Critical leaf S concentration and S requirement of stevia grown in two different soils of Bangladesh

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ABSTRACT

Sulphur is one of the essential nutrients for plant growth, protein and chlorophyll biosynthesis and can be supplemented by inorganic fertilizers like gypsum. To examine the effects of S on the growth, leaf biomass yield, S content and uptake and to estimate minimum S requirement and critical leaf S concentration of stevia, an experiment was conducted in the net house of the Department of Agricultural Chemistry, Bangladesh Agricultural University during March to July 2012 in two different soils of Bangladesh. Six levels of S (0, 10, 20, 30, 50 and 80 kg ha⁻¹) were applied. Plant sampling was done at 15, 30, 45 and 60 days after planting (DAP) to measure plant height, number of branches and leaves, dry weight of leaves, leaf area and leaf S concentration. All the studied parameters of stevia plant were significantly affected by different levels of S. The highest values of the parameters except S concentration were obtained from 30 kg S ha⁻¹ and the lowest from S control. Sulphur application increased leaf dry yield at harvest by 54 to 573% in acid soil and 75 to 545% in non-calcareous soil, respectively over control. The growth of the plant was rapid at the later stages (30 to 60 DAP). Leaf S content was directly proportional with the increased levels of S. Critical S concentration to achieve 80% of the maximum production of stevia leaf was estimated to be ca 0.125 and 0.137% in the leaves of stevia plants grown in acid and non-calcareous soils, respectively. The minimum amount of S for maximum leaf biomass production in the plants grown in acid and non-calcareous soils was also estimated to be ca 40 and 45 kg ha⁻¹, respectively. The overall results suggest that farmers can be advised to apply S @ 40 kg ha⁻¹ in acid soil for getting 80% production of stevia leaf under the agro-climatic conditions of Bangladesh Agricultural University.

INTRODUCTION

Stevia (Stevia rebaudianaBertoni) is an amazing plant contains the secret of stevioside, which make it the sweetest herb in the world (Soejarto et al. 1983). The stevia herb in its natural form is approximately 20 to 30 times sweeter than common table sugar. Extracts of stevia in the form of stevioside can range anywhere from 100 to 300 times sweeter than table sugar. Recently it has been introduced as a crop in a number of countries and has become a popular natural source of high potency sweetener and dietary supplement. Bangladesh being an agro-based country could easily introduce this plant as an industrial crop like sugarcane, sugar beet, tea or coffee and can commercially be cultivated in its relatively high land, char land, home stead area etc. as it grows well in open space having regular sun light.

Few sporadic trials on date of planting, pruning, stem cutting, growth and leaf yield of stevia have been conducted both at pot and field conditions (Khanom 2007; Nasrin 2008; Hasan 2008; Khan 2014). Recently, suitable soil for stevia cultivation (Zaman et al. 2015), N requirement and critical N content of stevia grown in two contrasting soils of Bangladesh has been reported by Zaman et al. (2016). It is expected that a higher and

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balanced nutrient supply will result in higher foliage yield.

Unfortunately no systematic and detailed study has yet been conducted on the inorganic fertilizer requirement (particularly S fertilizer) for stevia cultivation in Bangladesh. Optimum S requirement need to be screened out for achieving maximum leaf biomass yield of stevia in the country. Critical values are quite useful and are frequently referred to when interpreting a plant analysis result. The critical S concentration should be estimated. Though the level of S in the soil is one of the critical factors determining the growth and yield of the plants, no report has yet been published on the requirement of S and critical S level for the growth and leaf biomass yield of stevia in Bangladesh. Keeping in view the significant role of S in crop production systems, the present piece of research was undertaken to investigate the effects of different levels of S on the growth, leaf yield, S content and its uptake, determine S requirement and critical leaf S concentration of stevia under the agro climatic conditions of Bangladesh Agricultural University, Mymensingh was also estimated.

MATERIALS AND METHODS

The experiment was conducted at the net house of the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh during March to July, 2012 to examine the effects of different levels of S on the growth, leaf yield, S content and its uptake, determine S requirement and critical leaf S concentration of stevia. Two soils viz. acid and non-calcareous of contrasting physical and chemical properties were used (Zaman et al. 2015). Approximately 40 kg soils from each location (Madhupur for acid soil and BAU farm for non-calcareous soil) were collected from 0 - 15cm depth of selected fellow land for the experiment. The samples were made free from plant residues and other extraneous materials, air dried, ground and sieved through a 2mm sieve. 500g sieved soil from each source was preserved in a polythene bag and the physical and chemical properties were determined following standard procedure (Page et al. 1982). Eight kilograms processed soil was taken in each earthen pot of 23cm in height with 30cm diameter at top and 18cm at bottom leaving 3 cm from the top. In vitro produced 45 day old stevia seedlings were collected from brachitiotechnology laboratory, Joydebpur, Gazipur and used for the experiment. One stevia seedling was planted in each pot during 1st week of March, 2012. N, P, K, Zn and B were applied as basal doses @ 250, 100, 200, 3 and 1kg ha⁻¹ from prilled urea, TSP, MoP, zinc sulphate and boric acid, respectively (Zaman 2015). Six levels of S viz. 0 (S₀), 10 (S₁₀), 20 (S₂₀), 30 (S₃₀), 50 (S₅₀), and 80 (S₈₀) kg ha⁻¹ were applied from gypsum. Sulphur from ZnSO₄ was considered during S level calculation. Nitrogen was applied in equal three installments, 1/3rd during pot preparation, 1/3rd at 15 days after planting (DAP) and 1/3rd at 30 DAP. The experiment was laid out in completely randomized design with three replications. Intercultural operations like irrigation, soil loosening, weeding, insect pest control, removal of flowers etc. were done as and when necessary. Data were collected at 15, 30, 45 and 60 DAP. The crop was destructively harvested at 60 DAP. After harvesting the crop, leaf samples were separated, cleaned, dried for 72 hours, weighed, ground and stored. Plant heights, number of branches and leaves, leaf area and leaf dry weight were studied. S content was determined by turbidimetric method (Page et al. 1982). Uptake was calculated from S content and leaf dry yield. S requirement and critical S concentration of stevia was also estimated (Chowdhury 2000). The results obtained were subjected to statistical analysis using standard method of analysis (Steel et al. 1997). The differences among the treatment means were compared by using Duncan Multiple Range test (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Plant Height

The plant height of stevia as influenced by different levels of S are presented in figure 1. At harvest, plant height was significantly and rapidly increased with the increased levels of S up to 30kg ha⁻¹ and then slowly decreased with further increase in S levels (50 and 80kg ha⁻¹). S application at all levels increased plant height by 19 to 41cm in acid soil and 18 to 37cm in non-calcareous soil, respectively at 60 DAP. Plant height was significantly increased with the advancement of the growth period irrespective of S levels. However, the tallest plants of 97cm in acid soil and 92cm in non-calcareous soil were obtained from S₀ which was identical with S₀ and S₀ but statistically different from S₁₀ and S₅₀ and the shortest plant was obtained from the control treatment irrespective of the soils studied. Height increase was 74% higher in acid soil and 75% higher in non-calcareous soil over control. The increase in S levels increased the plant height progressively up to S₀ at 60 days of growth. Absolute control without S recorded significantly lowest plant height of stevia. The results are in accordance with the findings of Chalapathi et al. (1999) who also reported increased plant height of stevia with higher nutrient levels in sandy loam soils at Bangalore. Our result is in agreement with the findings of Chaubey et al. (2000) who reported that S fertilizer increased plant height of groundnut. Similar finding was also reported by Halder (2002) who observed highest plant height of wheat applying S @ 30kg ha⁻¹.

Branch Number

The branch number per plant of stevia as influenced by different levels of S are presented in figure 2. At harvest, number of branches per plant was significantly increased with the increased levels of S up to 30kg ha⁻¹ and then slowly decreased with further increase in S levels (50 and 80kg ha⁻¹). S application at all levels increased branch number by 100 to 300% in acid soil and 90 to 150% in non-calcareous soil, respectively at 60 DAP. Branches per plant was significantly increased with the advancement of the growth period irrespective of S levels and soils used. However, highest number of branches per plant in both soils was obtained from S₀ which was statistically identical with all S levels except control and the lowest value was obtained from the control treatment in both soils. Crop performance to a great extent is governed by the number of branches plant⁻¹. It is, therefore, imperative that if the number of branches per plantis higher, the numbers of leaves are expected to be higher; ultimately the leaf yield will be higher. This finding is also similar with the results of Islam et al. (2013) who reported that number of branch per plant of tomato was increased due to the application of different doses of inorganic fertilizers. Halder (2002) found highest number of tillers of wheat applying S @ 30kg ha⁻¹. This growth parameter might have possibly contributed positively to the higher leaf yield with higher S application.
the plant receiving 30kg S ha\(^{-1}\) which was significantly higher than other levels of S. Second highest values (1181cm\(^2\) in acid soil and 1635cm\(^2\) in non-calcareous soil) were obtained from S0. Identical leaf area was also obtained from the plants fertilized with S\(_{30}\) and S\(_{50}\) in non-calcareous soil. The lowest leaf area was found from the control treatment which was identical with S\(_{80}\) irrespective of soils used. S application at all levels increased leaf area by 88–856% in acid soil and 102 to 976% in non-calcareous soil, respectively at harvest. Leaf area is an important growth indices determining the capacity of plant to trap solar energy for photosynthesis and has marked influence on the growth and yield of plant. The leaf area was significantly influenced by varied levels of inorganic nutrients. Like yield attributes, leaf area also followed increasing trends with the progress of plant growth with maximum value at 60 DAP irrespective of treatments. Highest values were obtained from S\(_{50}\). Higher leaf area of stevia with higher S levels could be attributed to more number of branches and leaves per plant due to higher plant height. Khanom (2007) reported highest leaf area of stevia plant grown in non-calcareous soil applying chemical fertilizers.

Table 1. Effects of different levels of S on leaf area, dry weight and yield increase of stevia leaves over control at harvest

<table>
<thead>
<tr>
<th>S level</th>
<th>Acid soil</th>
<th>Non-calcareous soil</th>
<th>Acid soil</th>
<th>Non-calcareous soil</th>
<th>Acid soil</th>
<th>Non-calcareous soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(_1)</td>
<td>232d</td>
<td>270c</td>
<td>1.29e</td>
<td>1.43e</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S(_5)</td>
<td>435cd</td>
<td>546c</td>
<td>1.97d</td>
<td>2.50d</td>
<td>54</td>
<td>75</td>
</tr>
<tr>
<td>S(_{20})</td>
<td>642cd</td>
<td>920bc</td>
<td>2.58cd</td>
<td>3.65c</td>
<td>102</td>
<td>155</td>
</tr>
<tr>
<td>S(_{30})</td>
<td>2219a</td>
<td>2906a</td>
<td>8.61a</td>
<td>9.23a</td>
<td>573</td>
<td>545</td>
</tr>
<tr>
<td>S(_{50})</td>
<td>1181b</td>
<td>1635b</td>
<td>5.02b</td>
<td>5.60b</td>
<td>292</td>
<td>289</td>
</tr>
<tr>
<td>S(_{70})</td>
<td>783bc</td>
<td>1294bc</td>
<td>3.35c</td>
<td>5.01b</td>
<td>162</td>
<td>250</td>
</tr>
<tr>
<td>CV (%)</td>
<td>6</td>
<td>6</td>
<td>5.27</td>
<td>4.51</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>260</td>
<td>583</td>
<td>0.47</td>
<td>0.54</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SE±</td>
<td>166</td>
<td>237</td>
<td>0.60</td>
<td>0.62</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CV = Coefficient of variance, LSD = Least significant difference, SE± = Standard error of means

**Leaf Dry Weight**

The data pertaining to the dry weight of stevia leaves per plant at harvest as influenced by different levels of S fertilizer have been presented in Table 1. Results revealed that leaf dry weight significantly and progressively increased with the increased levels of S application up to 30kg ha\(^{-1}\) in both soils and then declined with further addition (S\(_{30}\) and S\(_{50}\)). The highest dry weight per plant (8.61g in acid soil and 9.23g in non-calcareous soil) at harvest was measured from the plant receiving 30 kg S ha\(^{-1}\) which was significantly higher than other levels of S. Second highest values (5.02g in acid soil and 5.60g in non-calcareous soil) were obtained from S\(_{80}\) in both soils. Identical dry weight was also obtained from the plants fertilized with S\(_{30}\) and N\(_{60}\) in non-calcareous soil. The lowest values were obtained from the control treatment (1.28g in acid soil and 1.43g in non-calcareous soil. S application at all levels increased leaf dry yield at harvest by 54 to 292% in acid soil and 75 to 289% in non-calcareous soil, respectively over control.

Dry matter accumulation by the crop is another important growth parameter to be considered for determining the economic yield while assessing the effects of different treatments. Sulphur fertilizers showed significant influence on the dry weight of stevia leaves. The leaf biomass yield was highest due to application of different levels of S fertilizers both in acid and non-calcareous soil. This is also in confirmation with Angkapradipta et al. (1986), where increased
biomass production was achieved due to application of higher levels of S. Murayama et al. (1980) in Japan experimentally proved that no fertilization resulted in lowest leaf yield of stevia. Increased dry leaf yield was also reported by Shock (1982) in Japan with moderate application of S. In conformity of these findings, growth and yield of stevia increased significantly with increasing rates of S up to 30 kg ha$^{-1}$ per crop with the highest dry leaf yield which was at par with 40 kg ha$^{-1}$ per crop in sandy loam soils at Bangalore (Chalapathi et al. 1999).

**Leaf S Concentration and Uptake**

The data on the S concentration and uptake by stevia leaves as influenced by different levels of S have been presented in Table 2. Both the concentration and uptake was significantly influenced by the application of S fertilizers. Sulphur concentration of the leaf was increased with the increased levels of S irrespective of soils used. The highest concentration (0.13% in acid soil and 0.14% in non-calcareous soil) was obtained when S was applied @ 80 kg ha$^{-1}$ in both soils which was statistically identical with the S contents of the leaves of stevia plant fertilized with $S_{20}$, $S_{80}$ and $S_{80}$ but significantly different from other treatments. The lowest S content was obtained from the plants receiving no S fertilizer in both soils. Sulphur uptake was also significantly affected by its additions. The uptake of S did not follow the same trend like S concentration of stevia leaves. S uptake varied from 0.90 to 9.47 mg pot$^{-1}$ in acid soil and 1.29 to 11.08 mg pot$^{-1}$ in non-calcareous soil. The uptake of S as expected increased as S levels increased up to 30 kg ha$^{-1}$ and then decreased with further additions ($S_{30}$ and $S_{60}$). The lowest S uptake was observed in the control treatment of both soils. The nutrient content of a plant varies not only among its various plant parts but changes with age and stage of development. S contents and its uptake by stevia leaf varied significantly in both soils with their additions.

The increase in concentration was proportional with the rate of application but the nutrient uptake did not follow the same trend. The highest nutrient contents were obtained from highest S addition ($S_{60}$) but the highest nutrient uptake was obtained from $S_{40}$. Higher nutrient uptake may be related to higher biomass yield. This may be due to the highest dry leaf yield harvested from that treatment. Because nutrient uptake was calculated from their concentrations and corresponding dry leaf yield. In contrast, the lowest content and uptake of nutrients was obtained from control treatments of both soils. These results are in conformity with those of Angkapraditpa et al. (1986). They reported that the stevia plant S content increased due to increased concentration in plant which could be attributed to higher availability and uptake. Shivraj et al. (1997) concluded that increased nutrient uptake by stevia resulted from increased application of S fertilizers.

**Critical S Concentration of Stevia Leaf**

To determine critical S concentration in stevia leaf we followed the “Critical nutrition concentration” concept advanced by Ulrich (1952) for plant. Critical values as used by Ulrich and Hills (1973) are determined from the relationship of nutrient concentration and relative yield at the time of sampling. The critical S concentration in stevia leaf was estimated from the relative amount of leaf biomass to achieve 80% of the maximum production of stevia leaf following the procedure of Kouno et al. (1999). For both the soil, relative leaf biomass yield was plotted on the ordinate (Y axis) against the respective S concentration of stevia leaf on the abscissa (X axis) in figure 4. The S concentration corresponding to the arbitrary point at 80% to achieve the maximum leaf biomass production was estimated by the fitted curve to be ca 0.125 and 0.137% in the leaves of stevia plants grown in acid and non-calcareous soils, respectively.

The critical value of S for grasses is around 0.10% (Bryson and Mills 2015). Sulphur deficiencies occur in corn do not generally occur until the S level is less than 0.13% in the leaves. Under certain conditions, legumes, cotton, tobacco, and tomatoes have a critical S level of about 0.20 to 0.25%. The S critical level for crops such as cabbage, spinach, turnips, and collards is around 0.30% (Bryson and Mills 2015). Kouno and Ogata (1988) reported critical S concentration associated with 60% of maximum yield of African millet as 160 µS g$^{-1}$ dry matter while Smith et al. (1985) found the critical concentration to achieve 90% of maximum yield for S in perennial ryegrass as 0.18%. Chapman (1997) estimated the critical S content for immobilization of barley straw to be 0.13%. Chowdhury (2000) reported the critical S content of African millet to achieve 80% of the maximum yield as 1.5mg g$^{-1}$ dry matter.

![Figure 4. Correlation between leaf S concentration and relative leaf biomass yield of stevia grown in acid and non-calcareous soils. Values are the means of all treatments. **Correlated significantly at P<0.01.](image)

**Sulphur Requirement of Stevia Plant**

To determine the requirement of S in soil to obtain 80% of maximum leaf biomass yield, the applied S was plotted on the X axis against the relative leaf biomass yield on the Y axis. From the fitted curve, the corresponding estimated minimum amount of S for leaf biomass production in the plant grown in acid soil and non-calcareous soil to be ca 40 and 45 kg ha$^{-1}$, respectively (Figure 5). A crop’s requirement for a specific nutrient is commonly defined as “the minimum content of that nutrient associated with the maximum yield” or “the minimum rate of intake of the nutrient associated with the maximum growth rate” (Loneragan 1968). S requirement vary greatly depending upon fertilizer applied and whether or not the investigation was performed in the field or green house, choice of crop etc. Sulphur deficiencies occur primarily on the very sandy soils and when low S containing fertilizers are used over several years.

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**Table 2.** Effects of different levels of S on nit’s content and uptake by stevia leaf at harvest

<table>
<thead>
<tr>
<th>S level</th>
<th>Sulphur</th>
<th>Acid soil</th>
<th>Non-calcareous soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Content (%)</td>
<td>Uptake (mg pot$^{-1}$)</td>
<td>Content (%)</td>
</tr>
<tr>
<td>$S_{0}$</td>
<td>0.07c</td>
<td>0.90e</td>
<td>0.09b</td>
</tr>
<tr>
<td>$S_{10}$</td>
<td>0.08b</td>
<td>1.58e</td>
<td>0.10b</td>
</tr>
<tr>
<td>$S_{20}$</td>
<td>0.10a</td>
<td>2.58d</td>
<td>0.11b</td>
</tr>
<tr>
<td>$S_{30}$</td>
<td>0.11a</td>
<td>4.97a</td>
<td>0.12a</td>
</tr>
<tr>
<td>$S_{40}$</td>
<td>0.12a</td>
<td>6.02b</td>
<td>0.13a</td>
</tr>
<tr>
<td>$S_{50}$</td>
<td>0.13a</td>
<td>4.35c</td>
<td>0.14a</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.45</td>
<td>5.76</td>
<td>2.08</td>
</tr>
<tr>
<td>LSD$_{0.05}$</td>
<td>0.03</td>
<td>0.52</td>
<td>0.03</td>
</tr>
<tr>
<td>SE±</td>
<td>0.01</td>
<td>0.72</td>
<td>0.01</td>
</tr>
</tbody>
</table>

CV = Coefficient of variance, LSD = Least significant difference, SE± = Standard error of means
The proper S level can be maintained in the plant by providing a S source near the germinating seed or by adding S with side dress and/or top dress applications particularly in sandy soils. If the pH is not too low when roots enter the subsoil, sufficient S will generally be available to satisfy the crop requirement.

### CONCLUSION

The results indicated that all the parameters examined in this study were significantly affected by different doses of S. The highest values of most parameters except S content were obtained from 30kg ha\(^{-1}\) and the lowest values from control. S application increased leaf dry yield at harvest by 54% to 292% in acid soil and 75 to 289% in non-calcareous soil, respectively over control. The increase of most parameters was fast at the later stages (30 to 60 DAP) of plant growth. Leaf S content proportionately increased with the increase of S level. The highest S content was obtained from its highest application.

The critical S concentration in stevia leaf was estimated from the relative amount of leaf biomass to achieve 80% of the maximum production of stevia leaf to be ca 0.125 and 0.137% in the plants grown in acid and non-calcareous soils, respectively. The minimum amount of S for maximum leaf biomass production in the plants grown in acid and non-calcareous soils was also estimated to be ca 40 and 45kg ha\(^{-1}\), respectively. The overall results suggest that farmers can be advised to apply S @ 40kg ha\(^{-1}\) in acid soil for getting 80% production of stevia leaf under the agro-climatic conditions of Bangladesh Agricultural University.

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### CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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