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Full Length Article

Sulfur Application Improved Leaf Yield and Quality of Flue-Cured Tobacco by Maintaining Soil Sulfur Balance

Quanren Cui, Wenming Wu, Yaocheng Qi, Lili Jing and Chaoqiang Jiang[*]
Tobacco Research Institute, Anhui Academy of Agricultural Sciences, Hefei 230031, China
[*]For correspondence: chaoqjiang@163.com
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Abstract

Sulfur (S) fertilization is critical for improving tobacco (*Nicotiana tabacum* L.) yield and quality; however, limited information is available about the movement and distribution of S in soil, and its effects on yield and quality of flue-cured tobacco. Therefore, this two-year field experiment was conducted to study the effects of S on tobacco growth, S accumulation, as well as the movement and distribution of S in soil layers in tobacco filed. Tobacco was grown under five levels of S *i.e*., CK (without S), 22.5, 45, 67.5, and 90 kg ha^{-1}. Results showed that the available S contents in different soil layers varied as follows: 0–20 > 20–40 > 40–60 > 60–80 cm. The available S contents in the 0–20 soil layer significantly increased with an increased S application rate during the tobacco growth period, while no significant difference was observed among different treatments before tobacco transplanting. The S concentrations of root and stem were highest in the clumping stage of tobacco, while the leaf S concentration was highest at harvest. When the rate of S application was 45 kg ha^{-1}, the leaf yield was highest and the S concentration was on average 6.4 mg kg^{-1}, which was within the optimal S concentration (2–7 mg kg^{-1}) for high-quality leaf. In conclusion, S application at 22.5–45 kg ha^{-1} seemed highly effective for sustainable increase in yield and quality of tobacco leaf and maintaining S balance in the soil. © 2020 Friends Science Publishers

Keywords: Sulfur; Flue-cured tobacco; Soil layer; Distribution; Accumulation

Introduction

Sulfur (S) is a necessary element for plant growth and development and plays an important physiological role in plants (Droux 2004; Rossini *et al.* 2018). Sulfur is a component of methionine and cysteine in plants, and also involved in the synthesis of proteins and enzymes (Stockmann *et al.* 2018; Bayoumi *et al.* 2019). It has been reported that S plays an important role in the growth and quality of tobacco (*Nicotiana tabacum* L.) leaves (Feng *et al.* 2018; Wang *et al.* 2018). In a S-deficient soil, flue-cured tobacco leaves became yellow and aged prematurely, showing chloroplast dysplasia and inhibition of photosynthesis, thus inhibiting the growth of tobacco plants (Zhu *et al*. 2013a; Ma *et al*. 2017). Moreover, leaves of S deficient plants had a light color, an uncoordinated chemical composition, and poor sensory quality (Zhu *et al*. 2013a; Ma *et al.* 2017). However, when the available S contents exceeded than optimum in soil, tobacco leaves absorbed a large amount of S; therefore, the cured leaves had a rough appearance, less oil than normal, lower quality, and reduced output and value (Xu *et al.* 2008; Zhu *et al.* 2013b). Meanwhile, the combustibility of the leaves had obviously deteriorated and the aroma quality, aroma quantity and taste had declined significantly (Qin *et al.* 2007; Ma *et al.* 2017). When the S concentrations of leaves exceeded 7 g kg^{-1}, the combustibility of leaves was obviously reduced, often to the point of not continuing to burn (Tso 1990; Chu *et al*. 2016).

Available S in the soil provides the main source for nutrient S to crops (Cui *et al.* 2016; Qian *et al.* 2018) and the S concentration in tobacco leaves was positively correlated with the available S content in the soil (Shao *et al.* 2017). When the S content in the soil was deficient or excessive, the S concentration in tobacco leaves was deficient or accumulated excessively, respectively, thus affecting the yield and quality of leaves (Liu *et al.* 2000). Generally, the S concentration in leaves was higher than that in root and stem, while it was lowest in roots (Tan 2006; Zhang *et al.* 2013). Having an appropriate S concentration in soil resulted in high yield and improved quality of crops, while a lack of S lead to reductions in yield and quality (Shah *et al*. 2013; Capaldi *et al.* 2015). Therefore, to meet the needs of crops, S application in soil has received an increasing amount of attention in recent years. The S concentrations in different organs of tobacco plants were significantly increased by increasing the amount of S applied in soil (Wang *et al.* 2004; Zou *et al.* 2004). However, this also led to a large accumulation of S in the soil under a high rate of S fertilizer application. It has been reported that the application of a large amount of S caused S leaching into deeper soil (Wang *et al.* 2004). Therefore, it is very important to determine the appropriate rate of S application for optimal crop yield and quality, S use efficiency and environmental pollution minimization. Especially for tobacco, excessive use of S fertilizer lead to excessive accumulation of S in leaves, which resulted in a significant decline in leaf quality (Tso 1990; Cui *et al.* 2016).

Tobacco fields are mainly the paddy fields in Anhui Province, where the tobacco-rice rotation system (TRRS) is carried out all year round. During the growth of flue-cured tobacco to meet the demand for potassium, a large amount of potassium sulfate is normally applied (Cui *et al.* 2016). We found that 45.5% of tobacco leaf samples in Anhui had S concentrations more than 7 g kg^{-1}, which exceeded the optimal S concentration for producing high-quality leaves (Cui *et al.* 2016). However, at present, migration affects the distribution of S in the soil of Anhui tobacco fields after it is applied; in addition, the distribution of S in different organs of plants is not very clear. Therefore, the primary objectives of the present study were to: a) clarify the distribution and accumulation of S in the soil of tobacco-growing fields; b) evaluate the effects of S application on S accumulation in tobacco plant, leaf yield and S use efficiency in a tobacco field in southern Anhui Province. The results will help farmers to optimize S fertilizer application technology and provide a theoretical basis for flue-cured tobacco fertilization and sustainable agricultural development.

Materials and Methods

Experimental site and growth conditions

The experiment was conducted in Huangdu Village, Xuanzhou District, Anhui Province, China (30°48′32″N, 117°49′17″E) from 2016 to 2018 in a typical flue-cured tobacco area of southern China. The physicochemical properties of the top soil (0–20 cm) and available S content in the 0–80 cm soil layers at the beginning of the experiment were showed in Table 1. The available S content of the topsoil soils was 12.53 mg kg^{-1}, which was deficient for tobacco growth and development (Liu *et al.* 2000). The region has a subtropical, humid monsoon climate with an average annual air temperature of 15.6°C and mean annual precipitation of 1500 mm.

Experimental design

Tobacco was grown under five levels of S *i.e.*, CK (without S), 22.5 (S_1), 45 (S_2), 67.5 (S_3) and 90 (S_4) kg ha^{-1} in the same experimental field for two consecutive years from 2016 to 2017. The S application of 45 kg ha^{-1} represented the current recommendation of agronomist (Cui *et al.* 2016). Each treatment was replicated three times, resulting in 15 plots each having an area of 72 m^2 (4.8 m × 15 m, *i.e.*, four 1.2 m spaced rows with 0.5 m distance between plants, 120 plants). To prevent the water and fertilizer from flowing between plots, four ridges (30 cm width, 30 cm height) were set up for each plot. Each plot was equipped with an independent water inlet and outlet. The fertilizers applied in the experimental field consisted of 97.5 kg N ha^{-1}, 195 kg P_2O_5 ha^{-1} and 341 kg K_2O ha^{-1}. These fertilizers included urea (N 46%), potassium dihydrogen phosphate (K_2O 27%, P_2O_5 24%), di-ammonium phosphate (N 18%, P_2O_5 46%), potassium nitrate (N 13.5%, K_2O 46%), calcium magnesium phosphate (P_2O_5 14%), potassium sulfate (K_2O 50%, S 18%), and magnesium sulfate (MgO 33.3%, S 26.6%).

All fertilizers were applied as base fertilizer by banding at one time before transplanting. The experiment employed flue-cured tobacco var. Yunyan 97. Tobacco seedlings were transplanted in mid-March, while harvested in late July. Except for the different rates of S application, other field management was the same for all treatments.

Sample collection and determination

Soil samples collection and determination: Before the experiment, a 0–20 cm soil layer sample was collected to determine the basic fertility. At the same time, the 0–80 cm soil layer (one sample per 20 cm) was used to determine the S contents. Based on the growth period of tobacco plants, soil samples were taken at nine periods during the experiment: before ridging [T_1, 0 day after transplantation (DAT)], clumping (T_2, 30 DAT), flourishing period (T_3, 45 DAT), and at the end of baking (T_4, 120 DAT) in 2016; before ridging (T_5, 0 DAT), clumping (T_6, 30 DAT), flourishing period (T_7, 45DAT), and at the end of baking (T_8, 120 DAT) in 2017 and before ridging (T_9, 0 DAT) in 2018. The sampling method was designed to select samples from the middle row of the plot and then dig a vertical section with a 120 cm width, 80 cm depth, and 100 cm length; the soil samples were divided into sections with depths of 0–20, 20–40, 40–60 and 60–80 cm. Each sampling layer was including five soil samples collected in a "Z" shape; these were mixed completely prior to available S content determination.

Soil organic matter was assayed using the dichromate oxidation method, alkaline hydrolysis N was determined using the diffusion method, available P was measured by the Olsen method and available K was extracted using NH_4OAc and determined by flame photometry (FP 640, Shanghai Xinyi Instrument Co., Ltd., China) (Li *et al.* 2014). Soil available S was determined by using $BaSO_4$ turbidimetry (Wang *et al.* 2018).

Plant sample collection and determination: Three representative plants per plot were collected at six stages: transplanting, clumping, flourishing, and at lower- (70 DAT), middle- (90 DAT), and upper-leaf-ripening (110 DAT). During the transplanting, clumping and flourishing stages, the plant samples were divided into root, stem, and leaf samples. During the stage of leaf maturity, the plants

Table 1: Physicochemical properties of the topsoil soil (0–20 cm) and available sulfur content in different soil layers

pH	Organic matter ($g\ kg^{-1}$)	Available N ($mg\ kg^{-1}$)	Available P ($mg\ kg^{-1}$)	Available K ($mg\ kg^{-1}$)	Available S content ($mg\ kg^{-1}$)			
					0–20 cm	20–40 cm	40–60 cm	60–80 cm
5.3	20.7	107.6	39.5	73.8	12.53	11.15	7.74	6.96

were divided into five parts: root, stem, and lower, middle, and upper leaf samples. All samples were first dried at 105°C for 30 min; after being dried to constant weight at 65°C. The root, stem and leaf samples were weighted separately at each stage for collect the growth data. Each sample was ground to powder, and passed through a 0.15 mm sieve in preparation for analyses. Sulfur was digested, and the concentration determined according to the method of Anderson (1996). Except for the sampled plants, all plants were harvested five times by hand at 8 day intervals, by removing three to five leaves each time, starting 70 days after transplanting. The leaves were cured immediately after harvest in a flue-curing barn for flue-cured tobacco. Tobacco leaf yield was the sum of the dry weights of each separate harvest.

Data processing and statistical analysis

Nutrient accumulation and apparent S recovery efficiency (ARE) were calculated using Eqs. (1) and (2), respectively:

$$\text{Nutrient accumulation (NA, kg ha}^{-1}) = \frac{\text{Dry weight} \times \text{Nutrient concentration}}{1000} \quad (1)$$

$$\text{ARE (\%)} = \frac{\text{NA by aboveground in fertilized} - \text{NA by aboveground in control}}{\text{nutrient application rate}} \times 100 \quad (2)$$

Data were processed using S.P.S.S. 13.0 software (S.P.S.S. Inc., Chicago, IL, USA). Statistically significant differences among means were performed using Least Significant Difference (LSD) test at $P < 5\%$ after conducting analysis of variance. Figures were drawn using Microsoft Excel 2010.

Results

Effects of S on root, stem and leaf biomass and leaf yield

Sulfur application had significant effect on root, stem, leaf and total dry weight of whole plants of tobacco (Table 2). The root dry weight of the CK plants was 269.9–270.5 g plant^{-1}, which was significantly lower than that of plants from all S treatments (284.2–312.9 g plant^{-1}). The total dry weight of the whole plants increased with an increase in the S application rate. The stem dry weights of 67.5 and 90 kg S ha^{-1} treatments were significantly higher than those of 22.5 and 45 kg S ha^{-1} treatments. Similarly, the root dry weight of S treatment samples was significantly higher than that of the CK, but no significant difference was observed between treatments 22.5 and 90 kg S ha^{-1}. Two years of results showed that the leaf dry weight was highest in 45 kg S ha^{-1} treatment (139.0–140.8 g plant^{-1}) among all treatments. The leaf yield was also highest in 45 kg S ha^{-1} treatment, which was significantly higher than that of 22.5, 67.5 and 90 kg S ha^{-1} treatments in both 2016 and 2017. However, there was no significant difference among 22.5, 67.5, 90 kg S ha^{-1} and CK treatment.

Effects of S on its concentration in roots, stems, and leaves at different growth stages

As tobacco plants grew, the S concentrations in leaves increased from 1.7–1.9 g kg^{-1} at transplanting to 5.1–9.8 g kg^{-1} at the mature stage of upper leaves (Fig. 1). Sulfur application resulted in a significant increase in leaf S concentration, which also increased as the rate of S application increased. The S concentrations of leaves from different parts of the plant could be ranked in the following order: upper > middle > lower. Similarly, S application resulted in an increased in the S concentrations of stems (Fig. 2). The S concentrations of stems peaked during the clumping stage and showed a downward trend after the lower leaves matured. Sulfur application resulted in an increase in the S concentrations of roots at different stages, especially during the clumping and flourishing stages (Fig. 3). The S concentration of root in S application treatments was 1.8- and 1.4-times of the CK during the clumping and flourishing stages, respectively. The root S concentration peaked at the clumping stage. After the lower leaves matured, the S concentration in root tended to stabilize. Comprehensive analysis showed that after the lower leaves matured, S absorbed by the root was mainly transported to and accumulated in the leaves.

Effects of S on its accumulation and use efficiency

Sulfur application resulted in significant increases in S accumulation in roots, stems and leaves (Table 3). Sulfur accumulation in 22.5, 45, 67.5 and 90 kg S ha^{-1} treatments increased by 24.9%, 49.3%, 74.1% and 95.5% when compared with control (13.78–15.32 kg ha^{-1}), respectively. In general, the total S accumulation of tobacco plants in 2017 was 3–5% higher than that in 2016, while the S accumulation in leaves increased by 3–10% than that in 2016. The S application rate had a significant effect on the ARE of S in flue-cured tobacco (Fig. 4). The ARE of S was 15.3–17.3% in all S treatments and was highest in 22.5 kg S ha^{-1} (17.3%) and lowest in 90 kg S ha^{-1} treatment (15.3%). The ARE of S decreased with an increase in the S application rate. The ARE was slightly lower in 2017 than that in 2016; that is, the use of S fertilizer decreased with an increase in S application years.

Effects of S on available s content in soil layers

During the tobacco growth period, the available S content in the

Table 2: Effects of sulfur application on dry weight of root, stem and leaf, and leaf yield of flue-cured tobacco

Treatments	Dry weight (g plant^{-1})								Leaf yield (kg ha^{-1})	
	Root		Stem		Leaf		Total			
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
CK	40.7 ± 2.2c	43.9 ± 1.1c	94.0 ± 3.1c	91.1 ± 2.1e	135.8 ± 3.0ab	134.9 ± 3.4b	270.5 ± 4.0e	269.9 ± 3.8d	2041.0 ± 56.7ab	2036.9 ± 46.2ab
S_1	54.5 ± 1.9ab	54.5 ± 1.7ab	97.1 ± 3.2c	99.6 ± 3.1d	132.6 ± 3.8b	136.2 ± 3.5ab	284.2 ± 8.9d	290.4 ± 6.3c	1992.5 ± 39.9b	1988.8 ± 43.9b
S_2	50.4 ± 2.3b	55.2 ± 1.0ab	104.9 ± 3.4b	109.1 ± 0.4c	139.0 ± 2.9a	140.8 ± 2.7a	294.4 ± 3.1bc	305.1 ± 2.7b	2119.3 ± 79.6a	2085.1 ± 55.9a
S_3	50.5 ± 1.9b	53.0 ± 0.3b	117.7 ± 2.7a	114.2 ± 2.5b	132.4 ± 2.7b	136.8 ± 2.9ab	300.5 ± 4.2ab	304.1 ± 5.1b	1991.3 ± 28.3b	1986.2 ± 24.2b
S_4	56.9 ± 2.7a	56.6 ± 0.8a	117.1 ± 4.4a	119.2 ± 1.4a	133.4 ± 2.2b	137.1 ± 3.0ab	307.4 ± 2.5a	312.9 ± 2.4a	1996.8 ± 41.4b	2001.2 ± 34.4b

Means sharing different letters, within a column, differ significantly from each other at $P < 0.05$
Here CK, S_1, S_2, S_3 and S_4 are respectively 0, 22.5, 45, 67.5 and 90 kg of S ha^{-1}

Table 3: Effects of sulfur application on sulfur accumulation in root, stem and leaf of flue-cured tobacco

Treatments	2016				2017			
	Root	Stem	Leaf	Total accumulation	Root	Stem	Leaf	Total accumulation
			(kg ha^{-1})				(kg ha^{-1})	
CK	1.20 ± 0.06d	2.93 ± 0.10d	9.66 ± 0.21e	13.78 ± 0.25e	1.37 ± 0.03d	3.24 ± 0.08e	10.72 ± 0.27e	15.32 ± 0.28e
S_1	1.63 ± 0.06c	3.67 ± 0.12c	12.37 ± 0.35d	17.68 ± 0.53d	1.91 ± 0.06c	3.71 ± 0.11d	13.01 ± 0.34d	18.63 ± 0.40d
S_2	1.82 ± 0.08b	4.95 ± 0.16b	14.60 ± 0.31c	21.37 ± 0.17c	2.12 ± 0.04b	4.79 ± 0.02c	15.10 ± 0.29c	22.00 ± 0.29c
S_3	1.82 ± 0.07b	6.08 ± 0.14a	16.91 ± 0.35b	24.81 ± 0.32b	2.15 ± 0.01b	5.46 ± 0.12b	18.16 ± 0.38b	25.77 ± 0.48b
S_4	2.34 ± 0.11a	6.24 ± 0.23a	19.12 ± 0.32a	27.70 ± 0.09a	2.30 ± 0.03a	6.09 ± 0.07a	20.72 ± 0.46a	29.10 ± 0.42a

Means sharing different letters, within a column, differ significantly from each other at $P < 0.05$
Here CK, S_1, S_2, S_3 and S_4 are respectively 0, 22.5, 45, 67.5 and 90 kg of S ha^{-1}

0–20 cm soil layer increased significantly after S application; as the rate of S application increased, more S became available in soil layers (Fig. 5). After clumping (30 DAT), the available S content in different soil layers of each treatment showed a decreasing trend. After harvest (120 DAT), except for the CK treatment, the available S content in the 0–20 cm soil layer of other treatments was significantly higher than that before ridging. This shows that S application significantly increased the available S content in topsoil.

After harvest (120 DAT) in 2016 and 2017, significant differences were observed in the available S content among the various treatments, while the available S content in the 0–20 cm of topsoil increased significantly with an increase in the S appli cation rate (Fig. 5A). Before ridging (T_5 and T_9) in 2017 and 2018, no significant difference was observed in available S content in the 0–20 cm soil layer. No significant difference was observed between the CK and 22.5, 45 and 67.5 kg S ha^{-1} treatments, but the difference between the CK and 90 kg S ha^{-1} treatments was significant.

The available S content in the 20–40 cm soil layer of S treatments increased with an increase in the S application rate (Fig. 5B). After clumping (30 DAT), the available S content in the 20–40 cm soil layer was decreased for all S treatments. After harvest (120 DAT) in both 2016 and 2017, compared with the initial value, no significant difference was observed in the increase of soil available S content among treatments, indicating that S application did not cause a significant accumulation of S in the 20–40 cm soil layer.

The available S content in the 40–80 cm soil layer of each treatment remained basically unchanged during plant growth, and no obvious difference from the initial value of available S was observed before tobacco planting in this soil layer (Fig. 5C and 6D). Sulfur application had no obvious effect on the available S content below the 40 cm soil layer and did not cause S accumulation in this soil layer. In general, the available S content in different soil layers of tobacco fields decreased in the flowing order: 0–20 > 20–40 > 40–60 > 60–80 cm.

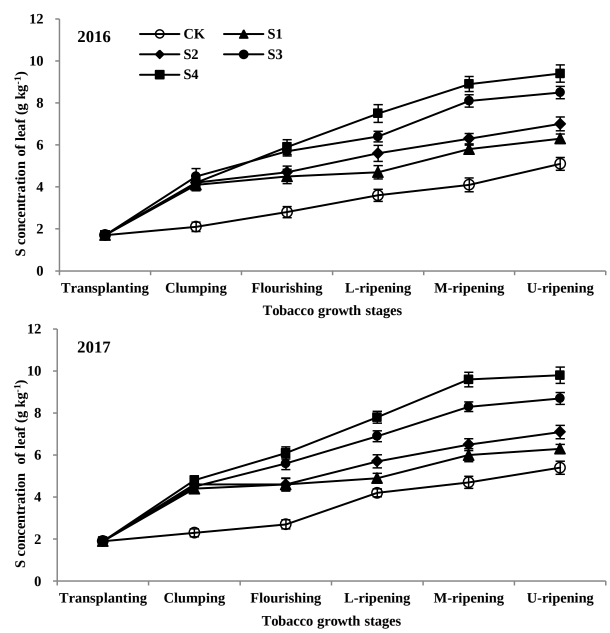

Fig. 1: Effects of sulfur application on sulfur concentration in leaf of flue-cured tobacco in 2016 and 2017 (mean ± SD)
Here CK, S_1, S_2, S_3 and S_4 are respectively 0, 22.5, 45, 67.5 and 90 kg of S ha^{-1}
Transplanting, clumping, flourishing, L-ripening, M-ripening, and U-ripening represent 0, 30, 45, 70, 90, and 110 days after transplantation, respectively

Discussion

Results of this two-year field study unveiled that soil available S contents was increased with gradual rise in S

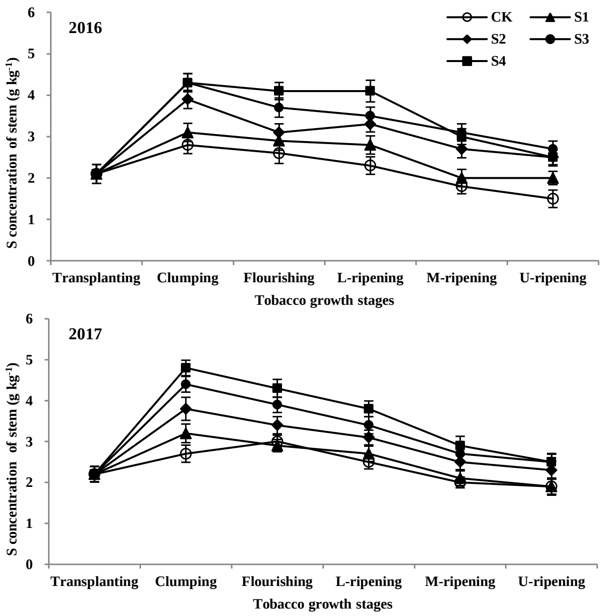

Fig. 2: Effects of sulfur application on sulfur concentration in stem of flue-cured tobacco in 2016 and 2017 (mean ± SD)
Here CK, S_1, S_2, S_3 and S_4 are respectively 0, 22.5, 45, 67.5 and 90 kg of S ha^{-1}
Transplanting, clumping, flourishing, L-ripening, M-ripening, and U-ripening represent 0, 30, 45, 70, 90, and 110 days after transplantation, respectively

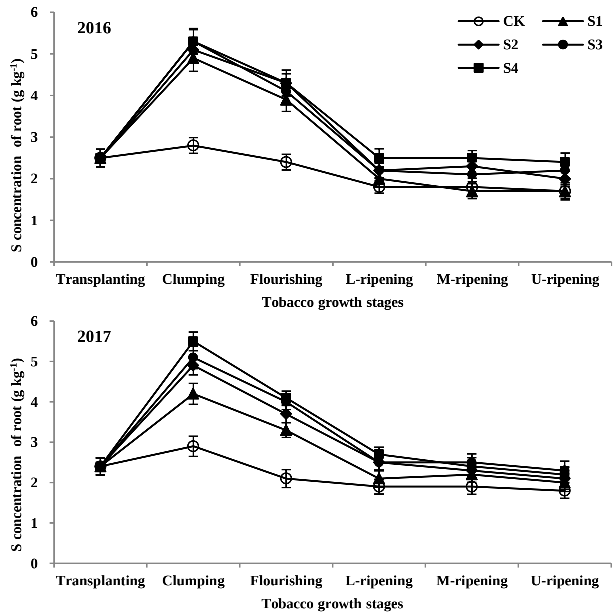

Fig. 3: Effects of sulfur application on sulfur concentration in root of flue-cured tobacco in 2016 and 2017 (mean ± SD)
Here CK, S_1, S_2, S_3 and S_4 are respectively 0, 22.5, 45, 67.5 and 90 kg of S ha^{-1}
Transplanting, clumping, flourishing, L-ripening, M-ripening, and U-ripening represent 0, 30, 45, 70, 90, and 110 days after transplantation, respectively

application rate and the S concentration of leaves was increased accordingly. Therefore, the recommended S application should not exceed 45 kg S ha^{-1} for tobacco in southern Anhui Province. The S absorbed by the root can easily be transported to the aboveground plant parts during transpiration (Zhu *et al.* 2013a) and eventually accumulated in the leaves. Therefore, the tobacco leaf may accumulate more S than its normal growth and development under the condition of extensive S application. However, the quality of tobacco leaves will be poor if the S exceeds a certain concentration. Therefore, optimizing the application of S fertilizer is very important to regulate S concentration in tobacco leaves.

Previous researches believed that the tobacco produced good quality leaves when the S concentration was 2–7 g kg^{-1} (Zhu *et al.* 2013b; Ma *et al.* 2017). Appropriate S concentration (3–6 g kg^{-1}) in leaves was important to enhance the combustibility of tobacco leaves (Wang *et al.* 2018). In contrast, excessive S resulted in the decline of combustibility, flavor, and usability of tobacco leaves (Tian *et al.* 2016). Sulfur fertilization is the most direct and effective method for increasing S accumulation in tobacco leaves. Wang *et al.* (2018) found that S concentration in tobacco leaves was kept within 3–6 g kg^{-1} under the soil available S content of 3.84–48.53 mg kg^{-1}. In this study, under 45 kg S ha^{-1} application, the S concentration in leaves was 6.4 g kg^{-1} (Fig. 1), and the available S content in the topsoil was 37.0–61.4 mg kg^{-1} (Fig. 5A). The soil available S content was increased with the increases of S application rate, and the S concentration of leaves was increased accordingly. Therefore, the recommended S application should not exceed 45 kg S ha^{-1} for tobacco in southern Anhui Province.

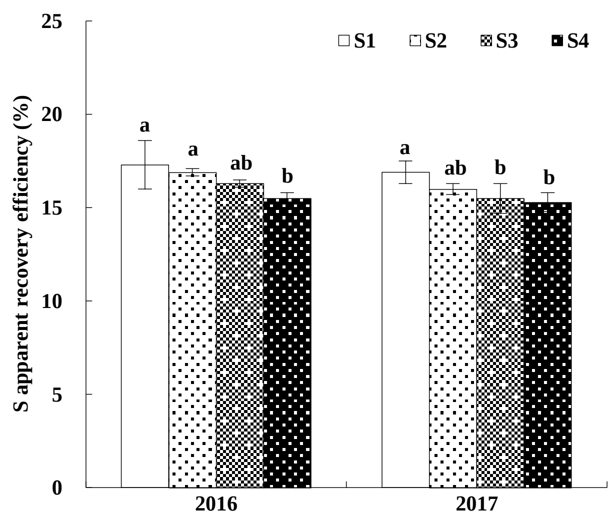

Fig. 4: Effects of sulfur application on sulfur apparent recovery efficiency of flue-cured tobacco in 2016 and 2017
Here S_1, S_2, S_3 and S_4 are respectively 22.5, 45, 67.5 and 90 kg of S ha^{-1}
Columns with different lowercase letters indicate a significant difference for different year ($P < 0.05$)

In this study, S application significantly increased the dry matter of tobacco roots and stems. However, the leaf yield of the CK was not significantly different from the S application treatments. Lin *et al.* (2000) found that S application could significantly increase rice, wheat and maize yield under the condition of soil available S content

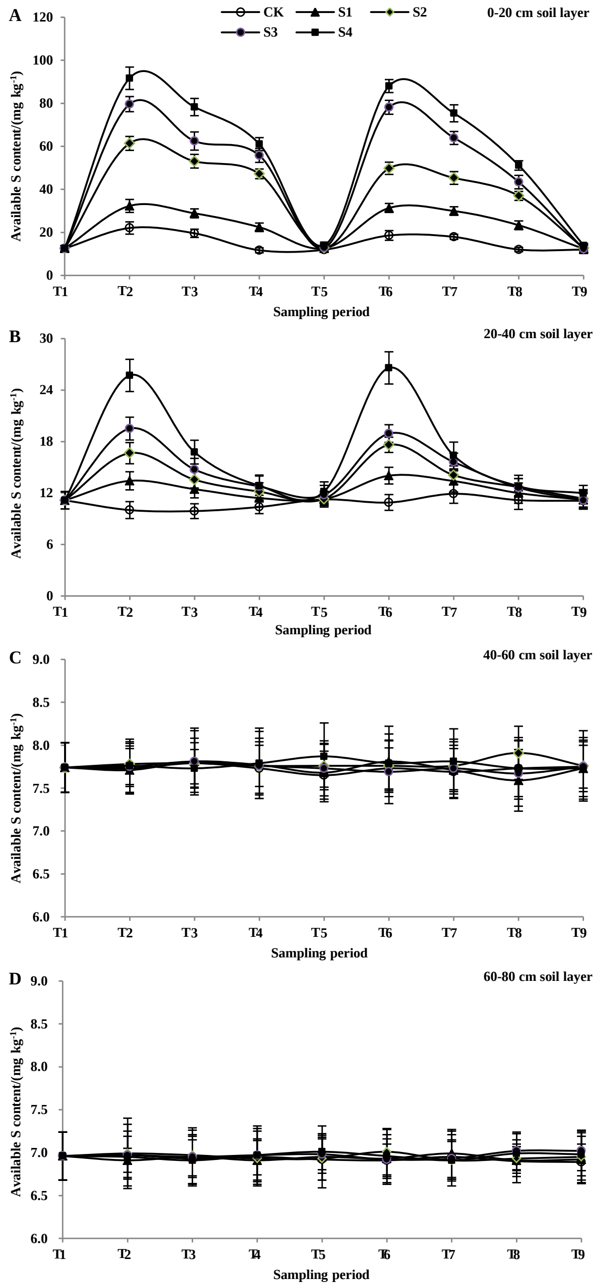

Fig. 5: Effects of sulfur application on sulfur content in different soil layers (mean ± SD)

Here CK, S_1, S_2, S_3 and S_4 are respectively 0, 22.5, 45, 67.5 and 90 kg of S ha^{-1}
T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8 and T_9 represent nine sample stages, *i.e*., before ridging [0 day after transplantation (DAT)], clumping (30 DAT), flourishing period (45 DAT), and at the end of baking (120 DAT) in 2016; 0 DAT, 30 DAT, 45 DAT and 120 DAT in 2017; and 0 DAT in 2018, respectively

of 40 mg kg^{-1} even as high as 94 mg kg^{-1}. Although soil available S content was deficient (12.53 mg kg^{-1}) in this study, the S content was averaged on 1.5 mg kg^{-1} in rainwater, which was an important S resource for tobacco growth. Therefore, the S content in the soil and rainwater found enough for the normal growth and development of tobacco plant. However, for the perspective of long-term sustainable production, at least 13.78 kg S ha^{-1} should be supplied for tobacco plants every year (Table 3), while the topsoil available S content less than 16 mg kg^{-1} is deficient for flue-cured tobacco (Liu *et al.* 2000). Therefore, S fertilizer should be applied for tobacco growth in this area. Based on the S requirement for high-quality and the leaf S concentration in this study, we concluded that the appropriate S application for flue-cured tobacco in southern Anhui was 22.5–45 kg ha^{-1}.

The distribution of available S in soil layer had the following order: 0–20 > 20–40 > 40–60 > 60–80 cm in the present study. Our results was similar to that of Zhang *et al.* (1996) in paddy fields, but different from that of Zou *et al.* (2004). The tobacco-growing soil is mainly paddy, acidic with a parent material of Quaternary red clay and the clay minerals are mainly kaolinite and hydromica (Ma *et al.* 2001). The sticky soil texture of the hydromorphic paddy soil is potentially deficient of available S (Cai *et al*. 1999; Qian *et al.* 2018). The different distribution of S in soil layers was probably due to the different depths of the clay layers of the studied soils. A hard clay plough layer composed of < 0.001 mm diameter clay particles appeared at about 20 cm of the tobacco field in southern Anhui. The soil available S content below 40 cm of all treatments was nearly the same and lower than 10 mg kg^{-1} in this study. However, the soil available S content below 80 cm was as high as 35.3 mg kg^{-1} and significantly higher than that in 20–60 cm soil layer in the clay layer of paddy soil (Zou *et al. 20*04). Therefore, Zou *et al.* (2004) found that the S was seriously leached into the deep soil layer. However, S leaching from tobacco soil is considered to be minimal in southern Anhui, due to the available S content is extremely low in the soil layer below 40 cm.

After harvest, the available S content in topsoil increased significantly with increasing S application rates. Zhang *et al.* (2013) also found that the available S content in the soil increased with an increase of the S application. However, S application did not significantly affect the available S in the 40–80 cm soil layer, which was consistent with the study by Zhang *et al.* (1963), who found that the S^{35} content in the 0–20 cm soil layer was 93.82%, but only 6.18% in 20–30 cm. Unlike the leaching of S studied by Wang *et al.* (2004), this may well be due to the irrigation amount and soil texture (Zhang *et al.* 1963). In the TRRS, the field was ploughed twice a year, resulting in a continuous sinking of fine clay minerals in soil, so that the plough layer was a tightly structure preventing the infiltration of water (Wang *et al.* 2014). The SO_4^{2-} hardly moved through the plough layer in the TRRS, while the soil could retain or cause surface runoff. However, in dryland, the structure of plough layer was loose so that the S moved downward with water easily, resulting in leaching (Wang *et al. 20*04). After two seasons of tobacco cultivation, the amount of available S in topsoil did not change obviously, this was primarily due to the tobacco-rice rotation system. During rice growth, irrigation and drainage were continuously carried out in the paddy fields, and the available S remaining in the soil will dissolve in irrigation water or rainwater and then runoff or be absorbed by the rice. Therefore, more attention should be pay on the pathway of S loss in the TRRS.

Conclusion

Sulfur application at the rate of 22.5–45 kg ha^{-1} resulted high-quality leaf with suitable leaf S concentration of 5.6–6.4 mg kg^{-1} along with higher leaf yield and S use efficiency of flue-cured tobacco. The available S content in the soil layers of tobacco field was as follows: 0–20 > 20–40 > 40–60 > 60–80 cm. Further studies are needed to evaluate the pathway of S loss, particularly for the period from harvesting to next year of transplanting of flue-cured tobacco.

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References

Anderson JW (1996). Distribution and redistribution of sulfur supplied as [^{35}S] sulfate to roots during vegetative growth of soybean. *Plant Physiol* 110:1151–1157

Bayoumi Y, N Taha, T Shalaby, T Alshaal, H El-Ramady (2019). Sulfur promotes biocontrol of purple blotch disease *via Trichoderma* spp. and enhances the growth, yield and quality of onion. *Appl Soil Ecol* 134:15–24

Cai Q, H Chu, D Cheng (1999). Study on the status of available sulfur in paddy soil in southern Anhui province. *Anhui Agric Sci Bull* 1:52–53

Capaldi FR, PL Gratão, AR Reis, LW Lima, RA Azevedo (2015). Sulfur metabolism and stress defense responses in plants. *Trop Plant Biol* 8:60–73

Chu X, X Zhang, B Yang, Z Zhang, Q Fu, W Wang, W Dong, J Dou, Y Dou (2016). Sulfur content and its relationship with quality of the middle tobacco leaves in Fujian. *J Fuj AgricFor Univ* (*Nat Sci Edn*), 45:496–500

Cui QR, WM Wu, LL Jing (2016). Status of soil sulfur content in Anhui tobacco area and its effects on sulfur content of flue-cured tobacco. *J Anhui Agric Sci* 44:121–123

Droux M (2004). Sulfur assimilation and the role of sulfur in plant metabolism: A survey. *Photosynth Res* 79:331–348

Feng X, W Liu, S Sehar, W Zheng, G Zhang, F Wu (2018). Application of sulfur fertilizer reduces cadmium accumulation and toxicity in tobacco seedlings (*Nicotiana tabacum*). *Plant Growth Regul* 85:165–170

Li J, J Lu, X Li, T Ren, R Cong, Li Zhou (2014). Dynamics of potassium release and adsorption on rice straw residue. *PLoS One*, 9:e90440

Lin B, ST Li, W Zhou (2000). Study on test methods for soil available S and critical levels of S deficiency. *Plant Nutr Fert Sci* 6:436–445

Liu Q, X Zhang, H Lai, ZH Cao (2000). On the sulphur nutrition of soil and flue-cured tobacco I. Sulphur nutrition of soil and its effects on growth and development of flue-cured tobacco. *Chin Tob Sci* 4:20–22

Ma YH, LG Zhang, YH Si, QR Cui, JZ Zhang, WM Zhu, RX Ding (2001). Effects of Se and S application on the uptake and accumulation of P in tobacco. *J Anhui Agric Univ* 28:18–23

Ma YQ, Y Xie, ZC Xu (2017). Analysis on the distribution of sulfur in flue-cured tobacco leaf and soil in the tobacco-growing area of Luliang. *Chin J Soil Sci* 48:882–886

Qian XH, P Yang, XJ Zhou, RG Hu, HL Sun, ZK Zhang, XJ Sun (2018). Current situation and spatial-temporal distribution of soil available sulfur in Anhui province. *J Plant Nutr Fert* 24:1357–1364

Qin S, ZY Wang, JX Shi (2007). Quality characteristics of tobacco leaves with different aromatic styles from Guizhou province, China. *Agric Sci Chin* 6:220–226

Rossini F, ME Provenzano, F Sestili, R Ruggeri (2018). Synergistic effect of sulfur and nitrogen in the organic and mineral fertilization of durum wheat: grain yield and quality traits in the Mediterranean environment. *Agronomy* 8:1–16

Shah MA, A Manaf, M Hussain, S Farooq, M Zafar-ul-Hye (2013). Sulphur fertilization improves the sesame productivity and economic returns under rainfed conditions. *Intl J Agric Biol* 15:1301–1306

Shao H, X Ren, N Qiao, Y Liu, W Wang, J Yu (2007). A review of sulphur nutrition in tobacco. *Chin Agric Sci* 23:304–307

Stockmann F, EA Weber, P Schreiter, N Merkt, W Claupein, S Graeff-Hönninger (2018). Impact of nitrogen and sulfur supply on the potential of acrylamide formation in organically and conventionally grown winter wheat. *Agronomy* 8:1–19

Tan Z (2006). Distribution analysis of chloride and sulfur in tobacco plantlet. *Chin Agric Sci Bull* 22:192–194

Tian A, S Wen, G Huang, Q Xu, H Li (2016. Research progress on the sulfur source and control measures of flue cured tobacco. *Crop Res* 30:600–606

Tso TC (1990). *Production, Physiology and Biochemistry of Tobacco Plant.* 1st edn. Ideal Publisher, USA

Wang WJ, XM Zhang, GH Ling, SX Wan (2014). Soil profile properties of gleyed paddy soils and improvement effects of inorganic amendment in South Anhui mountainous area. *J Soil Water Conserv* 28:237–241

Wang XD, ZC Xu, Y Xie, L Liu, XL Fan, XH Zhang (2018). Suitable soil available sulfur and tobacco leaf sulfur contents for high quality tobacco production in Qujing area, Yunnan province. *J Plant Nutr Fert* 24:528–534

Wang ZL, XH Zhang, PH Wang, L Xu, HY Zhou (2004). Study on sulphur (S) uptake pattern by flue-cured tobacco in the field. *J Yunnan Agric Univ* 19:105–109

Xu ZC, CK Liu, QW Bi, J Wang, JH Jiao, ZH Li (2008). Distribution of sulfur content in flue-cured tobacco leaves at main areas of China and its correlation analysis with other chemical components. *J Zhengzhou Univ Light Ind* (*Nat Sci Edn*), 23:1–5

Zhang J, H Liang, G Shen, J Dong, Q Zhang, X Chen, Y Wu, T Zha, H Yu (2013). Effects of successive fertilization of potassium sulfate on change of sulfur content in soil and tobacco plant. *Chin Tob Sci* 34:77–82

Zhang J, W Zhu, L Zhang, H Chu, C Liu, K Li (1996). Research on the status of soil available sulfur in Anhui province. *Chin J Soil Sci* 27:222–225

Zhang W, H Wang, F Qiu, W Tang (1963). Application of ^{35}S to study the movement of sulfur in the soil (Brown Soil) of poplar Canada. *Atom Ener Sci Technol* 1:936–940

Zhu YH, KJ Zhou, SL Wu, G Zhang, NM Tu (2013a). Effect of sulfur fertilization on agronomic traits and photosynthetic characteristics of flue-cured tobacco. *J Northwest A&F Univ* (*Nat Sci Edn*) 41:105–111

Zhu Y, K Zhou, H Xiao, G Zhang, N Tu (2013b). Preliminary study on available sulfur in tobacco-planting soils and sulfur content in flue-cured tobacco leaves in Hunan province. *Chin Tob Sci* 34:5–8

Zou CM, JS Gao, BR Wang, HP Shen (2004). Effects of long-term application of sulphur-containing chemical fertilizers on chemical properties of paddy soil and micro nutrients absorption of rice. *J Anhui Tech Teach Coll* 18:19–25