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Co-application of Phosphorus and Sulfur Improve Yield, Quality, and Nutrients Uptake in *Nicotiana tabaccum* L.

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A field experiment was carried out to evaluate the interactive effect of phosphorus (0, 30, 60, and 90 kg P_2O_5 ha^{-1}) and sulfur (0, 15, 30, and 45 kg ha^{-1}) on yield, quality, and uptake of nutrients by tobacco (*Nicotiana tabaccum* L.). The experiment was organized in a two-factorial randomized complete block design with three replications at Tobacco Research Station, Mardan, Pakistan in 2017-2018. Application of 90 kg P_2O_5 ha^{-1} improved tobacco leaf area (24.2%), green leaf weight (44.5%), cured leaf weight (44.7%), grade index (18.3%), nicotine (35.8%), sugar (12%), and P (24.5%) contents but decreased S content (35.3%) over control phosphorus plots. Similarly, application of 45 kg S ha^{-1} optimized leaf area (13.6%), grade index (12%), nicotine (15%), P (5.9%), and S (96%) contents over control sulfur plots. Interactively, P application antagonized leaf S content. However, it did not show any adverse effect on other agronomic and quality parameter. Both P and S application decrease leaf chloride content by 43.5% and 21.4%, respectively. Thus, 90 kg P_2O_5 ha^{-1} along with 45 kg S ha^{-1} is recommended for obtaining optimum yield of good quality tobacco crop.

Keywords: nutrient uptake, *Nicotiana tabaccum* L., phosphorus, quality, sulfur

Abbreviations: N—nitrogen, P—phosphorus, S—sulfur, RCBD—randomized complete block design, LSD—least significant difference

INTRODUCTION

Tobacco characterize to the genus *Nicotiana* of the family Solanaceae. Tobacco grown throughout the world belongs relatively to the species *Nicotina tabacum* L. and its leaves are used for making cigars and cigarettes. Its most productive species in the world is Flue cured Virginia and its world production is projected to reach 17 billion kg per year (Bortolini et al. 2019).

Tobacco is a cash crop, which lacks a large amount of phosphorus and also performs well with sulfur (Shafi et al. 2020). Phosphorus is a dominant nutrient which is indispensable exclusively for plant growth especially for cell division, root development, and fruit and seed formation. (Barly 1984). Most of Pakistani soils are P deficient and cannot cover the stipulation of the crop, making production low (Adnan et al. 2017). Phosphorus deficiency results in very slow growth, especially in the young stage. Consequently, the leaves are narrow with a dark green, even bluish color. Lower leaves often show many brown spots. Farmers of these areas use phosphatic fertilizers for fulfilling crops P requirement for obtaining optimum yield. Similarly, sulfur is a secondary nutrient that plays a crucial role in plant chlorophyll and is used for protein synthesis (Duke and Reisenaue 1986). In sulfur (S) deficiency, plants cannot obtain protein for chlorophyll (Zhao et al. 1999). Therefore, S is applied for the development and growth of the plant. In agriculture crops, mostly in tobacco, sulfur deficiency is seldom reported (Withers et al. 1995). Sulfur shortages are very similar to those induced by a nitrogen deficiency such as a pale green or even yellow color of the leaves. The chlorosis is systematic on the leaf blade and notably on young leaves. Sulfur deficiency mostly remains undetected mainly due to its resemblance with nitrogen (N). Approximately 9-12 kg ha^{-1} average sulfate is obtained from rainfall, and the requirement for wheat crops is less than others (Zhao et al. 1999).

Sulfur also interacts with phosphorus to form the phosphate ion bond that is stronger than sulfur. (Hedge and Murthy 2005; Ibrahim et al. 2016; Zahoor et al. 2016; Rafiullah et al. 2020). Application of phosphorus fertilizers show good results in increasing the sites of anion by phosphate, which then release sulfur ions in soil solutions (Tiwar and Gupta 2006; Khan et al. 2014). At low levels of sulfur application, there is a synergistic effect; at high levels, there is an antagonistic effect (Randhaw and Arora 2000). Moreover, it has been found that sulfur interacts with phosphorus as both are anions, and this interaction may be due to the anions' completion during uptake processes by plants. Phosphorous-sulfur interactions are conflicting as both synergistic and antagonistic effects are reported (Masood Ali 1989; Aulakh et al. 1990; Shinde and Saraf 1992; Basir et al. 2016; Adnan et al. 2017). The interactive effect of phosphorus and sulfur clearly suggests the importance of balanced fertilization of these two nutritive elements in the nutrition of the tobacco crop. Several studies reported both synergistic and antagonistic relationships between sulfur and phosphorus; however, their relationship depends on the rate of application and crop species. The synergistic effect of applied phosphorus and sulfur was observed by Kumawat et al. (2004) on number and nodules $plant^{-1}$, and an antagonistic relationship between phosphorus and sulfur was observed in moong and wheat (Islam et al. 2006). The interaction of these nutrient elements can affect the critical levels of phosphorus and sulfur available. Sulfur has a beneficial effect by lowering soil *pH* and improving physical conditions of the soil (Choudary et al. 1996). From the point of view of foreign exchange, domestic consumption, and other revenues, tobacco is the most important cash crop grown in Pakistan, especially in the Khyber Pakhtunkhwa province. The climatic conditions of this province are very suitable for growing tobacco, especially in the Swabi, Mardan, Swat, Mansehra, Bunner, and Charsadda districts. This experiment was executed to evaluate the main and interactive effects of sulfur and phosphorus on the yield, quality, and nutrient uptake by tobacco in the alkaline-calcareous soil of Mardan (34°14'01.1"N 72° 00'04.9"E) area.

MATERIALS AND METHODS

Experimental Site

This study was conducted at the Khan Gari Mardan Tobacco Research Station, Mardan-Pakistan (34°14'01.1"N 72°00'04.9"E). The soil of the experimental site was silt loam, alkaline, and slightly calcareous in nature. The soil was non-saline and low in organic matter (because of low vegetation and high temperature). It had available phosphorus and sulfur (Table 1) and is classified as Calcisol.

Experimental Procedure

Seeds of the main Tobacco variety cv TM-2008 were sown in the first week of December 2017 at the Tobacco Research Station, Khan Gari, Mardan, Pakistan and then transplanted to a field in mid-March of 2018 using randomized complete block design (RCBD) with split-plot arrangement replicated thrice. Phosphorus (0, 30, 60, 90 kg P_2O_5 ha^{-1}) was applied to the main plots while sulfur (0, 15, 30, 45 kg S ha^{-1}) was applied to subplots. The phosphorus source was a single super phosphate while elemental sulfur was used as the sulfur source. Recommended doses of nitrogen and potassium were applied in a basal dose to all plots. All recommended agronomic and cultural practices were carried out consistently. Leaves from each treatment were collected after picking for required agronomic parameters. Healthy leaves were taken after curing for the required qualitative

Table 1. Physico-chemical properties of the experimental site.

Parameter	Units	Values
Texture	----	Silt loam
Lime	%	10.6
Organic matter	%	0.14
pH (1:5)	----	7.46
Electrical conductivity (1:5)	d Sm^{-1}	0.14
Phosphorus (AB-DTPA)	mg kg^{-1}	2.05
Sulfur	mg kg^{-1}	19.38

and quantitative parametric study in tobacco. Tobacco response to the application of phosphorus, and sulfur was evaluated using some agronomic and qualitative parameters.

Agronomic and Qualitative Parameters

Agronomic Data

For leaf area, the length and breadth of the 5th, 10th, and 15th leaves were measured in centimeters from each selected plant. The method suggested by Eling (2000) was used to determine the leaf size in square centimeters. The mature leaves of each treatment were collected in four subsequent collections and then weighed separately for each treatment, after which, the data was expressed in kg ha^{-1}. The cured leaf weight from green leaves after curing was recorded. Data was taken in grams and then converted into kg ha^{-1} according to the formula as suggested by Eling (2000). Percentage of top grades which were collected by observing color of cured leaves.

$$\text{Grade index} = \frac{\text{Top grade leaves in a treatment} \times 100}{\text{Total number of leaves in treatment}}$$

Quality Attributes Studied

The nicotine content was determined using the nicotine method in environmental tobacco smoke using the gas chromatographic method (AOAC 2000). The sugar content of the curved tobacco leaves was determined using the method by Stellenbosch (2002). Chloride content in leaves was quantified using the method of Chapman and Pratt (AOAC 1990).

Nutritional Attributes

The cured leaves were ground followed by willy-mill and acid wet digestion (Isaac and Kerber 1971). P, S, and K concentration were determined after color development by spectrophotometer and flame-photometer, respectively (Benton Jones Jr. et al. 1991). Phosphorus content in the sample was determined using the method of Sultanpoor and Schewab (1977). Sulfur was determined in leaves in the form of sulfate using the turbidimetric method (Bardsely and Lencasteer 1960).

Statistical Analysis

The experimental data was electronically analyzed by using a statistic package (Statistic 2000) using analysis of variance (ANOVA) and the means were compared using the least significant difference (LSD) test as described by Steel et al. (1997).

RESULTS AND DISCUSSION

Agronomic Attributes The leaf area of tobacco was considerably affected by different levels of phosphorus (P) and sulfur (S), though interaction between P and S was recorded as non-significant (Table 2). Mean values for phosphorus levels indicated that increasing phosphorus levels increased leaf area and greater leaf area was observed with the addition of 90 kg P_2O_5 ha^{-1}, whereas lower leaf area was observed in control plots. Similarly, the increase in sulfur increased the leaf area and a greater leaf area was observed in the plots treated with 45 kg S ha^{-1}; meanwhile, a lower leaf area was observed in the control plots. A significant effect of P levels and the interaction between P and S were observed for green leaf weight, while S effect was non-significant (Table 3). The increase in P levels significantly increased the weight of the green leaves up to 90 kg P_2O_5 ha^{-1} while lower green leaf weight was recorded at control P. Interaction between P and S showed that green weight linearly increased with increasing phosphorus at all S application rates except control, which showed linear increase up to 60 kg P_2O_5 ha^{-1} and remained constant up to 90 kg P_2O_5 ha^{-1}.

The analyzed data revealed that the addition of 90 kg of P_2O_5 ha^{-1} increased the weight of the cured tobacco leaves (Table 4), while a lower weight of the cured leaves was noted in the control plots. Data showed that cured leaf weight linearly increased with increasing phosphorus levels with all sulfur levels. Grade index was significantly affected by phosphorus and sulfur levels, whereas their interactions were found unaffected (Table 5). Reported data regarding phosphorus levels indicated that grade index was higher with application of 90 kg P_2O_5 ha^{-1}, whereas a lower grade index was obtained in control P plots. Similarly, a higher-grade index was found at 45 kg

Table 2. Leaf area (cm^2) of tobacco as affected by different phosphorus and sulfur levels.

Phosphorus (kg ha^{-1})	Sulfur (kg ha^{-1}) 0	15	30	45	Means
0	895	896	1035	1128	989[b]
30	856	952	1078	1177	1016[b]
60	1251	1058	1140	1119	1142[a]
90	1176	1180	1238	1318	1228[a]
Means	1044[b]	1021[b]	1123[ab]	1186[a]	

LSD for phosphorus = 103.75, sulfur = 123.45, and P x S = NS. Means with different letters are significantly different at α = 0.05. P x S, NS, and LSD represent phosphorus and sulfur interaction, non-significant, and least significant difference, respectively.

Table 3. Green leaf weight (kg ha^{-1}) of tobacco as affected by various phosphorus and sulfur levels.

Phosphorus (kg ha^{-1})	Sulfur (kg ha^{-1}) 0	15	30	45	Means
0	9084	9164	9224	9261	9183[d]
30	11800	11564	11655	11725	11686[c]
60	12765	12565	12465	12566	12590[b]
90	13161	13206	13242	13457	13267[a]
Means	11703[ab]	11625[b]	11647[b]	11752 [a]	

LSD for phosphorus = 115, sulfur = 86, and P x S = NS. Means with different letters are significantly different at α = 0.05. P x S, NS, and LSD represent phosphorus and sulfur interaction, non-significant, and least significant difference, respectively.

Table 4. Cured leaf weight (kg ha^{-1}) of tobacco as affected by different phosphorus and sulfur levels.

Phosphorus (kg ha^{-1})	Sulfur (kg ha^{-1})				Means
	0	15	30	45	
0	1817	1818	1832	1832	1825 d
30	2360	2313	2331	2345	2337 c
60	2513	2513	2493	2513	2508 b
90	2632	2642	2648	2645	2642 a
Means	2330 ab	2321 b	2326 b	2334 a	

LSD for phosphorus = 13, sulfur = 7, and P x S = NS. Means with different letters are significantly different at α = 0.05. P x S, NS, and LSD represent phosphorus and sulfur interaction, non-significant, and least significant difference, respectively.

Table 5. Grade index (%) of tobacco as affected by different phosphorus and sulfur levels.

Phosphorus (kg ha^{-1})	Sulfur (kg ha^{-1})				Means
	0	15	30	45	
0	51.7	52.0	54.0	63.3	55.3 b
30	56.7	54.7	62.3	62.7	59.1 b
60	56.7	55.3	56.7	59.3	57.0 b
90	61.3	63.7	68.3	68.3	65.4 a
Means	56.6 b	56.4 b	60.3ab	63.4 a	

LSD for phosphorus = 5.54, sulfur = 5.39, and P x S = NS. Means with different letters are significantly different at α = 0.05. P x S, NS, and LSD represent phosphorus and sulfur interaction, non-significant, and least significant difference, respectively.

S ha^{-1} and a lower grade index was recorded with no sulfur fertilizer application.

Phosphorus and sulfur application significantly improved leaf area and gran leaf weight in tobacco crop. The probable reason for the increased leaf area of tobacco due to phosphorus application might be that increasing phosphorus increased crop growth and development by enhancing certain physiological process in plants, which increased assimilates in leaves and thus increased leaf area. Similar results were earlier reported by Turuko and Mohammed (2014). The increase in leaf area due to sulfur application might be the reason for it its increase of metabolic activities and nitrogen uptake. The application of phosphorus and sulfur to the soil could accelerate mitotic activities and cell enlargement as well as carbohydrate and fat metabolism in the plant, which may favor increased plant growth (Pathan et al. 2005). The probable reason for the increase in the weight of the green leaves due to P application could be due to a greater accumulation of dry matter and photosynthates with higher phosphorus content. Data was earlier observed by James et al. (2000) and Wahid et al. (2019). The data is also similar to the findings of Saran and Giri (1990) and Singh et al. (1998). The combined application of phosphorus and sulfur increasing the weight of green leaves may be due to the application of P and S to the soil that accelerates cell division and enlargement and also increases the metabolic activities of carbohydrates and fats in plants, which may be favoring a weight gain of the leaves similar to the weight of green leaves (Muhammad et al. 2019; Shafi et al. 2020). The results are further supported by the finding of Saran and Giri (1990) and Singh et al. (1996). In addition to the present findings for cured leaf weight, P/S ratio in leaf was plotted against the yield (Fig. 1). The graph shows how the P/S ratio increases the yield of tobacco curvingly and does not increase significantly. Moreover, it is evident from the graph that the maximum yield occurred at P/S ratio of 3.87 under the conditions of the experimental site. Similarly, the increased grade index with increasing P levels could be because phosphorus plays a vital role in crop maturity, as higher phosphorus improves maturity which leads to good tobacco quality and an improved grade index. The results agree with the early work of James et al. (2000).

Quality and Nutritional Parameters

The nicotine content in tobacco plants was considerably affected by different levels of phosphorus, sulfur, and their interaction (Table 6). Mainly, the increase in P and S levels significantly increased the nicotine content. Interaction between P x S was not significant for nicotine contents. The sugar content of tobacco plants was non-significantly affected by phosphorus, sulfur levels, and their interaction (Table 7). The chloride content of tobacco was significantly affected by various levels of phosphorus and sulfur, although their interactions were found to have responded negatively (Table 8). Mean values of phosphorus and sulfur revealed that increasing phosphorus and sulfur levels decreased the chloride content of tobacco leaves and higher leaf chloride contents were found in control, whereas lower leaf chloride content was found at the rate of 90 kg P_2O_5 ha^{-1} and 45 kg S ha^{-1}. Despite the fact that the response was statistically positive, the chloride content slightly decreased due to increased levels of phosphorus and sulfur applications reflecting the burning quality of tobacco.

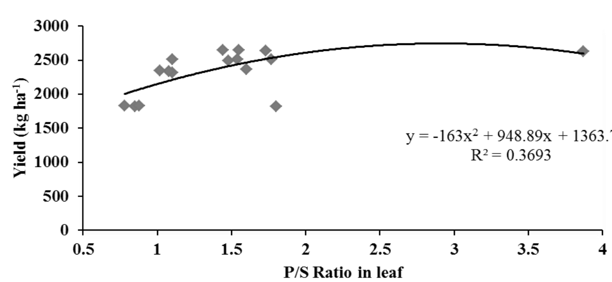

Fig. 1. Relationship between phosphorus-sulphur ratio and yield of tobacco.

Table 6. Nicotine content (%) in leaf of tobacco as affected by different phosphorus and sulfur levels.

Phosphorus ($kg\ ha^{-1}$)	Sulfur ($kg\ ha^{-1}$)				Means
	0	15	30	45	
0	1.58	1.58	1.58	1.59	1.59 [b]
30	1.61	1.62	1.64	1.65	1.63 [b]
60	1.65	1.68	1.73	1.74	1.70 [b]
90	1.84	1.88	2.23	2.70	2.16 [a]
Means	1.67 [c]	1.69 [c]	1.80 [b]	1.92 [a]	

LSD for phosphorus = 0.17, sulfur = 0.1, and P x S = NS. Means with different letters are significantly different at α = 0.05. P x S, NS, and LSD represent phosphorus and sulfur interaction, non-significant, and least significant difference, respectively.

Table 7. Sugar content (%) in leaf of tobacco as affected by different phosphorus and sulfur levels.

Phosphorus ($kg\ ha^{-1}$)	Sulfur ($kg\ ha^{-1}$)				Means
	0	15	30	45	
0	17.4	15.5	17.3	15.4	16.4
30	17.7	16.6	15.9	18.2	17.1
60	16.0	17.8	15.1	18.3	16.8
90	17.3	18.4	18.5	19.4	18.4
Means	17.1	17.1	16.7	17.8	

LSD for phosphorus, sulfur, and P x S are NS. P x S, NS, and LSD represent phosphorus and sulfur interaction, non-significant, and least significant difference, respectively.

Table 8. Chloride content (%) in leaf of tobacco as affected by different phosphorus and sulfur levels.

Phosphorus ($kg\ ha^{-1}$)	Sulfur ($kg\ ha^{-1}$)				Means
	0	15	30	45	
0	0.54	0.43	0.45	0.42	0.46 [a]
30	0.43	0.45	0.40	0.37	0.41 [b]
60	0.39	0.36	0.31	0.31	0.34 [c]
90	0.31	0.29	0.24	0.21	0.26 [d]
Means	0.42 [a]	0.39 [ab]	0.35 [b]	0.33 [b]	

LSD for phosphorus = 0.04, sulfur = 0.06, and P x S = NS. Means with different letters are significantly different at α = 0.05. P x S, NS, and LSD represent phosphorus and sulfur interaction, non-significant, and least significant difference, respectively.

A negative response was found for the potassium content in tobacco leaves when the phosphorus levels varied along with the interaction between phosphorus and sulfur. However, the application of sulfur did not affect the potassium content of the leaves (Table 9). The mean values of the data indicated that increasing phosphorus levels increased the leaf potassium content and higher leaf potassium contents were found with the addition of phosphorus at the rate of 90 kg ha^{-1}, while lower leaf potassium contents were observed in the control treatment with no phosphorus fertilizer applied. Data showed that potassium concentration in leaves increased with increasing P application with sulfur; however, in control, K concentration increased with 30 kg P_2O_5 ha^{-1} then decreased up to 60 kg P_2O_5 ha^{-1} and again increased. Similarly, leaf phosphorus content increased with increasing phosphorus application and higher phosphorus content in leaves were found when phosphorus at the rate of 90 kg P_2O_5 ha^{-1} was applied, while a lower phosphorus content was recorded in the leaves without the addition of phosphorus (Table 10). The sulfur levels increased up to 30 kg ha^{-1}; phosphorus content also increased and then declined. However, lower leaf phosphorus was observed in control plots. The application of phosphorus increased with the combination of sulfur levels, except for 15 kg S ha^{-1} which increased leaf phosphorus content up to 60 kg P ha^{-1}, while further increase in phosphorus slight decrease was observed. The data regarding phosphorus levels revealed that the sulfur content in the leaves was higher without the application of P, while the lower sulfur content was recorded with the application of phosphorous fertilizer at

Table 9. Potassium content (%) in leaf of tobacco as affected by different phosphorus and sulfur levels.

Phosphorus ($kg\ ha^{-1}$)	Sulfur ($kg\ ha^{-1}$)				Means
	0	15	30	45	
0	3.5	3.4	3.4	3.6	3.5
30	3.9	3.5	3.3	3.4	3.5
60	3.2	3.8	3.7	3.8	3.6
90	3.7	3.8	3.7	3.7	3.7
Means	3.6	3.6	3.6	3.6	

LSD for phosphorus, sulfur, and P x S are NS. P x S, NS, and LSD represent phosphorus and sulfur interaction, non-significant, and least significant difference, respectively.

Table 10. Phosphorus content (%) in leaf of tobacco as affected by different phosphorus and sulfur levels.

Phosphorus ($kg\ ha^{-1}$)	Sulfur ($kg\ ha^{-1}$)				Means
	0	15	30	45	
0	0.45	0.48	0.54	0.48	0.49 [c]
30	0.48	0.51	0.54	0.51	0.51 [bc]
60	0.48	0.62	0.55	0.55	0.55 [b]
90	0.62	0.59	0.62	0.62	0.61 [a]
Means	0.51 [b]	0.55 [a]	0.56 [a]	0.54 [ab]	

LSD for phosphorus = 0.035, sulfur = 0.033, and P x S = NS. Means with different letters are significantly different at α = 0.05. P x S, NS, and LSD represent phosphorus and sulfur interaction, non-significant, and least significant difference, respectively.

Table 11. Sulfur content (%) in leaf of tobacco as affected by different phosphorus and sulfur levels.

Phosphorus ($kg\ ha^{-1}$)	Sulfur ($kg\ ha^{-1}$)				Means
	0	15	30	45	
0	0.25	0.56	0.69	0.54	0.51 [a]
30	0.30	0.46	0.50	0.50	0.44 [ab]
60	0.31	0.35	0.37	0.50	0.38 [b]
90	0.16	0.34	0.40	0.43	0.33 [b]
Means	0.25 [c]	0.43 [b]	0.49 [a]	0.49 [a]	

LSD for phosphorus = 0.10, sulfur = 0.06, and P x S = NS. Means with different letters are significantly different at α = 0.05. P x S, NS, and LSD represent phosphorus and sulfur interaction, non-significant, and least significant difference, respectively.

90 kg P_2O_5 ha^{-1} (Table 11). In the case of sulfur levels, higher leaf sulfur contents were found in the plots applied with 45 kg ha^{-1} sulfur, while lower sulfur content of leaves was observed in control plots. The data showed that the sulfur content in the leaves decreased with increasing P levels and increasing sulfur application.

The combined application of phosphorus and sulfur increased the nicotine content because they accelerate cell division and enlargement and also increase the metabolic activities of carbohydrates and fats in plants, which may favor increasing the nicotine content and the quality of the crop (Pathan et al. 2005; Adnan et al. 2014; Ahmad et al. 2015). These results are similar to the studies of Saran and Giri (1990) and Singh et al. (1998). The present study also showed that increased nicotine contents in tobacco leaves occurred at higher phosphorus and lower sulfur due to antagonism. This is similar to the findings of Mehta and Patel (1969) who revealed that the nicotine content of the tobacco crop is improved through the application of phosphorus and sulfur. Potassium concentration in leaves increases due to the synergistic effect of P on K, and applied phosphorus increases potassium uptake (Lopez-Bucio et al. 2000). Sulfur also increases the potassium concentration in leaves because sulfur lowers soil *pH* and increases nutrient availability (Jamal et al. 2010). The higher concentration of potassium in leaves seems to be the consumption of potassium by tobacco in the present study (Hawks and Chahan 2014). However, these concentrations lower the toxic level as reported by Liang and Müller (1989). Phosphorus concentration in leaves increases due to inorganic fertilizer that is readily available for the plant (Lopez-Bucio et al. 2000). Sulfur increases the phosphorus concentration in the leaves because sulfur reduces the soil *pH* and increases the availability of nutrients (Jamal et al. 2010).

CONCLUSION

Tobacco leaf area, green and cured leaf weights, grade index, and nicotine, sugar, and chloride content were considerably improved by the co-application of both 90 kg P_2O_5 and 45 kg S ha^{-1}. Interactively, P application antagonized leaf S content but did not show any adverse effect on other agronomic and quality parameters. Both P and S application decreased leaf chloride content by 43.5% and 21.4%, respectively. Therefore, a combined application of 90 kg P_2O_5 and 45 kg S ha^{-1} beside N and K is suggested for achieving a high yield of good quality tobacco in alkaline soils.

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