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Evaluation of Different Indices of Sulfur Availability in Soils for Wheat (*Triticum aestivum* L.) Production in Ethiopia –II

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Authors' contributions

This was a collaborative work between all authors. Author AM designed the study, performed field-experiments, managed lab analysis, and wrote the first draft of the manuscript. Author NA wrote the protocol, reviewed and edited the manuscript and managed the literature searches. Authors TM and JMRS, monitored field works, edited data, reviewed and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Using high-analysis fertilizers lacking adventitious sulfur (S), coupled with traditional cropping-systems that mine S from native soil, leads to S deficiency. However, under field conditions, S deficiency symptoms are not easily identifiable in cereals, because they are mistaken with those of Nitrogen (N). Hence, S availability indicators are necessary for rational fertilizer use. Eighteen explorative field experiments were conducted in 2012/2013 seasons in Central Highlands (HLs) of Ethiopia, with the purpose of evaluating S deficiency indicators in wheat, with the ultimate aim of setting critical-thresholds. In the study organic carbon (OC), and SO_4-S in soils; total S and N/S ratio in grains were considered. Two levels of S (0 and 20 kg/ha); 2-levels of P (0 and 20 kg/ha);

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and 2-levels of N (0 and 69 kg/ha) as gypsum, triple-superphosphate (TSP) and urea, respectively were used. The experimental was laid out in randomized complete design (RCBD) in three replications. In the study, N/S ratio and S content in grain showed better association with S-uptake, with the degree of correlation, -0.83767 and 0.85547, respectively both significant at $P<0.001$. However, based on the minimum criteria set in literature, the total S in wheat grain showed better sensitivity, whereas N/S ratio was marginal. The critical-thresholds set at 90% Relative Yield (RY), were approximated to about 0.118% for total S, 14.7:1 for N/S ratio in grains; and 11.3 mg/kg for SO_4-S in native soil. Therefore, wheat grains from S responsive sites and/or treatments can be distinguished from un-responsive ones, in which case much S response is expected for sites and/or treatments with total S content of <0.118%, N/S ratio >14.7:1 and SO_4-S <11.3 mg/kg. In general, the results suggest that plant analysis, (in this case, grain), might be taken as a better tool for assessing S supply of soils or wheat crop than the soil analysis, and therefore, this preliminary result could be used as the basis for S research and as a provisional recommendation for wheat growers in Ethiopia.

Keywords: Sulfur; availability/deficiency indices; total sulfur; N/S ratio; SO_4-S and organic carbon.

1. INTRODUCTION

Crop production in Ethiopia has increased tremendously, primarily due to the growing food need triggered by the growing population pressure. To maintain soil fertility for sustainable crop production and productivity, however, small-holding farmers use intensive cropping systems (rotations and mixed/intercropping) that include legumes and oil crops with cereals. In such traditional practices, therefore, nutrient removal including sulfur (S) can be significantly high, especially when crop residues are removed from fields along with the produce. Organic matter (OM) such as crop-residues and farm yard manure (FYM), which are the possible sources of plant nutrients have many alternative uses like fuel-wood and fodder.

It is widely recognized that sufficient S to meet crop requirements can be obtained from incidental additions of S from low-analysis nitrogen (N) and phosphorous (P) fertilizers. However, the inorganic fertilizers available in markets and used for crop production are only urea and di-ammonium phosphate (DAP), high-analysis fertilizers, that contain little or no sulfur. This can lead to a considerable S depletion, if corresponding amount is not replenished through fertilizer, and thus can lead crops to S deficiency.

It has been reported that, continuous removal of S from soils through plant-uptake without replenishment has led to widespread S deficiency and affected soil S budget all over the world [1], even including the industrialized ones, areas where industrial pollutions (e.g., coal combustion) can contribute S for plants [2]. However, under field conditions S deficiency symptoms, particularly in cereals are not easily identifiable, because they can be confused with those of N, and yield losses may occur in crops with marginal deficiency showing no visual symptoms [3]. Consequently, sulfur availability indicators are needed for making fertilizer recommendations to avoid yield or quality losses, due to visible or hidden S deficiency.

To diagnose S deficiencies in wheat, methods based on soil and plant analysis including simulation models have been used [4,5]. Some of the candidate indices include Organic Carbon (OC), Total Sulfur (TS), Organic Sulfur (OS), and SO_4-S in soils; and SO_4^{2-}S, TS, N/S ratios, sulfate: Total S-ratio, malate: Sulfate ratio, and glutation etc at various growth stages of plants [6,7]. However, the critical values determined for those indices show wide variations depending on stages of growth, part of plant analyzed, experimental conditions(field or greenhouse) and method of analysis, all of which, limiting their use for routine recommendations [3]. For instance, according to [8] N/S ratio showed better sensitivity at one distinguishable node and visible flag leaf ligule stages. Consequently, N/S ratio was suggested to be a useful method for S deficiency diagnostics from the end of tillering to flag leaf in spring red wheat. But, the same authors reported, the lack of sensitivity of N/S ratio in stages between 2-4 tillers. Regardless, of these disparities, however, for spring red wheat, the authors recommended, N/S ratio in the advanced stages of crop cycle. In another similar work, it is reported that the indicators, like TS, sulfate and glutation lack stability for their critical values, and thus are not considered to be reliable tools for S deficiency in wheat [9]. In line with this, [10] made reviews on S availability indices, and concluded that plant analysis was better than soil-testing for predicting the need of S application and several diagnostic indices have been suggested, but there was no general consensus as to which index gives best results.

In current study, Organic Carbon (OC), SO_4-S in native soil; TS and N/S-ratio in wheat grain were considered, with the aim of evaluating their relationship with S uptake. The ultimate objective of this work was, therefore, to set critical levels (CLs), for those indices found suitable under native soils conditions. The possible questions intended by this set of experiments were: a) is native soil SO_4-S and/or OC; total S and N/S ratios in wheat seed best correlated with S uptake? b) What is the best estimate of critical thresholds for those indices found suitable?

2. MATERIALS AND METHODS

Eighteen explorative experiments were conducted in 2012/2013 cropping-season on farmer's fields in Central Highlands (HLs) of Ethiopia, representing major wheat growing areas: Arsi, East Shewa and Oromia Liyuu zones, covering different agro-ecological zones (AEZs) and soil types. The soil types in the study areas are typically vertisols and nitisols. Some of the relevant chemical contents of soils before planting were briefly discussed as follows. The pH (1:2.5, soil: Water ratio) of soils ranged from 5.1(more acidic) in some sites in O/Liyuu zone, followed by pH near neutral in Arsi; to about 8.1 in E/Shewa. The Calcium-orthophosphate (Ca $(H_2PO_4)_2$) extracted SO_4-S, ranged between 1.30-24.18 mg/kg. The total nitrogen (TN) determined by Kjeldahl digestion as described in [11] ranged between 0.06-0.25%. Available P extracted by [12] for E/Shewa, ranged between 7.55-10.99 mg/kg, while (Bray-I, [13] P for Arsi and O/Liyuu zones, ranged between 0.22-5.12 mg/kg. The OC contents of soils, ranged from 0.90%-2.99%.

The experiments were laid out in randomized complete block design (RCBD) in three replications, using a wheat cultivar, known as, "Kekeba". Evaluated treatments were 2-levels of S (0 and 20 kg/ha), 2-levels of P (0 and 20 kg/ha), and 2-levels of N (0 and 69 kg/ha). Each replication was sub-divided into, 3 m x 5 m =15 m^2 plots and there were 4-plots per block. Nitrogen (urea), P (triple superphosphate, TSP) and S as gypsum were applied. One third of N was incorporated into soils within rows just before seeding to enhance its use efficiency, whereas the remaining 2/3 was top-dressed at tillering stage. The entire sources of S and P were drilled within rows and incorporated into soils just before planting. The agronomic spacing for wheat between rows and plants, 25 cm x 5 cm was used. There were 12-rows of wheat per plot, two border rows and one used for plant sampling. The remaining middle rows were used for agronomic/yield data and seed sample collection. During crop's growing stage and/or before/after harvest agronomic parameters such as Number of Tillers per Plant (NTPP), Plant Height (PH), Spike Length (SL), Spike Weight (SW), Total above Ground Biomass (TAGB), Number of Grains per Spike (NGPS), Grain (GY) and Stover Yields (SY) were recorded.

2.1 Collecting Shoot and Grain samples

Representative plant shoots were taken from each plot at booting for Laboratory (Lab.) analysis. The, wheat shoot-tissue were collected from the upper 1/3 of 25-plants from a row next to the borders. After sampling, to remove dust or soil contamination, tissues were rinsed quickly in distilled water in fields and shaken to dry, placed in paper bags and air-dried in dust-free rooms. In Lab, the samples were oven-dried at 65-70ºC for 48 hrs to constant weight. Similarly, grain and stover samples were collected at harvest from each plot and oven-dried at 65-70ºC for 48 hrs. All samples, thus oven dried were finely-ground using Tecator CYCLOTEC-1093 sample mill and analyzed for total TN and TS. Finally, TN/TS ratio and S-uptake were determined. S-uptake were determined by multiplying the concentration of TS in grain by grain yield dry-matte (kg/ha) and dividing the whole by 100.

2.2 Analysis of Shoot and Grain Samples

In Lab, finely ground plant materials (grain/shoot) were wet-digested (using 68% HNO_3-30% H_2O_2 for TS determination) and read using a spectrophotometer. Whereas, TN was extracted by Kjeldahl wet-digestion (using conc.H_2SO_4) as described by [11]. The RY was calculated with levels of S as percentage. Relative Yield (RY) = [N/(N-1)]* 100 as described by [14]. Where: N is check treatment/without S, and (N-1) is the next higher level treatment containing S.

2.3 Data Analysis

Four indices for assessing sulfur supply: SO_4-S and OC in native soil; and TS and N/S ratio in wheat grain were correlated with S-uptake and slopes were compared through parallelism and coincidence test using PROC-REG procedure of SAS statistical package [15]. For those indices found suitable based on the coefficient of determination (R^2), critical levels were determined at RY = 90%, using Cate and Nelson procedure [14]. The method involves plotting values of a particular index against RY%. The horizontal and vertical lines were, then positioned on scatter-diagram to maximize number of points in positive quadrants for TS and OC (i.e., first and third quadrants); and in negative quadrants for N/S ratio to obtain critical levels (CLs). This can be verified statistically from the values of total variance (R^2) of observed values with the postulated critical values (CVs), where R^2 peaks at the CVs.

3. RESULTS AND DISCUSSION

Using only high-analysis fertilizers lacking adventitious S, coupled with traditional cropping systems that mine significant amount of S, sulfur deficiency is becoming a soil fertility constraint in Ethiopia. Therefore, to economize fertilizer use, S availability indices are necessary. In this respect, both soil and plant analysis are diagnostic tools for identifying S supply. Table 1 presents some selected indices of S deficiency: SO_4-S, OC and TN in soils; TN, TS and N/S ratio in grains for comparing against S-uptake. It is noticeable that, all the variables considered as indices of S supply to wheat showed a range of variations across the studied sites.

3.1 Correlation of Different S Indices with S Uptake

The relationship between a response variable, S-uptake, and different indices of S status are presented in Table 2. Though, the different indices evaluated in this study varied in their degree of association, all were well correlated with S-uptake/yield. In the association, TS (grain) > N/S ratio (grain) > SO_4-S (soil) > OC (soil), with coefficients of correlations 0.85547, -0.83767, 0.76378 and 0.43848, respectively. From the results, it is observed that TS, SO_4-S and OC had direct relationship, whereas N/S ratio had an inverse relationship with S-uptake. From the indices considered, the TS in grain and N/S ratios were highly significantly correlated (*P*<0.0001), with equal degree of association than SO_4-S and OC. Total S was still more strongly related wheat yield or S-uptake than N/S ratio based on the criteria set by [16]. Details of the results are discussed in the following sub-sections.

3.2 Soil Sulfur Indices

3.2.1 Organic Carbon (OC)

Organic carbon was well correlated with S-uptake with the coefficient of correlation (r= 0.43848), but weak as compared to other parameters or Indices considered (Table 2).

Table 1. Relative Yield (RY) of wheat grain and some selected Indices of S availability in wheat

Study area/zone	Farmer field/Site	SO_4-S in soil (mg/kg)	OC soil (%)	Total N soil (%)	Total N grain (%)	Total S in grain (%)	N/S ratio in grain	S uptake in grain (kg/ha)	Grain RY (%)
Arsi	A/Alko	6.94	1.11	0.126	1.637	0.08	19.95	1.09	67.39
Arsi	Dosha	10.44	2.04	0.252	1.657	0.11	15.19	2.04	90.35
Arsi	G/Silingo	7.77	1.17	0.14	1.670	0.09	19.37	1.21	73.42
Arsi	C/Misoma	22.13	2.75	0.133	1.533	0.12	12.46	1.92	96.47
Arsi	B/Edo	21.50	2.77	0.203	1.377	0.13	10.62	1.85	97.21
Arsi	B/Lencha	4.32	1.07	0.105	1.357	0.06	22.12	0.68	61.45
E/Shewa	C/Donsa	15.37	0.90	0.063	1.963	0.13	15.53	1.50	87.45
E/Shewa	Keteba	5.78	1.06	0.056	1.320	0.07	19.90	0.86	68.55
E/Shewa	Ude	12.37	1.23	0.098	1.683	0.11	16.10	1.03	89.16
E/Shewa	Bekejo	1.30	1.31	0.07	1.447	0.06	23.13	0.88	70.64
E/Shewa	Insilale	6.62	1.35	0.098	1.240	0.06	19.12	0.65	67.53
E/Shewa	Kilinto	8.27	1.39	0.056	1.563	0.08	20.91	0.78	69.92
O/Liyuu	N/Kersa	11.89	1.41	0.07	1.790	0.12	14.98	1.85	87.32
O/Liyuu	N/Suba	5.64	1.47	0.126	1.637	0.07	23.05	0.94	71.82
O/Liyuu	B/Tokofa	3.82	1.69	0.119	1.493	0.07	23.25	0.75	69.25
O/Liyuu	D/Lafto	10.83	1.71	0.14	1.587	0.08	20.25	0.88	79.09
O/Liyuu	W/Harbu	23.02	2.99	0.154	1.380	0.12	11.25	1.37	90.08
O/Liyuu	T/Harbu	24.18	1.31	0.14	1.403	0.12	11.43	2.19	92.68

Table 2. Pearson correlation coefficients (r), between S-uptake and different Indices of S availability in wheat and native soil related to check treatment (N =18)

	Site	Village	SO_4-S, (soil)	OC, (soil)	TN, (soil)	TN, (grain)	TS, (grain)	NS, (ratio), grain	Total S Uptake (grain)
Site	1.00000	0.00000	0.06135	-0.03656	-0.28725	0.01920	-0.02680	0.07268	-0.11014
		1.0000	0.8089	0.8855	0.2478	0.9397	0.9159	0.7744	0.6635
Village	0.00000	1.00000	0.25918	0.29265	0.08469	-0.68671	-0.11533	-0.13255	-0.10304
	1.0000		0.2990	0.2386	0.7383	0.0016	0.6486	0.6001	0.6841
SO_4-S, soil	0.06135	0.25918	1.00000	0.62671	0.37945	0.00405	0.86757	-0.94230	0.76378
	0.8089	0.2990		0.0054	0.1204	0.9873	<.0001	<.0001	0.0002
OC, soil	-0.03656	0.29265	0.62671	1.00000	0.60892	-0.26894	0.46977	-0.59705	0.43848
	0.8855	0.2386	0.0054		0.0073	0.2805	0.0492	0.0089	0.0687
TN, soil	-0.28725	0.08469	0.37945	0.60892	1.00000	-0.08742	0.35005	-0.43154	0.51562
	0.2478	0.7383	0.1204	0.0073		0.7301	0.1544	0.0737	0.0285
TN, grain	0.01920	-0.68671	0.00405	-0.26894	-0.08742	1.00000	0.40187	-0.02416	0.25828
	0.9397	0.0016	0.9873	0.2805	0.7301		0.0983	0.9242	0.3008
TS, grain	-0.02680	-0.11533	0.86757	0.46977	0.35005	0.40187	1.00000	-0.91060	0.85547
	0.9159	0.6486	<.0001	0.0492	0.1544	0.0983		<.0001	<.0001
NS, ratio, grain	0.07268	-0.13255	-0.94230	-0.59705	-0.43154	-0.02416	-0.91060	1.00000	-0.83767
	0.7744	0.6001	<.0001	0.0089	0.0737	0.9242	<.0001		<.0001
Total S uptake	-0.11014	-0.10304	0.76378	0.43848	0.51562	0.25828	0.85547	-0.83767	1.00000
	0.6635	0.6841	0.0002	0.0687	0.0285	0.3008	<.0001	<.0001	

Furthermore, its association to S-uptake was not significant at *P*=0.05 probability level. However, the OC's weak association to S-uptake, can be explained by the mount or quantity of available plant nutrients that may be released through mineralization to support plant needs.

It is reported that, S in soils is usually associated with organic fractions and its supply to crops is largely regulated by organic matter (OM), and thus the amount of labile OC is considered to be a good indicator of available sulfur [17]. It is also widely recognized that OC is not only the indicator of the supply of essential elements like C, N, P, K and S, but also considered to be one of the key indicators of soil health and/or quality [18]. However, controversies exist even in setting its critical-threshold for sustained soil functions and in quantifying plant available S that can be released [19]. This can hold true, because, during crop growth stages, OM mineralization can be slow and the amount of S released during critical growth stages of crops may not be sufficient enough to meet crop's S demand. Moreover, it will also be too late to satisfy crop's S need from SO_4-S that is coming through late mineralization vis-à-vis early growth stages, where crops are in greatest S need. In line with this, [10] reported the difficulty of predicting the amount of available SO_4-S that can come from added OM, because of the complicated soil dynamics in soil environment.

With respect to measuring the amount of essential nutrients including S that could be supplied through OM mineralization, even more controversies can exist under tropical climate and soil conditions [10]. Generally, all these conditions support the relative weak correlation of OC to S-uptake in wheat grain. Apart from its unpredictable quantity of available nutrients released through mineralization, the quantity of OC itself in the studied soils was very low, including TN and available P. This may indicate that the depletion of OM, in tropical soils can have significant effect on soils S status.

3.3 Sulfate Sulfur (SO_4-S)

The SO_4-S in soils varied significantly (*P*=0.0107) across sites. In the studied soils, extractable SO_4-S, ranged, 1.30 mg/kg to 24.18 mg/kg (Table 1). It is widely accepted that SO_4-S in soils can indicate S supply to crops. In this study, the SO_4-S in native soil was positively related to S-uptake in wheat grain with the coefficient of correlation, 0.76378. Furthermore, it had a higher level association (*P*=0.0002), than OC, but to lesser degree than N/S ratio and TS (Table 2). Its level of significance can be explained by the fact that, the available S in soils can be affected by many factors, (erosion, leaching, changes and/or balances between the activity of microbial biomass etc), before it can be absorbed by plants and/or losses other than plant-uptake. Despite its relative better correlation with yield in this study, however, inconsistencies between soil-test and crop performances have been reported widely and seasonal effects on its availability to plants and leaching restrict the usefulness of soil analysis to identify S responsive sites [20]. [21], share a similar idea in that, any analytical value on SO_4-S of soils to be more or less only for the moment, the time the sample had been taken.

In relation to the present results, in the case of S, both plant and soils S-status is assumed to be suitable parameters to calibrate different soil-test methods, but the suitability of any index depends on the degree of correlation to crop yields [16]. According to this author, a minimum coefficient of correlation (r=0.84) on field data is required for a reliable method and diagnosis of S deficiency to ensure efficient use of S fertilizers. Therefore, from the current study, the obtained value (r=0.76378) for the SO_4-S in native soil, is far below this criteria to declare its suitability. In accordance, this critical level approach for native soils, SO_4-S extracted by $CaCl_2$ or Ca $(H_2PO_4)_2$, was questioned by [22]. According to this author, SO_4-S may not be adequate to assess S status, as it is governed by many factors. To overcome this shortcoming, the author recommended considering OM of soils. By considering OM content of soils along with SO_4-S, [23] proposed the S availability index. According to this concept, if a soil contains SO_4-S, just above the critical limit and low in OM, it cannot be considered as sufficient in available S, since there is less OM to support the inorganic fraction in case of any depletion [24]. Similarly, the organic S is also in equilibrium with inorganic counterpart and, if there is any decline in inorganic SO_4-S level by means of crop uptake or leaching loss, it will be adequately replenished by the organic fraction. From this, therefore, it is noticeable that, OM content of the studied soils was also low to replenish SO_4-S in relation to the crop's S need and to declare the suitability of the soil's SO_4-S as a better index.

3.4 Plant Sulfur Indices

3.4.1 Nitrogen/Sulfur (N/S) ratio in grain

Total N/S ratio in grain was better related to S-uptake, with the coefficient of correlation (r= -0.83767); and significant at ($P<0.0001$) (Tables 2 and 3). It is known that the useful tools for diagnosing S deficiency are soil and plant analysis. In the case of S, plant S status is assumed to be a suitable parameter to calibrate soil-test methods, and the suitability of any index depends on the degree of correlation to crop yields. Interestingly, this coefficient of correlation for N/S ratio, though marginal, it is close to the minimum data set by [16]. As a result, the N/S ratio in grain can be considered, as a satisfactory tool for S deficiency in wheat.

[7] reported that N/S ratio is not an appropriate diagnostic tool for S deficiency in the early stages of wheat growth, and affirmed that in appropriate NS availability conditions, N/S ratio is not stable during the beginning of tillering to stem elongation end in wheat. According to these authors, this lack of stability was attributed to the lower S dilution in relation to N, which is related to a lower initial accumulation rate of sulfur. From this report, therefore, it can be assumed that wheat tissue sampling in later growth stages can give better index of S availability. [25] proposed the use of TS and N/S ratio in grain as a satisfactory indicators or tools of S supply for wheat based on the thresholds, 0.12% (TS) and 17:1(N/S ratios) they determined. Of course, this is in close conformity to the current result.

However, controversial views and arguments have emerged regarding the suitability of N/S ratio and TS in plants. According to [10], one of the problems of using N/S ratio is that a surplus of one of these elements may be interpreted as a deficiency with the other. Another problem with N/S ratio is that, S is a rather immobile nutrient in

Table 3. Mean, Std deviation and the range of variables considered in the correlation (N=18)

Variable	Mean	Std Dev	Sum	Minimum	Maximum	Range
SO_4-S,(native soil)	11.23	7.17	202.19	1.30	24.18	22.88
OC,(native soil)	1.60	0.63	28.73	0.90	2.99	2.09
TN,(native soil)	0.12	0.05	2.15	0.06	0.25	0.196
TN,(grain)	1.54	0.18	27.74	1.24	1.96	0.72
TS,(grain)	0.09	0.03	1.68	0.06	0.13	0.07
NS-ratio(grain)	17.70	4.33	318.61	10.62	23.25	12.63
S uptake,(grain)	1.25	0.51	22.47	0.65	2.19	1.54

plants and older leaves tend to be higher in S than young leaves, while N is mobile and young leaves tend to have higher N than old leaves [24]. Based on this, therefore, the authors opposed the stability of N/S ratio concepts.

In general, from results obtained in this study, it can be concluded that the N/S ratio could be used as a satisfactory index of S deficiency in wheat, when grain is considered in the analysis, because, though marginal, the value was close to the minimum criteria set in literature.

3.5 Total Sulfur (TS) in Grain

Total S in grain is much better correlated to S-uptake with coefficient of correlation (r=0.85547), and significant at (*P*<0.0001), than the rest of the variables considered (Tables 2 and 3). It is widely recognized that critical S concentration depends mainly on the plant species, sampled part of plants, development stage and yield level [21,26]. In accordance, [27,28] reported that total S in the above grown plant or in specific parts is widely used to assess S status of plants. In agreement with the current finding, [25] proposed the use of TS followed by N/S ratio in grain to be a reliable tool of S availability for wheat based on the thresholds they developed, 0.12%(TS) and 17:1(N/S ratio) in grains. But these thresholds were now modified by [26] and new thresholds developed, 0.15% (TS) and 13.3:1 (N/S ratio), the values suggested to be showing a good behavior. In general, from the above discussions and the present results, therefore, it can be concluded that, the plant analysis offered a better tool than soil-testing (SO_4-S and OC) in predicting S deficiency in wheat and/or studied soils, because the grain analysis in wheat showed better sensitivity of S deficiency.

3.6 Critical Levels for Selected Indices

3.6.1 Nitrogen/Sulfur (N/S) ratio in grain

The scatter diagram for relative yield (RY) and the N/S ratios in grain are shown in Fig. 1. This relationship was used to determine critical level using Cate and Nelson procedure [14]. The N/S ratio varied over sites depending on native soil sulfur (Fig. 1 and Table 3). Unlike other variables, the N/S ratio was inversely related to RY or native soil's S. Interestingly, all points lie in a straight line and nearly all are in negative quadrants indicating that there is no abnormal case in the behavior of RY in relation to S status. The coefficient of regression (R^2=82.3%) and the regression equation was Y = -2.449X+122.7.

The regression line indicates that maximum RY, 90% was obtained when the N/S ratio was nearly, 14.7:1, and as S deficiency becomes more sever, N/S-ratio was increased to about 23.25:1. This critical value 14.7:1 for N/S ratio can be used to separate sulfur responsive sites and/or treatments from non-responsive ones. Though the obtained N/S ratio is lower than that determined by [25], (17:1), but is comparable to that reported by [29], which was 14.8:1.

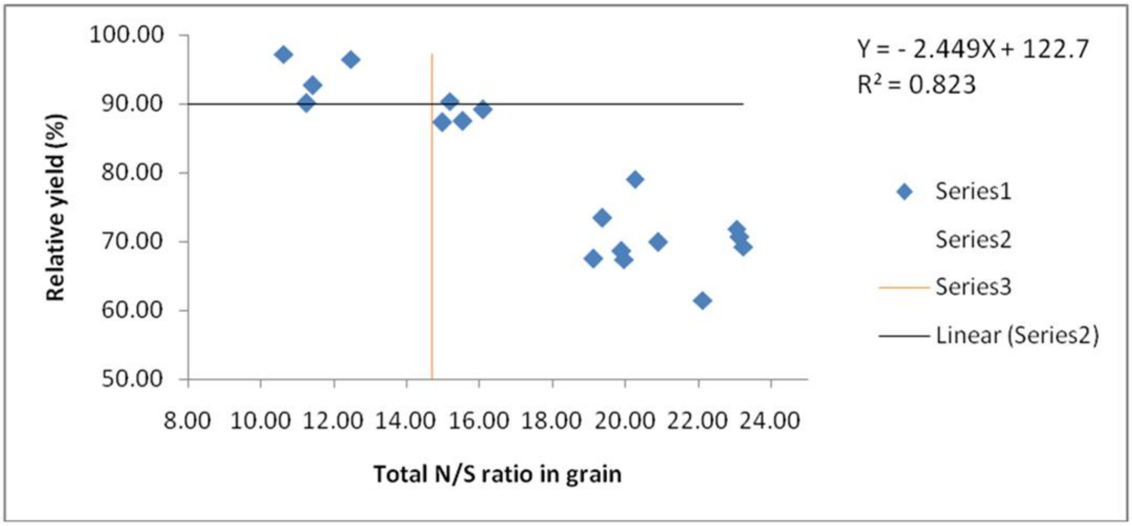

Fig. 1. Relationship between RY and N/S ratio in wheat grain for the native soil. The horizontal line depicts 90% maximum grain yield and the vertical line depicts N/S ratio threshold

However, regarding the suitability of N/S ratio as indicator of S supply in wheat, different views have emerged. For instance, [9,30] questioned the usefulness of N/S ratio concept, as it reflects relative proportions of N and S, than the actual magnitude of either of the elements. According to the authors, low N/S ratio suggests S sufficiency when both nutrients might be deficient, whereas high N/S ratio might mean excessive N instead S deficiency. Furthermore, TS concentration is less sensitive to S availability variations in soil, in relation to plant sulfate levels at early stages of growth [31], all of which would limit the use of N/S ratio for that stage. Consequently, they suggested that the critical value of N/S ratio to be determined empirically. However, from present study, though marginal, the N/S ratio in wheat grain was found to be a satisfactory index of S deficiency.

3.7 Total Sulfur Content in Grains

The scatter diagram for RY and TS in grain are shown in Fig.2. The critical level of the total S was estimated to be, 0.118%, and this value is comparable with that reported by other workers [25,26]. In general, the TS in grain was found to be a better index of S deficiency than other variables considered in this study, based on the criteria developed by [16] with a coefficient of regression (R^2=89.3%). The RY is always increasing with TS, with the regression equation of Y=427.3X+39.63. Interestingly, all the scatter diagram points lie in a straight line and nearly all are in positive quadrants, which means that there is no abnormal case in the behavior of RY in relation to soil's S supply. [30] determined a critical concentration of TS in grain of about 0.15%. However, this value is higher than that obtained by [25], 0.12%, but equal to that determined by [26].

In accordance [25], recommended that grain analysis can be used to diagnose, retrospectively, the S status of plants from which that grain came, and suggested that the information derived can be used to decide fertilizer applications to the succeeding crop(s).

In general, from the present study, it is important to note that, TS of wheat grain is found to be a better index as a diagnostic tool, and the critical level, those obtained, 0.118% could be used as a provisional recommendation for wheat growers in Ethiopia. As the critical level determined by the Cate and Nelson procedure divides only low and high levels, it is important to note that the marginal levels of TS can stretch up to 0.125% or higher in grains.

3.8 SO_4-S in Soils

The scatter diagram of RY and SO_4-S in native soils are shown in (Fig.3). The critical level of the SO_4-S in soils was estimated to be, 11.30mg/kg, with a coefficient of regression (R^2=77.00%). However, it was not a better index of S deficiency based on the criteria set by [16]. Furthermore, its coefficient of regression was the least as

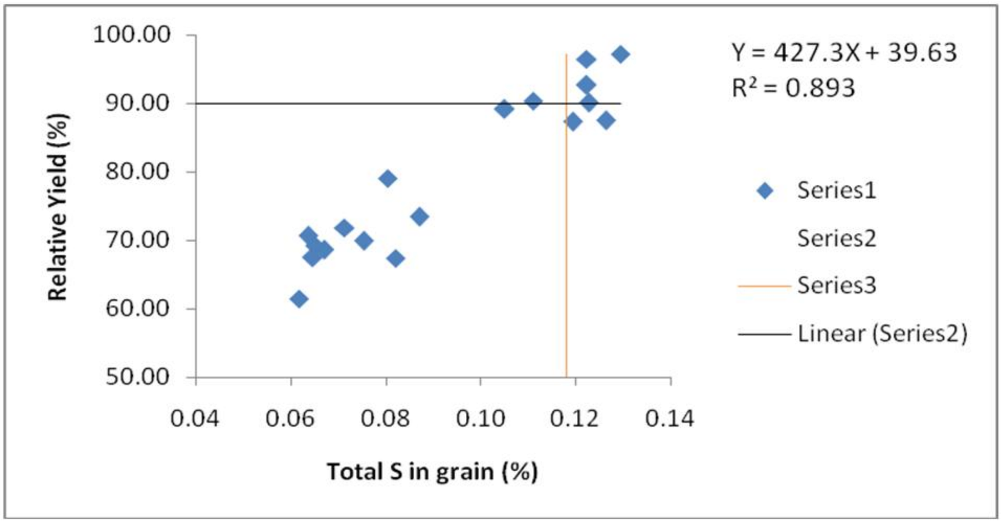

Fig. 2. Relationship between RY and total S in grain. The horizontal line depicts 90% maximum grain yield and the vertical line depicts N/S ratio threshold

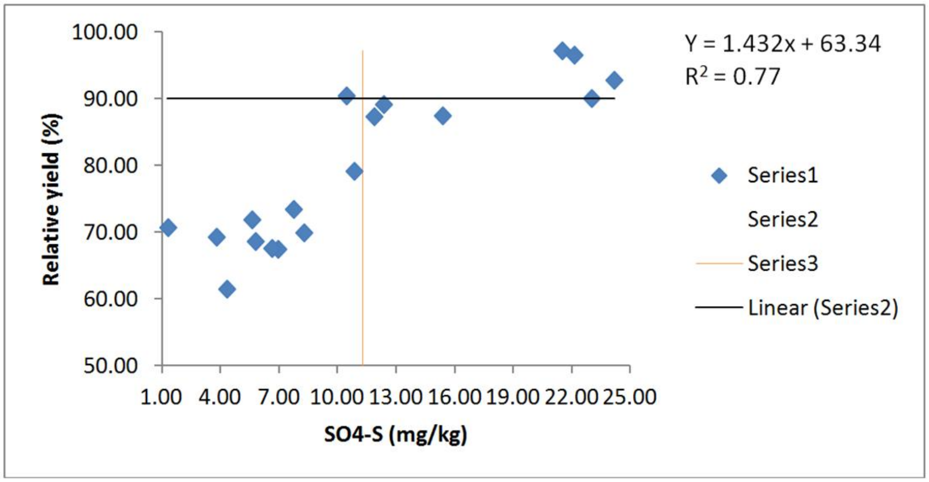

Fig. 3. Relationship between RY and SO_4-S in native soil. The horizontal line depicts 90% maximum grain yield and the vertical line depicts SO_4-S ratio threshold

compared to N/S ratio and TS in the grain. In the studied areas, the SO_4-S in soils ranged, from 1.30 mg/kg to 24.18 mg/kg. From the present study, it is observed that about, 66.7% of the studied soils were below this critical level, 11.30 mg/kg. However, as the critical level divides only lows and highs, it is important always to note that marginal/medium levels can stretch up some levels above 11.30 mg/kg. Considering this marginal level approach and the critical limit 10-13 mg/kg SO_4-S proposed by [22] for cereals (wheat, maize etc), however, over 70% of the studied soils can be S limiting for wheat production.

Similar to the other indices considered in this study, all the scatter diagram points lie in a straight line and nearly all are in positive quadrants with the regression equation of Y=1.432X+63.34, with no abnormal case in RY.

4. CONCLUSIONS

From the present study, it was learnt that both plant and soil analysis can be used as the diagnostic tools in predicting S availability to plants. But, from the variables evaluated, though can't be used to make interventions in advance, the plant analysis (TS followed by N/S ratio in grains) gave better sensitivity as index of S supply than soil, because of their better correlation with S-uptake or yield. However, based on the minimum data set in literature, still TS in grain could be taken as a better index or tool in predicting S deficiency than N/S ratios in wheat. Linear regression analysis showed that the critical threshold for grain S were estimated at 90% RY, and, thus, the wheat grain from S responsive sites or treatments can be distinguished from those un-responsive ones, in which case much S response is expected for sites or treatments with TS less than 0.118%; N/S ratio above 14.7:1; and soils SO_4-S below 11.3 mg/kg. In general, the critical levels obtained for the different variables in the present study were in close agreement with those reported by other workers, but standardizing the values as well as setting the best index of S deficiency may need further investigations. It is also important to note that the indices of S availability considered in this study as well as the various candidates proposed by other workers have comparative usefulness or advantage and, therefore, much is expected to be done to locate the most suitable indicator for wheat and/or other crops in Ethiopia. In conclusion, however, as this critical level approach is the first work in cereals (only one cultivar considered), the obtained preliminary results could be used as the basis for further S research and could be used as provisional recommendation for wheat growers in Ethiopia.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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