

Evaluation of more Indices of Sulfur Availability in Soils for Wheat Production in Ethiopia

ABSTRACT

Sulfur(S) deficiency is becoming one of the soil health challenges in the Ethiopian crop production systems. However, visual identification of its deficiency, especially in cereals is difficult, because the symptoms are nearly identical with those of nitrogen. Hence, deficiency indicators are necessary for balancing fertilizer use. For this purpose, 18 sulfur response experiments conducted in 2012-14 were considered. Major aim was identifying more suitable indices of S supply and setting their critical thresholds. The treatments were: absolute control(CK); nitrogen(N); nitrogen and sulfur(NS); and nitrogen, phosphorus and sulfur(NPS). The levels of nutrients tested were: S(0 and 20kg S/ha), P(0 and 20kg P/ha) and N(0 and 69kg N/ha) in the form of gypsum, triple-super phosphate(TSP) and urea, respectively. Treatments were arranged in randomized complete block(RCB) design and replicated 3 times. In the study, from the selected indices: N/S-ratio and S concentration in wheat at booting showed better sensitivity as indicators of S deficiency than the organic carbon(OC) in native soils. Critical levels(CLs) were set at 90% relative yield(RY), using the Cate and Nelson model, and estimated to be 16.5:1(N/S-ratio), and 16%(S concentration); and 2.07% (for the soil OC). Therefore, sulfur responsive soils/treatments in wheat at booting can be separated from un-responsive ones, in which case much sulfur response is expected for sites/treatments with N/S-ratio >16.5:1; TS <0.16%; and the soil OC <2.07%. This study further affirmed that, plant analysis could be used as a better tool for assessing sulfur deficiency in wheat than soils. Thus, the results could be used as provisional recommendations for wheat growing and as the basis for further sulfur research in Ethiopia. However, differences between the estimated values and those reported in literature have been observed. Therefore, the follow-up research should focus in identifying/standardizing a more reliable index of S deficiency and CLs, through a more reliable research condition.

Keywords: Sulfur deficiency indices, total sulfur, N/S-ratios, wheat shoot, booting and plant analysis.

1. INTRODUCTION

Continuous removal of plant nutrients from native soils through plant-uptake without replenishment, coupled with different losses has led to sulfur(S) deficiency, particularly in annually cropped-lands in Ethiopia, and affecting soils sulfur budget [1,2,3,4]. However, visual identification of S deficiency in cereals, (e.g., wheat) under field conditions is difficult, since the deficiency symptoms are nearly identical with those of nitrogen(N). As a result, yield losses may occur with marginal deficiency showing no visual symptoms. Consequently, S availability indicators are required to balance fertilizer recommendations in order to avoid or reduce yield and quality losses due to visible or hidden S deficiency.

To diagnose deficiencies of S in crops methods based on soil and plant analysis including simulations models have been used [5,6]. Among others, such indices may include organic carbon(OC), total sulfur(TS), organic sulfur(OS) and $\text{SO}_4\text{-S}$ in soils; and SO_4^{2-}S , TS, N/S-ratio, $\text{SO}_4^{2-}\text{S}:\text{TS}$ ratio, malate: SO_4^{2-}S ratio and glutation etc. at various stages of plants growth [7,8]. The critical values determined for those indices, however, show a range of variations depending on factors including experimental conditions and method of analysis. For instance, according to [9,10], N/S-ratio in wheat showed better sensitivity at one distinguishable node and visible flag leaf ligule stages. Consequently, N/S-ratio was suggested to be a useful method from the end of tillering to flag leaf in spring red wheat. But, the same authors reported, lack of stability of N/S-ratio in the stages between 2-4 tillers. Regardless, of these disparities, for spring red wheat, the authors recommended, N/S-ratio in advanced stages of crop cycle. In line with this, [11] made reviews on various S deficiency 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 no general consensus has been reached.

56 A. Menna *et al.*, [2] considered SO₄-S in native soils; TS and N/S-ratio in wheat grain and indicated
 57 that, plant variables showed better correlation with S-uptake than soil variables. The authors
 58 concluded that, TS in wheat seed followed by its N/S-ratio was found to be a better tool of S supply
 59 than SO₄-S in soils. However, [12] recommended the youngest fully-developed leaves, if critical S
 60 concentrations in plant are to be developed for wheat. That was the most likely stage to produce
 61 satisfactory results, because leaf tissues contain the highest nutrient concentrations, which facilitate
 62 analysis. Furthermore, the deficiency symptoms are suggested to be more pronounced in younger
 63 developing leaves.

64
 65 Also, according to [13] S deficiency was best identified by determining the total N/S-ratio followed by
 66 S concentration in vegetative tissue in wheat. The authors further noted that, S content in the whole
 67 plant tissue was not as reliable as N/S-ratio for determining S deficiency, because S content declined
 68 rapidly with growth, (0.25% at tillering to 0.12% at heading). This was also reported to vary
 69 significantly between years at a comparable growth stage and as a result, determination of critical S
 70 concentration was reported to be difficult.

71
 72 Based on the above backgrounds, therefore, the objectives of this work were: 1) correlate some
 73 selected indices of S deficiency with yield, and 2) to estimate critical levels(CLs) for the selected
 74 indices: OC in soils; N/S-ratio and TS in wheat. The possible questions intended by this set of
 75 experiment were: a) Is OC in native soils; TS and N/S-ratios in wheat at booting, best correlate with S-
 76 uptake data? If so, b) what are the CLs for those indices?

77
 78 **2. MATERIALS AND METHODS**

79 **2.1. Description of the Study Areas**

80 The study was conducted in Arsi(Ar), East Shewa(ES) and Oromia Liyuu(OL) zones, in the Central
 81 Highlands(HLs) of Ethiopia. The areas cover different agro-ecological zones(AEZs) and soil types.
 82 Some specific locations and salient features of the study areas are presented in Table 1.

83
 84 **Table 1. Geographic locations of the selected study sites for sulfur response trial**

Farmer field/Sites	Latitude(N)		Longitude(E)		Altitude m	Soil type
	degree	mm.mm	degree	mm.mm		
Abosara-Alko (A/Alko),(AA)	7	49.454	39	1.661	2297.02	Chromic Vertisol
Dosha,(Do)	7	53.813	39	6.176	2418.32	Nitrosol
Gora-Silingo (G/Silingo),(GS)	8	0.792	39	8.436	2151.10	Chromic Vertisol
Chefe-Misoma (C/Misoma),CM	7	59.067	39	3.964	1768.98	Nitrosol
Boneya-Edo (B/Edo),BE	8	3.507	39	17.184	2359.95	Chromic Vertisol
Boru Lencha (B/Lencha),(BL)	8	7.476	39	17.722	2186.37	Nitrosol
Chefe Donsa (C/Donsa),CD	8	57.113	39	6.087	2426.53	Pellic Vertisol
Keteba(Ke)	8	53.553	39	1.913	2224.37	Pellic Vertisol
Ude(Ud)	8	40.767	39	2.197	1873.86	Pellic Vertisol
Bekejo(Bk)	8	38.376	38	55.322	1874.16	Pellic Vertisol
Insilale(In)	8	51.647	38	53.214	2211.30	Chromic Vertisol
Kilinto(Ki)	8	54.099	38	49.133	2204.00	Pellic Vertisol
Nano-Kersa (N/Kersa),(NK)	8	55.605	38	31.062	2123.74	Chromic Vertisol
Nano-Suba (N/Suba),(NS)	8	57.287	38	29.756	2229.54	Nitrosol
(Berfeta-Tokofa) B/Tokofa,(BT)	8	59.605	38	30.98	2252.64	Nitrosol
Dawa-Lafto, (D/Lafto),(DL)	8	59.147	38	26.92	2173.60	Nitrosol
Wajitu-Harbu (W/Harbu),(WH)	9	1.457	38	28.731	2335.63	Nitrosol
Tulu-Harbu (T/Harbu),(TH)	9	2.571	38	28.817	2349.62	Nitrosol

86
 87 **2.2. Methodology**

88 Eighteen explorative sulfur response field experiments were conducted in 2012-14 cropping-seasons
 89 in the central HLs of Ethiopia, representing major cereal(wheat) and legume growing three
 90 representative locations, namely Ar, ES and OL zones. Soil-types in the studied areas are typically
 91 vertisols and nitisols. The pH(1:2.5, soil:water ratio) of soils ranged from 5.1(strongly acidic) in OL
 92 followed by a pH near neutral in Ar; to 8.1(moderately alkaline, with the observed gray nodules of
 93 CaCO₃(calcareous) in ES. The Calcium-ortho-phosphate(Ca(H₂PO₄)₂) extractable SO₄-S range was
 94 1.30–24.18mg/kg. The total nitrogen(TN), determined by micro-Kjeldlehl digestion as described in [14]
 95 ranged, 0.06-0.25%. Available P extracted by [15] for ES ranged from 7.55 to 10.99mg/kg; and the
 96 Bray-I P, [16] for Ar and OL, ranged 0.22–5.12mg/kg. The OC contents of soils ranged from 0.90% to
 97 2.99% [1].

98
 99 The test crop used was, "Kekeba", a newly released wheat cultivar. The treatment combinations were:
 100 absolute control/check(CK); nitrogen(N) only; nitrogen plus sulfur(NS); and nitrogen, phosphorus plus

101 sulfur(NPS). Two levels of each of the nutrient were tested: S(0 and 20kg S/ha), P(0 and 20kgP/ha)
102 and N(0 and 69kgN/ha). Nutrient sources were gypsum, triple-super phosphate(TSP) and urea. The
103 treatments were arranged in randomized complete block(RCB) design and replicated 3 times. Each
104 replication was sub-divided into a 3m x 5m =15m² experimental units, and there were 4 plots per
105 block. One third of N was incorporated into soils within rows before seeding to enhance its use
106 efficiency, whereas the remaining 2/3 was top-dressed at tillering, a stage where wheat is considered
107 to be in greater N demand. Entire sources of SP were drilled within rows and incorporated into the
108 soils just before planting, as both SP deficiencies affects plant development in its early stages of
109 growth. The agronomic spacing for wheat 25(rows) x 5cm(plants) was used. There were 12-rows of
110 wheat per plot, two borders and one row next to a border was used for plant sampling. The remaining
111 rows were used for agronomic/yield data collection.

112

113 2.3. Plant Tissue Sampling and Analysis

114 At booting stage 54 representative healthy wheat plant samples were collected from each plot from
115 the rows next to a border for laboratory(Lab) analysis. The samples were collected with clean hands,
116 by cutting with scissors to avoid contaminations. Samples were rinsed quickly using distilled water
117 and shaken to dry right in fields and thereafter put in paper bags. The sampling points were geo-
118 referenced using Global Positioning System(GPS) assisted by Google earth-(2011), and were
119 classified by elevation and soil-type when known. The GARMIN model number GPS-60 made in USA
120 in 2007 was used. Then in the Labs, samples were oven-dried at 65-70°C for 48hrs. On dry-weight
121 basis the RY% and S-uptake were calculated. Finally, 27 plants were selected randomly and cut at
122 the upper 1/3 part of each of the plants and ground using Tecator-CYCLOTEC-1093 sample mill.

123

124 In Labs, finely ground materials were wet-digested using 68%HNO₃-30%H₂O₂ for TS determination
125 (turbidimetric). The contents were then read using spectrophotometer. The TN was determined by
126 stem distillation [17] after extracting by micro-Kjeldahl wet-digestion (using conc.H₂SO₄) in digestion
127 tubes [18] and back-titrated against 0.05N:H₂SO₄, from which S uptake and N/S-ratios were
128 calculated. The relative yield(RY) was calculated with levels of S as percentage. $RY = [N/(N+1)] * 100$
129 [19]. Where: N is wheat yield from treatments without sulfur; and (N+1) is yield of wheat at next higher
130 level treatments containing S fertilize.

131

132 2.4. Data Analysis

133 For augmenting the works of [2] three more sulfur supply indices: OC in native soils; and TS and N/S-
134 ratio in wheat at booting stage were correlated with S uptake and the slopes were compared through
135 parallelism and coincidence test using PROC-REG for SAS statistical package [20]. Based on the
136 coefficient of determination(R²) for the indices, CLs were set at the RY of 90%, using the Cate and
137 Nelson model [19]. The method involved plotting of the values for the indices against RY. The
138 horizontal and vertical lines were then positioned on scatter-diagram points to maximize the number
139 of points in positive quadrants for S and OC(1st and 3rd quadrants); and in the negative quadrants for
140 N/S-ratio. This can be verified statistically from the values of total variance(R²) of the observed values
141 with postulated critical values, where R² peaks at CLs. The analysis of variance(ANOVA) for yield and
142 yield components data was done using PROC-MIXED of generalized linear model(GLM) of SAS
143 protocols [20] to evaluate the differences between treatments. When the differences between
144 treatments were significant, least significant difference(LSD) was used to separate the means, with a
145 significant level of 0.1%, 1% and 5%.

146

147 3. RESULTS AND DISCUSSION

148 Using high-analysis fertilizers lacking adventitious sulfur coupled with traditional farming and cropping
149 systems that mine plant nutrients, particularly S from native soils is becoming one of the soil health
150 problems in the agricultural crop production systems in Ethiopia. To reduce yield and quality loss of
151 crops due to S deficiency, therefore, S supply indices are necessary. Table 2 presents some more
152 selected indices: TN and OC in native soil; and TS and N/S-ratio in wheat shoot at booting for
153 investigating against yield.

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159 **Table 2. Some more selected indices of sulfur supply in wheat at booting (native soil**
 160 **conditions).**

Study area/zone	Farmer field	SO ₄ -S in soil (mg/kg)	OC Soil (%)	Total N Soil (%)	Total N in Wheat (%)	Total S in wheat (%)	N/S-ratio in wheat	S-uptake in wheat (kg/ha)	RY of wheat (%)
Arsi	A/Alko	6.94	1.11	0.126	2.618	0.11	23.80	2.28	68.40
Arsi	Dosha	10.44	2.04	0.252	2.705	0.15	18.03	4.58	91.36
Arsi	G/Silingo	7.77	1.17	0.14	2.467	0.11	22.43	2.52	74.43
Arsi	C/Misoma	22.13	2.75	0.133	2.131	0.18	11.84	3.99	97.48
Arsi	B/Edo	21.50	2.77	0.203	2.311	0.18	12.84	3.75	98.22
Arsi	B/Lencha	4.32	1.07	0.105	2.594	0.11	23.58	1.46	62.46
E.Shewa	C/Donsa	15.37	0.90	0.063	3.103	0.18	17.24	3.15	88.46
E/Shewa	Keteba	5.78	1.06	0.056	3.056	0.13	23.51	1.83	69.56
E/Shewa	Ude	12.37	1.23	0.098	2.793	0.15	18.62	2.19	89.17
E/Shewa	Bekejo	1.30	1.31	0.07	2.635	0.11	23.95	1.79	71.65
E/Shewa	Insilale	6.62	1.35	0.098	2.646	0.12	22.05	1.35	68.54
E/Shewa	Kilinto	8.27	1.39	0.056	1.624	0.08	20.30	1.66	70.93
O/Liyuu	N/Kersa	11.89	1.41	0.07	2.010	0.12	16.75	3.75	88.33
O/Liyuu	N/Suba	5.64	1.47	0.126	2.557	0.12	21.31	1.98	72.83
O/Liyuu	B/Tokofa	3.82	1.69	0.119	1.823	0.09	20.26	1.55	70.26
O/Liyuu	D/Lafto	10.83	1.71	0.14	2.603	0.11	23.66	1.86	80.10
O/Liyuu	W/Harbu	23.02	2.99	0.154	2.541	0.16	15.88	2.79	91.09
O/Liyuu	T/Harbu	24.18	1.31	0.14	2.386	0.16	14.91	4.78	93.69

161

162 **3.1. Relation of Selected Indices**

163 The relationship between S uptake and S supply indices are presented in Table 2. It is shown that,
 164 the indices under investigation are positively related to S uptake in the order of importance: N/S-ratio
 165 >TS >OC with the coefficients of correlations, 0.78, 0.71 and 0.41 respectively. As shown, S content
 166 and OC had direct relationships, whereas, the N/S-ratio had an inverse relationship. Indeed, the N/S-
 167 ratios and S concentration were relatively more strongly related with yield than the OC, based on the
 168 criteria set in literature [21]. Details of the results are discussed in the following sub-sections.
 169

170 **Table 3. Pearson correlation coefficients(r), between S-uptake and different indices of**
 171 **S availability in wheat at booting, (N =18)**

	Site	Village	SO ₄ -S	OC	TN(soil)	TN	TS	NS-ratio	S-uptakes
Site	1.00000 0.00000 1.0000	0.00000 1.0000	0.06135 0.8089	-0.03656 0.8855	-0.28725 0.2478	-0.16471 0.5137	-0.18006 0.4746	0.00447 0.9860	-0.11813 0.64060
Village	0.00000 1.0000	1.00000	0.25918 0.2990	0.29265 0.2386	0.08469 0.7383	-0.33936 0.1683	-0.03766 0.8821	-0.14146 0.5755	-0.09846 0.69750
SO₄-S(soil)	0.06135 0.8089	0.25918 0.2990	1.00000	0.62671 0.0054	0.37945 0.1204	-0.07343 0.7722	0.80584 <.0001	-0.87894 <.0001	0.74266 0.00040
OC(native soil)	-0.03656 0.8855	0.29265 0.2386	0.62671 0.0054	1.00000	0.60892 0.0073	-0.30129 0.2244	0.48091 0.0433	-0.66597 0.0026	0.40798 0.0928
TN(native soil)	-0.28725 0.2478	0.08469 0.7383	0.37945 0.1204	0.60892 0.0073	1.00000	0.00407 0.9872	0.37197 0.1285	-0.36552 0.1358	0.53809 0.0212
TN(at booting)	-0.16471 0.5137	-0.33936 0.1683	-0.07343 0.7722	-0.30129 0.2244	0.00407 0.9872	1.00000	0.37362 0.1267	0.28074 0.2591	-0.04774 0.8508
TS(at booting)	-0.18006 0.4746	-0.03766 0.8821	0.80584 <.0001	0.48091 0.0433	0.37197 0.1285	0.37362 0.1267	1.00000	-0.77604 0.0002	0.70675 0.0010
NS ratio (at booting)	0.00447 0.9860	-0.14146 0.5755	-0.87894 <.0001	-0.66597 0.0026	-0.36552 0.1358	0.28074 0.2591	-0.77604 0.0002	1.00000	-0.78397 0.0001
Uptakes (at booting)	-0.11813 0.6406	-0.09846 0.6975	0.74266 0.0004	0.40798 0.0928	0.53809 0.0212	-0.04774 0.8508	0.70675 0.0010	-0.78397 0.0001	1.00000

172

173 **3.1.1. Soil Organic Carbon**

174 The soil OC is positively related to S uptake, with coefficient of correlation(r), 0.41. But it is weak as
 175 compared to the N/S-ratio and TS (Table 3). Organic carbon's weak correlation is not unexpected,
 176 because of its unpredictable quantity of nutrients that can be released through mineralization.
 177

178 Sulfur in soils is usually associated with organic fractions, and its supply to crops is largely regulated
 179 by soil organic matter(SOM). It is reported that the amount of labile OC is considered to be a good
 180 indicator of plant available S [22]. It is also widely recognized that, OC is not only the indicator of the
 181 supply of essential elements like C, N, P, K and S, but also considered to be one of the key indicators

182 of soil health or quality [23,24,25]. However, controversies exist in quantifying the amount of S that is
 183 released through mineralization and in setting its CLs for sustained soil functions. This can hold true,
 184 because during various growth stages of crops, the mineralization can be slow or late and the amount
 185 of S released during critical stages of plant growth, may not be sufficient enough to meet S demand,
 186 especially when accounting for the different losses. In line with this, [11] reported difficulty of
 187 predicting the amount of SO₄-S that can come from added OM, because of complicated dynamics in
 188 the soil system.
 189

190 **Table 4. Simple Statistics for the variables considered in correlation (N=18)**
 191

Variable	N	Mean	SD	Sum	Min	Max	R
Site	18	2.00000	0.84017	36.00000	1.00000	3.00000	2.00
Village	18	3.50000	1.75734	63.00000	1.00000	6.00000	5.00
SO ₄ -S(soil) (mg/kg)	18	11.23278	7.16712	202.19000	1.30000	24.18000	22.88
OC (soil) (%)	18	1.59611	0.63195	28.73000	0.90000	2.99000	2.09
TN (soil) (%)	18	0.11939	0.05118	2.14900	0.05600	0.25200	0.196
TN (in wheat at booting) (%)	18	2.47794	0.38513	44.60300	1.62400	3.10300	1.479
TS (in wheat at booting) (%)	18	0.13167	0.03111	2.37000	0.08000	0.18000	0.10
NS ratio in wheat at booting	18	19.49778	3.91614	350.96000	11.84000	23.95000	12.11
S uptake	18	2.62556	1.10833	47.26000	1.35000	4.78000	3.43

Where: SD =standard deviation; min =minimum, max =maximum, and R =range.

192
 193 This can especially hold true, under tropical climatic/soil conditions. In addition, the organic resources,
 194 in the studied areas have alternative uses and not returned into soils [4]. Furthermore, the quantity of
 195 OC itself, including nitrogen in the studied soils was critically low for sustaining soil quality (Table 1).
 196

197
 198 **3.1.2. Total Sulfur**

199 The total sulfur(TS) content in wheat was also positively related to S-uptake with coefficient of
 200 correlation(*r*), 0.71, and level of significance, (*P*<0.0010) (Tables 2 through 4). But, it is less strongly
 201 related to yield compared to the N/S-ratio, as its *r* value is lower. This may suggest that, the S content
 202 at vegetative stage is less reliable diagnostic tool of S deficiency than N/S-ratio. Indeed, this is in
 203 agreement with the works of [13]. The authors suggested determining the total N/S-ratio followed by S
 204 content in vegetative tissue as a better tool for identifying S deficiency in wheat. The authors further
 205 noted that, sulfur concentration is less reliably indicated S-deficiency as compared to N/S-ratio in
 206 vegetative stage, because of the differences in S levels between S-deficient and S-sufficient wheat.
 207 According to, [13], sulfur distribution among various plant organs suggests that critical S levels might
 208 best be obtained by utilizing green leaf tissue, as vegetative stages are in greatest nutrients demand
 209 and with higher S content in tissues.
 210

211 **3.1.3. N/S-Ratio in Wheat**

212 The N/S-ratio in wheat at booting was better correlated with yield than both sulfur and OC contents.
 213 Its *r* value is -0.78 and significant at *P*<0001 (Tables 2 through 4). It is known that, useful diagnostic
 214 tools for S deficiency are the soil and plant variables. Further, it is well recognized that, the S status of
 215 plants is assumed to be a suitable parameter to calibrate soil-test methods and its suitability should
 216 depend on the degree of its association with yield. However, its coefficient of correlation determined is
 217 slightly lower than the minimum data set by [21], i.e. greater or equals to 85%.
 218

219 In accordance, [13] suggested determining the total N/S-ratio followed by S concentration in
 220 vegetative tissue as a better tool for identifying S deficiency. But, [8] opposed the idea. The authors
 221 reported, that N/S-ratio is not an appropriate diagnostic tool for S deficiency in the early stages of
 222 wheat growth, and affirmed that in the appropriate nitrogen and sulfur availability conditions, the N/S-
 223 ratio is not stable during, the beginning of tillering to stem elongation end in wheat. According to these
 224 authors, this lack of stability was attributed to lower S dilution in relation to N, which is related to a
 225 lower initial accumulation rate of S. In any case, however, from the present study it is learnt that, plant
 226 analysis offered a better tool than soil-testing (in this case, the OC) in predicting S deficiency in wheat
 227 and/or the studied soils.
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 229

230 4. ESTIMATION OF CRITICAL LEVELS

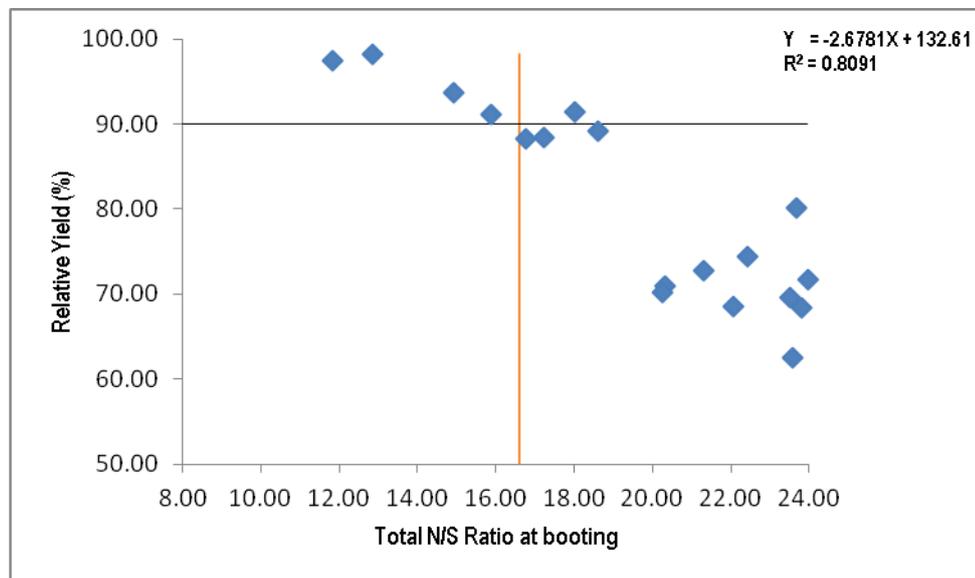
231 Critical values for TS, N/S-ratio and OC contents were derived from yield-composition curves fitted by
 232 eye and represent the value of the index corresponding to 90% of maximum yield. The horizontal
 233 lines depict 90% maximum dry-matter yield and vertical lines depict the critical thresholds. However, it
 234 should be noted that, critical values are only useful for differentiating between deficiency and
 235 sufficiency levels, and does not describe the degree of deficiency, as there are no exact break points
 236 between a nutrient being sufficient, deficient, or toxic. Further, it is important to note that, as CLs
 237 separates only the lows and highs, marginal levels can go some points above or below the values that
 238 are being estimated.

239

240 4.1. Nitrogen to Sulfur Ratio

241 The scatter diagram for relative yield(RY) and N/S-ratios in wheat at the booting stage of growth are
 242 shown in Fig.1. This relationship was used to determine the critical levels using the Cate and Nelson
 243 model [19]. As shown, the N/S-ratios varied over sites depending on native soil conditions (Fig.1 and
 244 Table 3). Unlike the S and OC contents, N/S-ratio was inversely related to the RY. All the scatter
 245 diagram points lie in a straight line and all are in negative quadrants, except only for one point,
 246 indicating that the RY was behaving normally in relation to S status of soils. Its regression equation is,
 247 $Y = -2.6781X + 132.61$ with the coefficient of regression(R^2), 81%. The regression line indicates that,
 248 maximum RY, 90% was obtained when the N/S-ratio was nearly 16.5:1; and as the S deficiency
 249 becomes more severe, the ratio increased to above 24:1. In general, this critical threshold 16.5:1 could
 250 be used to distinguish S responsive sites/soils or treatments from non-responsive ones. Wheat is
 251 likely to suffer from sulfur deficiency when the N/S-ratio goes above this CL. This is nearly close to the
 252 value reported by [26], 17:1, in the upper fully developed leaves at flag leaf stage to anthesis. The
 253 values is also falls in a range reported by [28] for the total N/S-ratio in wheat, that varied between
 254 14.8:1 to 16:1, during tillering to heading. Reussi *et al.*, [28], reported that, between 90 and 100% of
 255 wheat samples were correctly diagnosed by total N/S-ratio during tillering and the critical N/S-ratio
 256 varied from 14.8:1 to 16:1. But, the obtained value in the present study is higher than that reported by
 257 [27], 14.9.

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259

260

Fig.1. The relationship between RY and N/S-ratio in wheat at booting (native soil).

261 Rasmussen, *et al.*, [13] also suggested 17 as a CL for the N/S-ratio in the early stages of wheat
 262 growth. The authors further noted that, vegetative growth generally decreased from tillering to
 263 booting, when the whole plant N/S-ratio exceeded 17. According to their report, the N/S-ratio in S-
 264 sufficient plants declined gradually with age, implying that the critical N/S-ratio may decline with
 265 advancing growth up to harvest. Also, both N and S concentrations in the advanced stages of growth,
 266 including grain were reported to be low, due to dilution effects. The authors, further stressed that, the
 267 changes in stem:leaf ratio could have been responsible for the decline, since the N/S-ratio in stem
 268 tissue at heading was less than that of green leaf.

269

270 In any case, however, the suitability of N/S-ratio as indicator of S supply in wheat is still subject to
 271 strong debate. For instance, [29] questioned the usefulness of N/S-ratio concept, as it reflects the
 272 relative proportions than the actual magnitude of either of the elements. According to the authors, low
 273 N/S-ratio suggests S sufficiency when both nutrients might be deficient, whereas high N/S-ratio might
 274 mean excessive N instead S deficiency. Furthermore, S concentration is less sensitive to S availability
 275 variations in soil, in relation to plant sulfur levels at early stages of growth [30], which would further
 276 limit its use at that stage. From this reason, the authors suggested determining the CLs for the N/S-
 277 ratio empirically or to be reviewed cautiously. According to [11], one of the problems of using N/S-
 278 ratio is that a surplus of one element may be interpreted as a deficiency with the other. Another
 279 problem with N/S-ratio is that, S is a rather immobile nutrient in plants and older leaves tend to have
 280 higher S than the young ones, while N is mobile and young leaves tend to have higher N than old
 281 leaves.

282

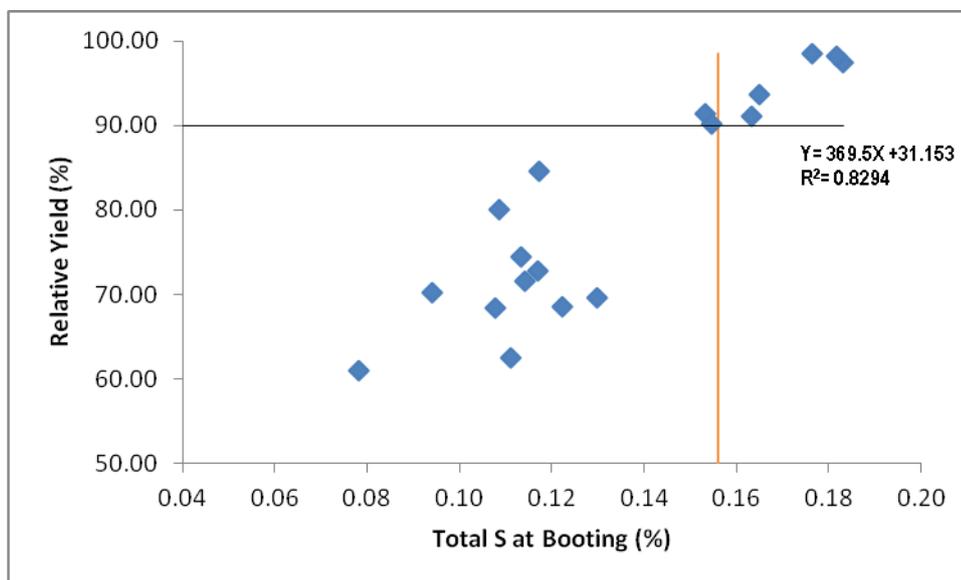
283 **4. 2. Total Sulfur Content**

284 The scatter diagram for RY% and S contents in wheat at booting are presented in Fig.2. This
 285 relationship was used to determine CL using the Cate and Nelson procedure [19]. As depicted, the CL
 286 for the S content was estimated to be about, 0.16%. This falls in a range for the TS content, 0.23 to
 287 0.08% between the first and third harvests, reported by [27]. But, it is much higher than that reported
 288 by [26], 0.20% below which the wheat crop is reported to suffer from S deficiency. Ryan *et al.*, [31],
 289 also reported a much closer value. Based on the report, for young wheat plants, 0.15-0.40% is
 290 considered to be the sufficiency range, with concentrations below 0.15% suggesting deficiency.

291

292 In general, from the results thus obtained, following the N/S-ratio, the S content in wheat at the early
 293 flowering stage was found to be a better index of S deficiency. Its coefficient of regression(R^2) is 83%
 294 (Fig.2). As can be seen in the figure, the RY is always increasing with sulfur content in native soils,
 295 with the regression equation, $Y = 369.5X + 31.153$. More interestingly, all the scatter diagram points lie
 296 in a straight line and all fall in the positive quadrants, which means that the behavior of RY in
 297 relation to the soil's S supply was normal.

298



299

300 **Fig.2.** The Relationship between RY and TS in wheat at booting (native soil).

301

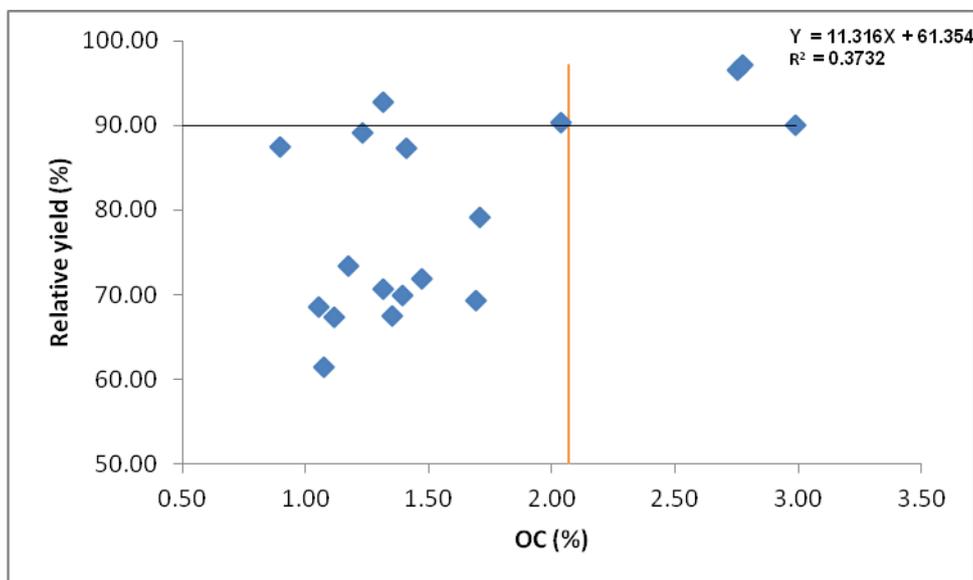
302 In general, from the study, it is noted that, the CL thus estimated for the TS in wheat at booting, could
 303 be used as a provisional recommendation for wheat growing in Ethiopia. As this critical level
 304 determined by [19] model, separates only the low and high levels, the marginal or medium levels can
 305 stretch up some point above or below 0.16%.

306

307 **4.3. Organic Carbon**

308 The scatter diagram for the RY% and organic carbon(OC) contents in the native soils just before
 309 planting wheat are presented in Fig.3. The Cate and Nelson model [19] identified the critical threshold
 310 for the OC to be, about 2.07%. The regression equation was, $Y = 11.316X + 61.354$, with the coefficient

311 of regression(R^2), 37%. Indeed, this value is in accordance with that reported by other workers
 312 [32,33]. From the coefficient of regression(r) value, however, the OC is not a better index of S
 313 deficiency based on the criteria set in literature [21]. Furthermore, the r value was the least when
 314 compared with N/S-ratio and the S concentration. Similar to the other indices considered in this study,
 315 all the scatter diagram points lie in a straight line except for one point, and all are in positive
 316 quadrants, indicating that there were no abnormal cases in the behavior of RY vis-à-vis the OC
 317 contents in the studied areas soils.
 318



319
 320
 321 **Figure 3.** The relationship between RY and OC% in wheat at booting (native soil).
 322 The OC contents of studied soils ranged from 0.90% to 2.99% (Table 2). From the data presented, in
 323 about, 83.3% of the soils, the SOC content was very low, far below the CLs suggested by [32,33,34].
 324 This may indicate that, some of the key soil quality indicators like structural stability could be at risk,
 325 because up to 98% of the total soil S in the sub-humid Ethiopian highlands is considered to be
 326 present as the organic S compounds. It is worth mentioning that, soil OC is also reported to be a
 327 promising indicator for guiding N fertilizer management under the challenges of soil heterogeneity
 328 among smallholder farming systems, given its integrative benefits that are leading to a high N supply
 329 and soil health.

330
 331 According to [4], a root cause for the alarmingly low levels of soil OC in the studied soils was the
 332 traditional farming and cropping systems of the areas. Therefore, it is not surprising that, the soils in
 333 the studied areas are regarded as deficient in major plant nutrients, notably nitrogen, phosphorus and
 334 sulfur.

335
 336 **5. CONCLUSIONS AND RECOMMENDATIONS**

337 From the indices evaluated, owing to their relative higher degree of correlation with yield, the N/S-ratio
 338 followed by S concentration in wheat during the field growth stage of booting, gave better sensitivity
 339 as an index of S deficiency than the soil organic carbon. Their critical thresholds were estimated to be
 340 0.16% for the TS content; 16.5:1 for the N/S-ratios; and 2.07% for the soil OC. Thus, for the wheat
 341 plant at its early flowering or booting stage, sulfur responsive soils or treatments can be separated
 342 from non-responsive ones, in which case much sulfur response is expected for sites or treatments
 343 with the N/S-ratio > 16.5, S content < 0.16%, and the soil OC < 2.07%. The results, thus obtained
 344 could be used as provisional recommendations for wheat growing in Ethiopia, and as the basis for
 345 further S research in the country. However, it is noted that, the indices of sulfur availability considered
 346 in this study as well as the various candidates suggested in literature have comparative usefulness or
 347 limitations. Furthermore, disparities between the CLs determined in present study as well as those
 348 reported in literatures have been observed. So, the follow-up research agendas should focus on
 349 identifying and/or standardizing a more reliable index of S supply and their CLs by installing a more
 350 reliable research condition (e.g., at lath house or green-house level). Furthermore, as this CL
 351 approach is the first work, only one cereal cultivar was considered; therefore, much is expected to be
 352 done to locate the most suitable indicator of S deficiency for wheat or other crops in the country.

353 **COMPETING INTERESTS**

354 I declare, that no competing interests exist.

355

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