations in Şanlıurfa province (Table 7).

Considering the flag leaf samples, no S nutrition problem existed in wheat production areas of Şanlıurfa province. In contrast, 32% of the whole shoot samples indicated S deficiency. The results obtained from Şanlıurfa confirm the contradiction between flag leaf and whole shoot samples for S nutrition.

Based on flag leaf samples collected from all three regions and whole shoot samples collected from central Anatolia and GAP regions, it can be concluded that there is no significant S problem in wheat production areas according to the S concentration in the flag leaf. However, according to the S concentrations whole shoot samples, it is concluded that 49% of wheat plants in central Anatolia and GAP regions have S deficiency.

3.4. Sulfur concentration of soils in wheat production areas of central Anatolia, Çukurova and GAP regions

A total 1009 surface (0–20 cm) and 938 subsurface (20–40 cm) soil samples were collected from wheat production areas in central Anatolia, Çukurova and GAP regions. Soil sampling sites were classified on the basis of provinces and districts.

The available SO₄-S concentration in the tested soil samples had a very wide range. The SO₄-S concentration range for soil samples collected from Konya was 6.4–3202.1 mg kg⁻¹ (Table 8), 8.9–22.9 mg kg⁻¹ for Karaman (Table 9), 4.9–38.1 mg kg⁻¹ for Aksaray (Table 9), 2.7–26.4 mg kg⁻¹ for Niğde (Table 10), 4.5–28.6 mg kg⁻¹ for Nevşehir (Table 10), 6.7–133.1 mg kg⁻¹ for Adana (Table 11) and 8.0–1446.9 mg kg⁻¹ for Şanlıurfa (Table 12).

The highest variation in available SO₄-S concentration range was recorded for Konya and Şanlıurfa provinces. The sampled locations within these provinces also had quite different S concentrations from each other. The highest SO₄-S concentration (134.1 mg kg⁻¹) in the surface soil samples was recorded for Çumra district, whereas Ilgın district recorded the lowest (10.7 mg kg⁻¹) SO₄-S concentration (Table 8). Akçakale district in Şanlıurfa province had the highest (87.0 mg kg⁻¹) SO₄-S concentration, whereas the lowest SO₄-S concentration (14.2 mg kg⁻¹) was noted for Ceylanpinar district (Table 12).

The highest average S concentration was recorded for the surface and subsurface soil samples collected from Çumra, Konya, Merkez and Çihanbeyli districts in Konya province and Akçakale district in Şanlıurfa. Another finding obtained from soil samples is that the S concentration of soils increased with increasing depth. This increase was noted for surface and subsurface soil samples taken from all provinces. (Figs. 1 and 2).

According to literature, 6.5 mg kg⁻¹ is the critical S value for soil samples. The surface and subsurface soil samples with 6.5 mg kg⁻¹ S are sufficient in with 99% accuracy. According to limit value for light soils, Niğde and Nevşehir soils had no significant S nutritional problems. While the rate of samples with sufficient S concentration in the surface soil samples in two provinces is 83%, this value is 17% in soils containing insufficient S. The same values for the subsurface soil samples were 87% and 13%, respectively.

Blair et al. (1991) found that available S concentration in the soil having the highest relationship between the dry matter yield and available SO₄-S concentration was 6.5 mg kg⁻¹. According to the results soil samples collected from Konya, Karaman, Aksaray, Adana and Şanlıurfa were S-sufficient.

4. Discussion

The results of the plant sample analyses indicated that there is no S deficiency in the studied regions. However, according to the

| Location               | Sample     | n   | S concentration (%) | Mean | Range    | N concentration (%) | Mean | Range    | N:S ratio | Mean | Range |
|------------------------|------------|-----|---------------------|------|----------|---------------------|------|----------|-----------|------|--------|
| Ceyhan                 | Flag leaf  | 124 | 0.32                | 0.22–0.45 | 3.72     | 2.03–5.40          | 12   | 6–15     |
| Ç.Ü.Z.F.Çiftliği         | Flag leaf  | 13  | 0.39                | 0.33–0.45 | 4.64     | 4.14–4.93          | 12   | 10–13    |
| İmamoğlu               | Flag leaf  | 22  | 0.28                | 0.22–0.39 | 3.16     | 1.43–4.27          | 12   | 4–16     |
| Karasak                | Flag leaf  | 51  | 0.35                | 0.25–0.53 | 3.93     | 3.03–4.61          | 11   | 8–13     |
| Karataş                | Flag leaf  | 44  | 0.35                | 0.24–0.58 | 4.36     | 3.30–5.77          | 13   | 6–18     |
| Kozan                  | Flag leaf  | 7   | 0.31                | 0.22–0.43 | 3.52     | 2.73–4.41          | 12   | 10–13    |
| Adana-Osmaniye         | Flag leaf  | 12  | 0.32                | 0.25–0.39 | 3.52     | 2.16–4.54          | 11   | 8–13     |
| Yenice                 | Flag leaf  | 53  | 0.37                | 0.25–0.53 | 3.94     | 2.41–5.31          | 11   | 5–13     |
| Yüreğir                | Flag leaf  | 36  | 0.34                | 0.11–0.59 | 3.07     | 2.42–4.96          | 12   | 7–27     |
| Mean                   | Flag leaf  | 362 | 0.34                | 0.11–0.59 | 3.87     | 1.43–5.77          | 12   | 4–27     |
critical limit value, 49% of the samples collected from Central Anatolia and GAP region had S deficiency. Similar results have been reported for S nutrition status in wheat crop grown in Ankara province of Turkey (Inal et al., 2003).

The contradictory results for S concentration between flag leaf and whole shoot samples observed in this study are actually not too surprising. Generally, critical S concentration depends on plant species, part of the plant sampled, developmental period and crop yield level (Bouranis et al., 2020; Wen et al., 2003; Wilson et al., 2020). Researchers recommend that younger parts of the plants must be sampled in order to determine the S nutritional status during the period of high S requirement. This time is period from flowering to heading and vegetative organs are not ideal for determining S nutrition status (Courbet et al., 2019). Sampling whole shoot before the appearance of the flag leaf in monocotyledons is regarded as the best period for the determination of S nutrition status.

### Table 7
Average sulfur (S) and nitrogen (N) concentrations and N:S ratio in flag leaf and whole shoot samples collected from wheat production areas in different locations of Şanlıurfa province.

| Location | Sample         | n  | S concentration (%) Mean | Range | N concentration (%) Mean | Range | N:S ratio Mean | Range |
|----------|----------------|----|--------------------------|-------|--------------------------|-------|----------------|-------|
| Akçakale | Flag leaf      | 16 | 0.4                      | 0.23–0.82 | 3.61                      | 2.55–4.91 | 10             | 4–15   |
| Bozova   | Flag leaf      | 20 | 0.29                     | 0.17–0.47 | 3.16                      | 1.67–4.40 | 11             | 7–14   |
| Bozova   | Whole shoot    | 6  | 0.19                     | 0.14–0.25 | 1.79                      | 1.46–2.56 | 10             | 8–12   |
| Çeylanpınar | Flag leaf   | 15 | 0.36                     | 0.29–0.42 | 3.56                      | 1.69–4.57 | 10             | 4–13   |
| Harran   | Flag leaf      | 21 | 0.34                     | 0.25–0.44 | 3.48                      | 2.74–4.19 | 10             | 7–13   |
| Harran   | Whole shoot    | 2  | 0.19                     | 0.18–0.19 | 2.09                      | 1.77–2.40 | 11             | 10–12  |
| Hilvan   | Flag leaf      | 66 | 0.29                     | 0.19–0.41 | 3.67                      | 2.81–4.37 | 13             | 9–17   |
| Hilvan   | Whole shoot    | 10 | 0.2                      | 0.13–0.28 | 2.46                      | 1.48–3.26 | 12             | 9–15   |
| Siverek  | Flag leaf      | 5  | 0.33                     | 0.26–0.42 | 3.11                      | 1.94–3.80 | 9              | 7–11   |
| Siverek  | Whole shoot    | 2  | 0.24                     | 0.22–0.26 | 2.28                      | 2.18–2.38 | 10             | 8–10   |
| Suruç    | Flag leaf      | 22 | 0.3                      | 0.23–0.39 | 3.29                      | 1.96–3.99 | 11             | 6–14   |
| Suruç    | Whole shoot    | 1  | –                        | 0.2      | –                        | 1.92     | –              | 10     |
| Viranşehir | Flag leaf   | 15 | 0.35                     | 0.20–0.57 | 3.52                      | 2.35–4.34 | 11             | 6–16   |
| Viranşehir | Whole shoot | 1  | –                        | 0.22     | –                        | 2.21     | –              | 10     |
| Mean     | Flag leaf      | 180| 0.32                     | 0.17–0.82 | 3.5                       | 1.67–4.91 | 11             | 6–17   |
| Mean     | Whole shoot    | 22 | 0.2                      | 0.13–0.28 | 2.19                      | 1.46–3.26 | 11             | 8–15   |

### Table 8
Average SO₄-S concentration of 230 surface (0–20 cm) and 220 sub-surface (20–40 cm) soil samples collected from different locations in Konya province.

| Location | Depth (cm) | n  | SO₄-S Concentration (mg kg⁻¹) Mean | Min-Max values |
|----------|------------|----|-----------------------------------|----------------|
| Akören   | 0–20       | 4  | 12.3                              | 9.6–13.8       |
|          | 20–40      | 4  | 11.9                              | 9.7–13.5       |
| Aksaray  | 0–20       | 11 | 10.9                              | 6.8–14.6       |
|          | 20–40      | 11 | 13.1                              | 5.8–37.6       |
| Altınkına | 0–20      | 27 | 15.4                              | 8.4–27.7       |
|          | 20–40      | 26 | 20.2                              | 11.2–56.2      |
| Beyşehir | 0–20       | 8  | 14.7                              | 8.4–18.4       |
|          | 20–40      | 8  | 14.5                              | 8.4–17.9       |
| Cihanbeyli | 0–20     | 43 | 17.0                              | 6.5–174.5      |
|          | 20–40      | 41 | 43.7                              | 4.8–874.1      |
| Çumra    | 0–20       | 28 | 134.1                             | 8.1–3203.1     |
|          | 20–40      | 27 | 209.4                             | 7.5–3663.9     |
| Emirgazi | 0–20       | 7  | 12.4                              | 6.4–17.2       |
|          | 20–40      | 7  | 15.7                              | 6.9–35.0       |
| Eregi    | 0–20       | 7  | 13.4                              | 9.3–19.4       |
|          | 20–40      | 7  | 15.8                              | 10.6–26.8      |
| Hüyük    | 0–20       | 4  | 13.6                              | 11.1–16.2      |
|          | 20–40      | 3  | 12.4                              | 9.9–14.0       |
| Ilgın    | 0–20       | 5  | 10.7                              | 7.4–12.8       |
|          | 20–40      | 5  | 12.1                              | 8.4–15.8       |
| Kadmıhan  | 0–20      | 28 | 15.8                              | 9.1–48.3       |
|          | 20–40      | 26 | 20.6                              | 8.9–88.9       |
| Karapınar | 0–20     | 15 | 17.6                              | 6.6–52.4       |
|          | 20–40      | 14 | 20.6                              | 7.0–66.7       |
| Konya Merkez | 0–20   | 47 | 39.7                              | 6.6–453.7      |
|          | 20–40      | 45 | 102.3                             | 6.9–1138.7     |
| Kulu     | 0–20       | 11 | 11.6                              | 9.7–14.0       |
|          | 20–40      | 11 | 12.3                              | 9.5–16.4       |
| Sarayönü | 0–20       | 22 | 13.3                              | 10.2–15.9      |
|          | 20–40      | 20 | 14.3                              | 9.3–28.9       |
| Seydişehir | 0–20   | 6  | 11.3                              | 8.1–15.2       |
|          | 20–40      | 6  | 12.0                              | 8.9–15.6       |
| Tuzluççu | 0–20       | 8  | 12.3                              | 8.9–15.2       |
|          | 20–40      | 8  | 12.8                              | 10.5–16.3      |
| Yunak    | 0–20       | 24 | 13.5                              | 8.1–39.2       |
|          | 20–40      | 23 | 13.9                              | 8.8–35.0       |
It is understood from the literature that sampling times were carefully selected in this study. The S concentration decreases with increasing plant age (Robson et al., 1995). In dicotyledons, sampling is recommended for young leaves that have fully grown. It is further suggested that the sampled plant part and sampling time should be standardized in order to reduce the difficulties in interpreting analytical data (Jones, 1986).

### Table 9
Average SO$_4$-S concentration of 54 surface (0–20 cm) and 51 sub-surface (20–40 cm) soil samples collected from different locations in Karaman and Aksaray provinces.

| Province   | Location  | Depth (cm) | n | SO$_4$-S Concentration (mg kg$^{-1}$) |
|------------|-----------|------------|---|-------------------------------------|
|            |           |            |   | Mean | Min-Max values                      |
| Karaman    | Ayrancı   | 0–20       | 3 | 13.6 | 11.5–16.4                           |
|            |           | 20–40      | 3 | 19.1 | 12.9–27.6                           |
|            | Kazımkarabekir | 0–20   | 15 | 13.7 | 8.9–22.9                            |
|            |           | 20–40      | 14 | 15.5 | 8.3–32.5                            |
|            | Suduğaş    | 0–20       | 6 | 13.0 | 11.6–14.9                           |
|            |           | 20–40      | 6 | 15.4 | 13.0–22.8                           |
|            | Ağızkaraahan | 0–20    | 2 | 13.8 | 13.7–13.9                           |
|            |           | 20–40      | 2 | 14.9 | 13.0–16.7                           |
|            | Aksaray Merkez | 0–20  | 7 | 19.5 | 11.0–29.8                           |
|            |           | 20–40      | 6 | 32.2 | 16.0–78.9                           |
|            | Eskil      | 0–20       | 12| 22.7 | 11.6–38.1                           |
|            |           | 20–40      | 11| 28.5 | 14.3–50.5                           |
|            | Güzelyurt  | 0–20       | 9 | 11.7 | 4.9–22.1                            |
|            |           | 20–40      | 8 | 17.0 | 4.0–29.3                            |

### Table 10
Average SO$_4$-S concentration of 59 surface (0–20 cm) and 54 sub-surface (20–40 cm) soil samples collected from different locations in Nevşehir and Nigde provinces.

| Province   | Location  | Depth (cm) | n | SO$_4$-S Concentration (mg kg$^{-1}$) |
|------------|-----------|------------|---|-------------------------------------|
|            |           |            |   | Mean | Min-Max values                      |
| Nevşehir   | Acıgöl     | 0–20       | 5 | 12.4 | 4.5–25.5                            |
|            |           | 20–40      | 5 | 15.5 | 5.3–30.6                            |
|            | Avanos     | 0–20       | 2 | 11.6 | 10.3–12.8                           |
|            |           | 20–40      | 2 | 15.7 | 14.6–16.8                           |
|            | Derinkuyu  | 0–20       | 3 | 9.6  | 6.4–12.8                            |
|            |           | 20–40      | 3 | 14.9 | 9.4–22.8                            |
|            | Gülşehir   | 0–20       | 4 | 17.5 | 12.1–28.6                           |
|            |           | 20–40      | 4 | 23.3 | 15.2–35.4                           |
|            | Hacbeketaş | 0–20       | 8 | 16.2 | 13.0–18.4                           |
|            |           | 20–40      | 7 | 15.7 | 13.0–18.5                           |
| Nigde      | Altunhisar | 0–20       | 3 | 7.6  | 3.3–11.1                            |
|            |           | 20–40      | 3 | 8.0  | 3.3–11.4                            |
|            | Bor        | 0–20       | 6 | 16.2 | 8.6–26.4                            |
|            |           | 20–40      | 6 | 19.9 | 9.6–27.9                            |
|            | Çiftlik    | 0–20       | 5 | 7.9  | 3.8–10.6                            |
|            |           | 20–40      | 4 | 10.7 | 6.7–14.5                            |
|            | Misli Ovası| 0–20       | 23| 9.5  | 2.7–23.6                            |
|            |           | 20–40      | 20| 9.8  | 3.1–21.5                            |

### Table 11
Average SO$_4$-S concentration of 309 surface (0–20 cm) and 288 sub-surface (20–40 cm) soil samples collected from different locations in Adana province.

| Location      | Depth (cm) | n | SO$_4$-S Concentration (mg kg$^{-1}$) |
|---------------|------------|---|-------------------------------------|
|               |            |   | Mean | Min-Max values                      |
| Ceyhan        | 0–20       | 90| 12.8 | 7.8–19.1                            |
|               | 20–40      | 89| 12.9 | 7.6–33.8                            |
| Ç.U.Ziraat Fak. Çiftliği| 0–20  | 13| 12.2 | 9.3–15.0                            |
|               | 20–40      | 13| 12.9 | 9.3–18.4                            |
| İmamoğlu      | 0–20       | 22| 12.4 | 9.1–18.5                            |
|               | 20–40      | 21| 12.2 | 8.1–15.9                            |
| Karaisalı      | 0–20       | 38| 11.1 | 6.8–27.9                            |
|               | 20–40      | 34| 14.0 | 7.2–85.2                            |
| Karataş        | 0–20       | 44| 17.1 | 8.7–133.1                           |
|               | 20–40      | 44| 19.9 | 7.2–81.9                            |
| Kozan          | 0–20       | 17| 13.1 | 10.3–15.6                           |
|               | 20–40      | 6 | 14.5 | 10.7–21.9                           |
| Adana-Yenice   | 0–20       | 47| 12.1 | 7.3–19.0                            |
|               | 20–40      | 39| 14.7 | 7.7–46.1                            |
| Yüreğir        | 0–20       | 36| 13.3 | 8.0–25.7                            |
|               | 20–40      | 30| 16.2 | 53.7–9.1                            |
| Adana-Osmaniye | 0–20      | 12| 10.6 | 6.7–13.8                            |
|               | 20–40      | 12| 10.5 | 6.5–14.0                            |
The S concentration in green parts of the plant changes depending on the growth period. This means it is not possible to sample from plants that are at the same growth point or period, especially in the flag leaf, under farmer conditions. It is impossible that flag leaf at heading initiation and grain-filling period contain same amounts of S or other nutrients. For example, Spencer et al. (1984) reported that although total S was used as a criterion for the nutritional status of plants, the most important controversy was the S concentration during the growth period. Freney et al. (1978) found that the total S concentration of wheat in all green parts at the end of germination period was 2.5 g kg\(^{-1}\) dry weight, which reduced to 1.1 g kg\(^{-1}\) during middle development and 0.8 g kg\(^{-1}\) during grain-filling period. Generally, critical total S concentration is 2 g kg\(^{-1}\) dry matter, but can vary between 1 and 3 g kg\(^{-1}\).

Table 12
Average SO\(_4\)-S concentration of 282 surface (0–20 cm) and 254 sub-surface (20–40 cm) soil samples collected from different locations in Şanlıurfa province.

| Location     | Depth (cm) | n  | SO\(_4\)-S Concentration (mg kg\(^{-1}\)) |
|--------------|------------|----|--------------------------------------------|
|              |            |    | Mean | Min-Max values |
| Akçakale     | 0–20       | 25 | 87.0 | 11.0–1446.9 |
|              | 20–40      | 16 | 295.0| 13.2–2397.9 |
| Bozova       | 0–20       | 49 | 15.6 | 8.4–18.9    |
|              | 20–40      | 43 | 15.8 | 11.3–19.2   |
| Ceylanpınar  | 0–20       | 15 | 14.2 | 12.6–15.7   |
|              | 20–40      | 12 | 17.0 | 13.8–23.9   |
| Harran       | 0–20       | 33 | 19.2 | 12.5–57.7   |
|              | 20–40      | 33 | 24.9 | 13.8–58.9   |
| Hıvvan       | 0–20       | 92 | 15.1 | 8.0–29.7    |
|              | 20–40      | 87 | 15.4 | 8.0–39.5    |
| Siverek      | 0–20       | 9  | 15.5 | 12.9–16.6   |
|              | 20–40      | 8  | 15.1 | 13.2–16.2   |
| Sırıç        | 0–20       | 41 | 15.0 | 10.6–18.3   |
|              | 20–40      | 37 | 15.0 | 10.1–21.4   |
| Viransehir   | 0–20       | 17 | 16.1 | 11.6–29.2   |
|              | 20–40      | 16 | 16.8 | 14.1–25.5   |

Fig. 1. Total SO\(_4\)-S concentration in surface (0–20 cm) and sub-surface (20–40 cm) soil samples collected from different provinces.
Some researchers have argued that the conflicting results in determining the S nutritional level of plants may be due to the total S concentration; thus, determining SO₄-S may be more accurate. Large differences were reported between the concentrations of SO₄²⁻-S in plant tissues with and without S-deficiency. It is reported that SO₄-S concentration in plants is compatible with S concentration in soil in legumes (Wen et al., 2003), annual grasses and rape-seed (Grant et al., 2012; Robson et al., 1995). When the SO₄ concentration in vacuoles is close to the phosphorus concentration, S is a reliable parameter for determining nutrition status (Bouranis et al., 2020). Moreover, after the removal of the stored SO₄ in the roots and green parts, S is mobilized for a few days. Consequently S concentration is increased in old leaves, while decrease is noted in young leaves (Bouranis et al., 2020).

Apart from these two parameters, it is suggested that the ratio of SO₄ to total S should be considered as an indicator of S-deficiency (Bouranis et al., 2020). However, considering this ratio would not provide much advantage, it is argued that this ratio is impractical since the difference is due to changes in the SO₄ concentration and requires at least two separate analyses (Blake-Kalff et al., 2000).

In this study, S and N concentrations in the green part of the plant and N:S ratio were determined to reveal S nutritional status in wheat from different regions. According to this ratio, it is revealed that the plants do not have S-deficiency, but this is related to insufficient available N. Several studies have reported similar results. When factors other than S are optimum, it is assumed that the critical N:S ratio values are more stable than total S in the plant. Therefore, N:S ratio is a much better indicator than total S in wheat. Besides, one of the problems in using N:S ratio is that when one of these elements is in excess, the other is interpreted as deficient. Another problem is that S is relatively less mobile in plants and its concentration is higher in older leaves than in younger leaves, whereas N concentration remains opposite. For this reason, it is not correct that N:S ratio gives more accurate results than total S or SO₄ concentration, regardless of plant age and sampling period. In contrast, Spencer et al. (1984) reported that N:S ratio was less affected by the age of the plant. Thus, N amount would be a more useful indicator than S concentration for determining S-deficiency in cereals and legumes.

The use of critical values used in determining S nutritional level may be misleading. These values should be considered as more guiding and represent an approximate range (Melsted et al., 1969). However, successful diagnosis of S deficiency depends on selected critical limit value. If the critical limit value chosen for S is too high, many production areas will be unnecessarily fertilized. On the other hand, if the critical limit value is too small, it will not be possible to determine the S-deficiency. An additional problem is that for a successful critical limit value correct analysis must be done. It has been reported that small deviation from the required critical limit value has great impact on determining whether the samples are S-sufficient or deficient. For example, a standard could not be set among laboratories for determining total S concentration in plant samples. Even samples analyzed from different commercial plant and soil analysis laboratories give varying results for the same samples (Crosland et al., 2001). Total S concentration for grasses varied between 0.15% and 0.23%, while in wheat grain samples variation was between 0.8% and 0.14%. The critical limit value for wheat grain is accepted as 0.12% (Westfall et al., 1990). According to this limit value, S values of 3 commercial laboratories show...
that the concentration is sufficient, while the other 7 laboratories indicated that samples were S-deficient. These results show that besides the difficulties caused by the plant, there may be some technical difficulties in determining the nutritional level of plants with S.

Sulfur fertilization is the leading measure against S-deficiency. Significant yield increase has been noted with S fertilization in different plant species and different soils or regions. Although S requirement is lower for rapeseed, S-deficiency in grains was reported in Scotland 15 years ago (and studies showed that S application increases yield. In the trials conducted in 44 locations in North Oregon, when grain yield of wheat <2 tons S application had no impact on wheat yield. However, when yield was >2 tons, 27% yield increase was reported in the region with S application. The yield increase was achieved with S application in other plants except wheat. For example, studies conducted in the north-east of Scotland where atmospheric S intake is extremely low, four-fold increase in yield was reported with correct S application in rapeseed (Walker and Booth, 1994).

In pot experiments conducted by Scherer and Lange, (1996) with different legume plant species, S-deficiency was observed during early growth phase where S was not applied. In the late growth period plant growth was adversely affected. On the other hand, the yields of legumes in optimum S application were different from each other. This shows that S requirements of legumes are different from each other.

5. Conclusion

The results revealed that the studied region had no S-deficiency problem according to flag leaf samples. However, whole shoot samples indicated that central Anatolia and GAP regions had S-deficiency problem. In conclusion, S-containing fertilizer use in central Anatolia and GAP regions must be considered as an important approach for the prevention of yield and quality losses. Furthermore, rapid and sensitive plant and soil analysis methods are needed, which must also consider the local and site-specific conditions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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