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NITROGEN REQUIREMENT AND CRITICAL N CONTENT OF STEVIA GROWN IN TWO CONTRASTING SOILS OF BANGLADESH

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ABSTRACT

Nitrogen is recognized as one of the most limiting nutrient for crop growth in Bangladesh and can be supplemented with inorganic fertilizers like urea. The experiment was conducted in the net house of the Department of Agricultural Chemistry, Bangladesh Agricultural University during March to July 2012. The objective was to examine the effects of different levels of N on the growth, leaf biomass yield, N content and to estimate minimum N requirement and critical N content of stevia. The treatments included six N rates (0, 100, 150, 200, 250 and 300 kg ha^{-1}). Plant sampling was done at 15, 30, 45 and 60 days after planting (DAP) to measure plant height, number of branches and leaves, fresh and dry weight of leaves, leaf area and N concentration. The results revealed that all the characters were significantly affected by different N rates. The highest values of all parameters except plant height and N concentration were obtained from 250 kg N ha^{-1} and the lowest values from N control. Nitrogen application at all levels increased leaf dry yield at harvest by 99 to 505% in acid soil and 69 to 438% in non-calcareous soil, respectively over control. The growth of most parameters was rapid at the later stages (30 to 60 DAP). Leaf N content proportionately increased with the increasing rates of N. The highest N concentration was obtained from its highest application (300 kg N ha^{-1}). The minimum amount of N for maximum leaf biomass production in the plants grown in acid and non-calcareous soils was estimated to be ca 273 and 257 kg ha^{-1}, respectively. The critical N concentration to achieve 80% of the maximum production of stevia leaf was also estimated to be ca 1.43 and 1.50% in the leaves of stevia plants grown in acid and non-calcareous soils, respectively.

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INTRODUCTION

Stevia, as a new crop to be domesticated in Bangladesh, a package regarding the cultivation aspects, need to be standardized under different agro climatic conditions of Bangladesh. The stevia cultivation has an immense scope for intensive agriculture and precision farming and fits well for high return agriculture (Barathi, 2003). Few sporadic trials on the growth and leaf yield of stevia have been conducted both at pot and field conditions (Khanom, 2007; Nasrin, 2008 and Hasan, 2008). Recently Khan (2014) conducted few experiments on different agronomic aspects like date of planting, pruning, stem cutting etc on the growth and yield of stevia. It is expected that a higher and balanced nutrient supply will result in higher foliage yield. Unfortunately no detailed study has yet been conducted on inorganic fertilizer requirement for large scale cultivation of stevia in Bangladesh. Incorporation of stevia into agricultural production systems depends upon details information regarding the plant, its agronomic potential and nutritional requirement (Ramesh *et al*., 2007).

Nitrogen (N) is recognized as one of the most limiting nutrients for crop growth. All vital processes in plant are associated with protein, of which N is an essential constituent. It also involved in chlorophyll synthesis, and influences stomatal conductance and photosynthetic efficiency. Consequently, getting more crop production, N application is essential in the form of chemical fertilizer (Ali *et al*., 2000). Nitrogen is applied to the crop for higher vegetative growth, productivity and quality (Gwal et al., 1999 and Iqbal et al., 2012). Balanced use of N is a key point for higher land profitability and healthy environment. Optimum N requirement need to be screened out for achieving maximum leaf biomass yield of stevia in Bangladesh. Critical values are quite useful and are frequently referred to when interpreting a plant analysis result. The critical N concentration -that is the minimum leaf N concentration required to reach maximal accumulated leaf biomass should be estimated. To the best of our knowledge, the critical N content of stevia is yet to be estimated elsewhere including Bangladesh. Keeping in view the significant role of N in crop production systems, the present research project was designed to study the effects of different levels of N on the growth, leaf yield, N content and its uptake and to estimate minimum N requirement and critical leaf N concentration of stevia under the agro climatic conditions of Bangladesh Agricultural University, Mymensingh-2202.

MATERIALS AND METHODS

A pot 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 N on the growth, leaf yield, N content and its uptake, determine N requirement and critical leaf N 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 kg processed soil was taken in each earthen pot of 23 cm in height with 30 cm diameter at top and 18 cm at bottom leaving 3 cm from the top. *In vitro* produced 45 day old stevia seedlings (*Stevia rebaudiana* Bertoni) were collected from *brac* biotechnology laboratory, Joydebpur, Gazipur and used for the experiment. One stevia seedling was planted in each pot during 1st week of March, 2012. P, K, S, Zn and B were applied as basal doses @ 100, 200, 30, 3 and 1 kg ha^{-1} from TSP, MoP, gypsum, zinc sulphate and boric acid, respectively (Zaman, 2015). Six levels of N viz. 0, 100, 150, 200, 250, and 300 kg ha^{-1} were applied from prilled urea, 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. Total number of pots was 36 (6 treatment X 2 soil X 3 replication). 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 at $60^{O}C$ for 72 hours, weighed, ground and stored. Plant height, branches $plant^{-1}$, leaves $plant^{-1}$, leaf area $plant^{-1}$, fresh and dry leaf weight of stevia leaves were studied. N content was determined by micro Kjeldahl method (Page *et al*., 1982). Uptake was calculated from N content and leaf dry yield. N requirement and critical N concentration 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

Effects of different levels of N on various parameters of stevia are described under the following heads

Effect of N on plant height

The data on plant height as affected by N rates in acid and non-calcareous soil is given in Fig 1. Different N rates had significant effect on plant height of stevia. The application of N influenced plant height variably from 15 days after planting (DAP) to 60 DAP. There was a general trend of increase in plant height with increase in N fertilizer at 15, 30, 45 and 60 DAP with the control treatment registering the least.

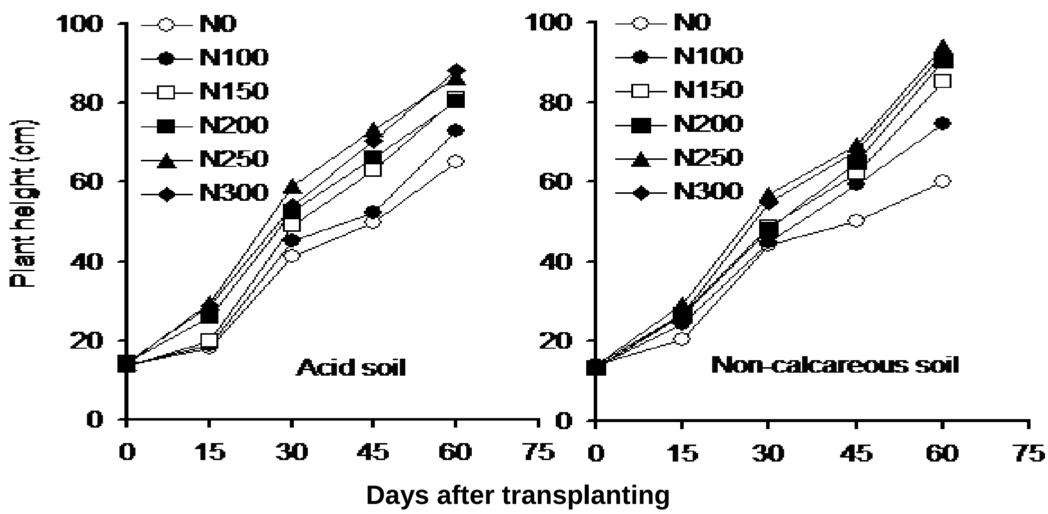

Figure 1. Effects of different levels of N on the plant height of stevia at various DAP

In the first stage of growth, differences between N levels were not significant. After this stage, increased plant height and difference between treatments could be observed. An increase in plant height was observed from planting stage to harvesting in both soils irrespective of treatments. N application at all levels increased plant height by 8 to 23cm in acid soil and 15 to 34cm in non-calcareous soil, respectively. It was observed that the pots subjected to N_{250} and N_{300} were significantly higher than control at 60 DAP although identical to those subjected to N_{200} and N_{250} at the same time. It was also noted that N_{100} and N_{150} were not significantly different at harvest. The highest level of N (N_{300}) produced the tallest plant (88.3cm) in acid soil where as N_{250} produced tallest plant (94cm) grown in non-calcareous soil and the shortest plants formed in the control (without N) in both soils. Height increase was 36% higher in acid soil and 57% higher in non-calcareous soil over control.

Effect of N on branch number

The data on branch number are presented in Figure 2. Branch number plant^{-1} responded significantly due to the application of different levels of N. The result revealed that branches plant^{-1} progressively increased with increasing levels of N application up to 250 kg ha^{-1} in both soils and then declined with further addition. The application of N influenced the number of branches plant^{-1} variably from 15 to 60 DAP irrespective of soils and treatments. Rapid increase in branch number was observed between 15 and 45 DAP and then remained constant or very slowly increased in both soils. The highest number of branches plant^{-1} (7.7 in acid soil and 9.3 in non-calcareous soil) at 60 DAP was counted from the plant receiving 250 kg N ha^{-1} which was identical with N_{200} and N_{300} but significantly different from N_{100} and N_{150}. The lowest branch number was counted from control. N application at all levels increased branch number by 89-156% in acid soil and 60 to 180% in non-calcareous soil, respectively at 60 DAP.

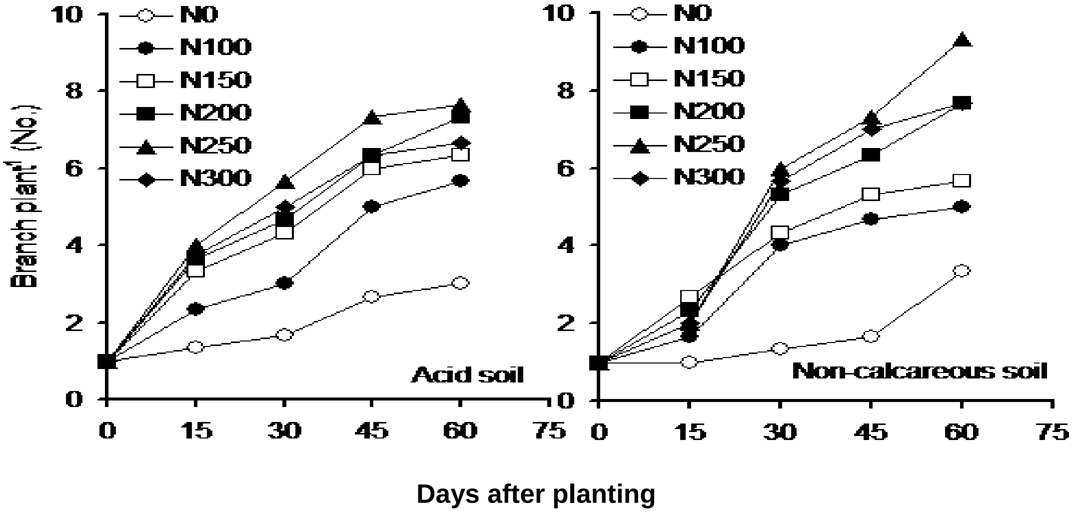

Figure 2. Effects of different levels of N on the branch number of stevia at various DAP

Effect of N on leaf number

The data pertaining to the number of leaves plant^{-1} as influenced by different levels of N in both acid and non-calcareous soils at various DAP have been presented in Fig. 3. Application of N at different doses significantly influenced the number of leaves of stevia plants at all growth stages except 0 DAP irrespective of soils and treatments used.

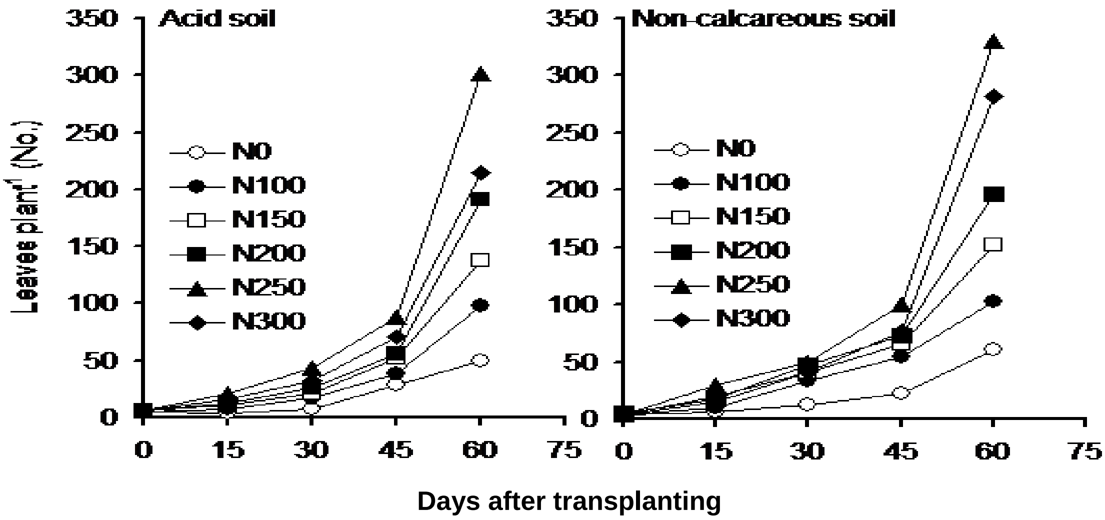

Figure 3. Effects of different levels of N on the leaf number of stevia at various DAP

Leaf number was increased with the increased levels of N up to 250 kg ha^{-1} and then declined by 40% in acid soil and 17% in non-calcareous soil with further addition (N_{300}). Leaf number increase was very slow at the early growth stages (0-30 DAP) while it was rapid between 30 and 60 DAP irrespective of N levels except control. N application at all levels increased the number of leaves by 49 to 251 in acid soil and 42 to 269 in non-calcareous soil, respectively. Maximum number of leaves was recorded with N_{250} which was significantly higher than all other levels of N in both soils. Plants fertilized with N_{200} and N_{300} produced identical number of leaves in acid soil. The minimum number of leaves plant^{-1} was harvested from the plants fertilized with no N irrespective of soils and growth period.

Effect of N on leaf area

The data on total leaf area plant^{-1} at harvest as influenced by different levels of N are presented in Table 1. Leaf area plant^{-1} responded significantly due to the application of different levels of N. The result revealed that leaf area progressively increased with increasing levels of N application up to 250 kg ha^{-1} in both soils and then declined with further addition (N_{300}). The highest total leaf area plant^{-1} (2512cm^2 in acid soil and 2805cm^2 in non-calcareous soil) at 60 DAP was measured from the plant receiving 250 kg N ha^{-1} which was significantly higher than other levels of N. Second highest values (1769cm^2 in acid soil and 2157cm^2 in non-calcareous soil) were obtained from N_{300}. Identical leaf area was also obtained from the plants fertilized with N_{200} and N_{150} in both soils. The lowest leaf area was found from the control treatment irrespective of soils used. N application at all levels increased leaf area by 111-794% in acid soil and 84 to 546% in non-calcareous soil, respectively at harvest.

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

N level	Leaf area plant^{-1} (cm^2)		Leaf dry weight (g plant^{-1})		Yield increase over control (%)	Yield increase over control (%)
	Acid soil	Non-calcareous soil	Acid soil	Non-calcareous soil	Acid soil	Non-calcareous soil
N_0	281e	434e	1.52e	1.84f	-	-
N_{100}	592de	799de	3.02d	3.11e	99	69
N_{150}	1003cd	1186cd	4.22c	4.66d	178	153
N_{200}	1401bc	1570c	5.80b	5.92c	282	222
N_{250}	2512a	2805a	9.20a	9.90a	505	438
N_{300}	1769b	2157b	6.57b	8.57b	332	366
CV (%)	4.5	5	4.07	4.13	-	-
$LSD_{0.05}$	240	320	0.61	0.64	-	-
SE±	186	204	0.62	0.70	-	-

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

Effect of N on dry weight

The dry weight of stevia leaves plant^{-1} at harvest varied significantly due the application of different levels of N fertilizer (Table 1). Results revealed that dry weight progressively increased with increasing levels of N application up to 250 kg ha^{-1} in both soils and then declined with further addition (N_{300}). The highest dry weight plant^{-1} (9.20g in acid soil and 9.90g in non-calcareous soil) at harvest was measured from the plant receiving 250 kg N ha^{-1} which was significantly higher than other levels of N. Second highest values (6.57g in acid soil and 8.57g in non-calcareous soil) were obtained from N_{300}. Identical dry weight was also obtained from the plants fertilized with N_{200} and N_{300} in acid soil. The lowest values were obtained from the control treatment (1.52g in acid soil and 1.84g in non-calcareous soil. N application at all levels increased leaf dry yield at harvest by 99 to 505% in acid soil and 69 to 438% in non-calcareous soil, respectively over control.

Effect of N on fresh weight

The fresh weight of stevia leaves plant^{-1} at harvest varied significantly due the application of different levels of N fertilizer (Figure 4). Results revealed that fresh weight progressively increased with increasing levels of N application up to 250 kg ha^{-1} in both soils and then declined with further addition (N_{300}).

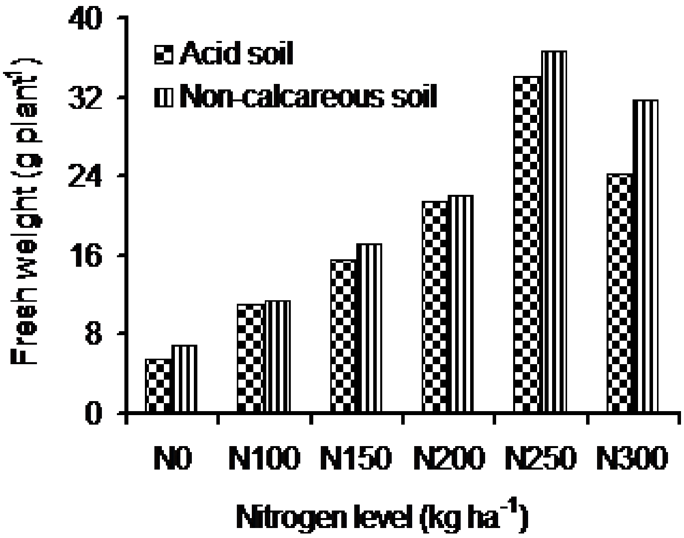

Figure 4. Effects of different levels of N on the fresh weight of stevia leaves at harvest

The highest fresh weight plant^{-1} (34.10g in acid soil and 36.73g in non-calcareous soil) at harvest was measured from the plant receiving 250 kg N ha^{-1} which was significantly higher than other levels of N. Second highest values (24.19g in acid soil and 31.70g in non-calcareous soil) were obtained from N_{300}. Identical fresh weight was also obtained from the plants fertilized with N_{200} and N_{300} in acid soil. The lowest values were obtained from the control treatment (5.61g in acid soil and 6.84g in non-calcareous soil irrespective of soils used. N application at all levels increased fresh weight at harvest by 5.46 to 28.49g plant^{-1} in acid soil and 4.68g to 30.25g plant^{-1} in non-calcareous soil, respectively.

N content and uptake

There was a significant effect of different levels of N on it's content and uptake by stevia leaf (Table 2). N content of the leaf was increased with the increased levels of N irrespective of soils used. The highest N content (1.51% in acid soil and 1.62% in non-calcareous soil) was obtained when N was applied @ 300 kg ha^{-1} in both soils which was statistically identical with the N contents of the leaves of stevia plant fertilized with N_{200} and N_{250} but significantly different from other treatments. The lowest N content was obtained from the plants receiving no N fertilizer in both soils.

The effects of different levels of N on its uptake were significant (Table 2). The trend was similar like N contents of stevia leaves. The N uptake varied from 16.48 to 131.56 mg pot^{-1} in acid soil and 20.42 to 150.48 mg pot^{-1} in non calcareous soil. N uptake as expected increased as N levels increased up to 250 kg ha^{-1} and then decreased with further addition (N_{300}). Like N content, the lowest uptake was observed in the control treatment of both soils.

Table 2. Effects of different levels of N on its content and uptake by stevia leaf at harvest

N level	Nitrogen			
	Acid soil		Non-calcareous soil	
	Content (%)	Uptake (mg pot^{-1})	Content (%)	Uptake (mg pot^{-1})
N_0	1.08c	16.48f	1.11c	20.42d
N_{100}	1.10c	33.22e	1.15c	35.77d
N_{150}	1.23bc	51.92d	1.28bc	59.65c
N_{200}	1.29abc	74.82c	1.38abc	81.70b
N_{250}	1.43ab	131.56a	1.52ab	150.48a
N_{300}	1.51a	99.21b	1.62a	138.83a
CV (%)	1.20	4.72	1.32	4.93
$LSD_{0.05}$	0.13	8.05	0.15	9.73
SE±	0.05	9.61	0.05	12.01

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

Critical leaf N concentration of stevia leaf

Critical values are quite useful and are frequently referred to when interpreting a plant analysis result. To determine critical N 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.

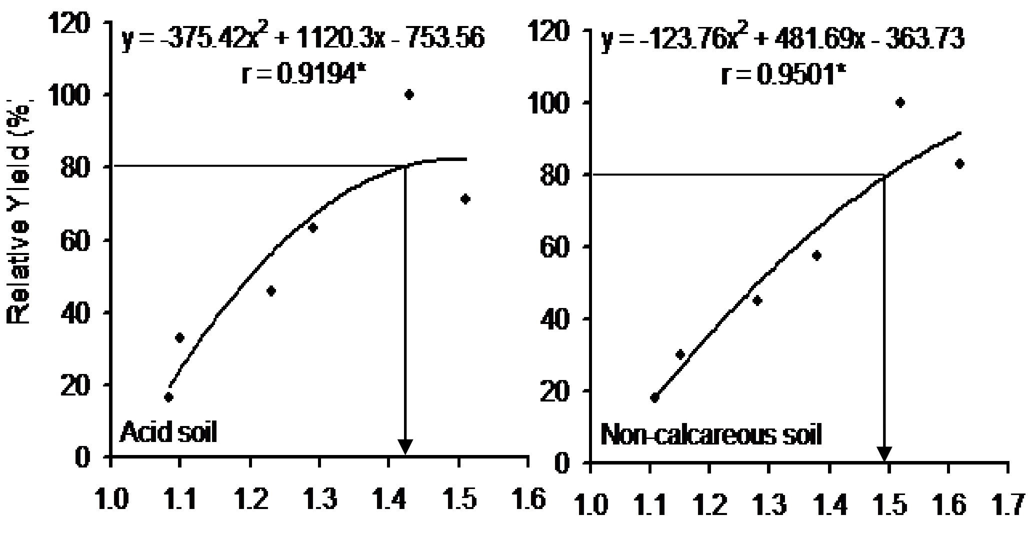

Figure 5. Correlation between leaf N concentration and relative leaf biomass yield of stevia grown in acid and non-calcareous soils. Values are the means of all treatments. *Correlated significantly at P<0.01

The critical N concentration in stevia leaf was estimated from the relative amount of leaf biomass to achieve 80% of the maximum production of stevia leaf (Kouno et al., 1999). For both the soil, relative leaf biomass yield was plotted on the ordinate (Y axis) against the respective N concentration of stevia leaf on the abscissa (X axis) in fig. 5. The N concentration corresponding to the arbitrary point at 80% to achieve the maximum leaf biomass production was estimated by the fitted curve to be ca 1.43 and 1.50% in the leaves of stevia plants grown in acid and non-calcareous soils, respectively.

Nitrogen requirement of stevia

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). Critical N levels vary greatly depending upon fertilizer applied and whether or not the investigation was performed in the field or green house, choice of crop etc. To determine the requirement of N in soil to obtain 80% of maximum leaf biomass yield, the applied N 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 N for leaf biomass production in the plant grown in acid soil and non-calcareous soil to be ca 273 and 257 kg ha^{-1}, respectively (Figure 6)

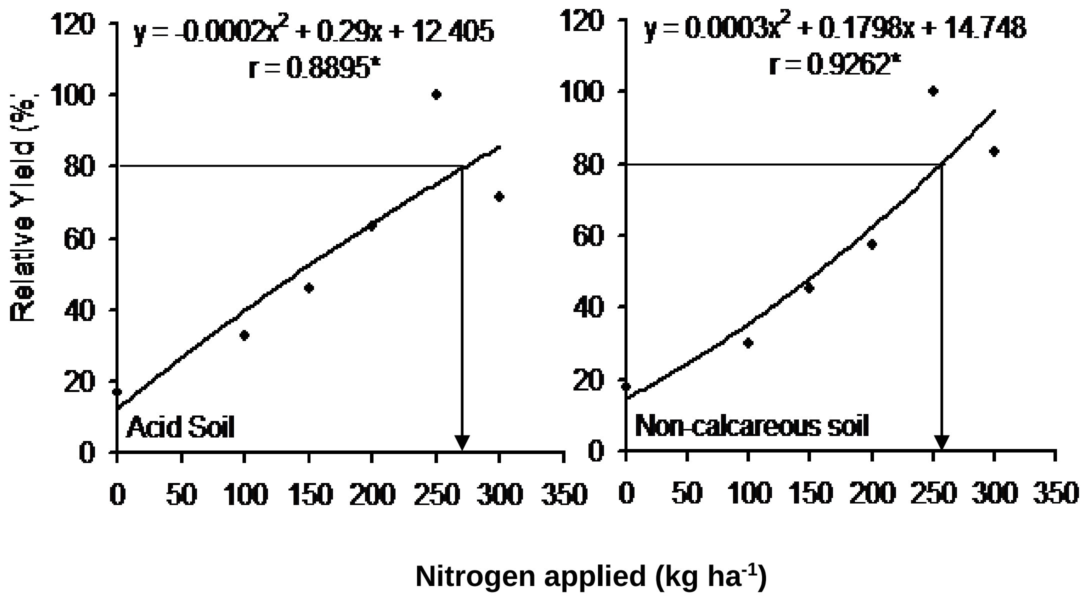

Figure 6. Correlation between applied N and relative leaf biomass yield of stevia grown in acid and non-calcareous soils. Values are the means of all treatments. *Correlated significantly at P<0.01.

DISCUSSION

Leaf yield and yield attributes

The results show that the studied parameters of the stevia plant gradually increased with the progress of growth up to 60 DAP irrespective of treatments. The changes in yield and yield contributing characters varied with treatments. Plant height is an indicator of vegetative growth which is directly influenced by plant production management strategies. The increase in N levels increased the plant height, number of branches and leaves $plant^{-1}$ progressively up to N_{250} at 60 days of growth. Absolute control without N recorded significantly lowest plant height, least number of branches and leaves $plant^{-1}$. Plant height at harvest was significantly influenced by higher levels of N which in turn was responsible for higher number of branches $plant^{-1}$ and number of leaves $plant^{-1}$ resulting into higher leaf yield. The results are in accordance with the findings of Chalapathi et al. (1999) who also reported increased plant height and number of branches $plant^{-1}$ with higher nutrient levels in sandy loam soils at Bangalore.

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 $plant^{-1}$ is 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 $plant^{-1}$ was increased due to application of different doses of inorganic fertilizers. These growth parameters might have possibly contributed positively to the higher leaf yield with higher N application.

Green leaves are the site of photosynthetic activity taking place in the plants. The number of leaves $plant^{-1}$ would also substantiate the fact that increased number of leaves $plant^{-1}$ would contribute to the final yield of the plant particularly the crops like stevia in which only leaves are used for commercial product. Kawatani *et al* (1980) at Japan had also reported the increased number of branches and leaves $plant^{-1}$ of stevia with higher N nutrition. Increased number of leaves plant^{-1} with increased levels of N fertilizers was also reported by Buana and Goenadi (1985) in Brazil. Nasrin (2008) obtained higher values of yield and yield parameters of stevia with N @ 250 kg ha^{-1}. This finding is also similar with the results of Islam *et al*. (2013) who reported that number of leaves plant^{-1} in tomato was increased due to application of different doses of inorganic fertilizers.

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 N_{250}. Higher leaf area of stevia with higher N levels could be attributed to more number of branches and leaves plant^{-1} due to higher plant height. Significantly lower leaf area, fresh and dry leaf yield was obtained with the absolute control as against all other levels of N due to the lowest number of branches and leaves plant^{-1}. Khanom (2007) reported highest leaf area of stevia plant grown in non-calcareous soil applying chemical fertilizers. From another experiment conducted by Nasrin (2008) using different levels of N, she obtained highest leaf area applying 250 kg N ha^{-1}.

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. Different levels of N fertilizers showed significant influence on fresh and dry weight of stevia leaves. The biomass yield was highest due to application of different levels of N fertilizers both in acid and non-calcareous soil. This is also in confirmation with Angkapradipta *et al.* (1986a), where increased biomass production was achieved due to application of higher levels of N. 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 N. He also reported lower leaf dry yield with absolute control without any fertilizer which was 62% less as compared with higher levels of N. Research conducted at Egypt showed a significant increase in dry leaf biomass yield of stevia when N fertilizer was increased from 100 to 300 kg ha^{-1} wherein the dry leaf yield increased by 64% compared to lower dose (Allam *et al*, 2001). In conformity of these findings growth and yield of stevia increased significantly with increasing rates of N up to 60 kg ha^{-1} per crop with the highest dry leaf yield which was on par with 40 kg ha^{-1} per crop in sandy loam soils at Bangalore (Chalapathi *et al*, 1999).

N content and uptake

The nutrient content of a plant varies not only among its various plant parts but changes with age and stage of development. There are also varietal differences which will affect the nutrient content found in various plant parts. A plant analysis interpretation is based on a comparison of the nutrient concentration found in a particular plant part taken at a specific time with known desired value or ranges in concentration. N 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 N addition (N_{300}) but the highest nutrient uptake was obtained from N_{250}.

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. These results are in conformity with those of Angkapradipta *et al*. (1986). They reported that the stevia plant N content increased due to increased concentration in plant which could be attributed to higher availability and uptake. Nasrin (2008) also reported higher N content and uptake with higher levels of N fertilizer. Shivraj *et al*. (1997) concluded that increased nutrient uptake by stevia resulted from increased application of N fertilizers.

Critical leaf N concentration and N requirement of stevia

The critical level of N in many plants is around 3 percent. For several crops, when the N level in leaves drops below 2.75 percent, N deficiency symptoms appear and yield and quality decline. The primary exceptions are for the very young plants when the critical level may be 4 percent or more, and for leguminous plants, such as soybeans, peanuts, alfalfa, etc., where the critical N percentage is 3 to 4.25 percent. For some tree fruits and ornamentals, N levels may be as low as 2 percent before deficiency occurs. Deficiencies as well as excesses can be a problem. Nitrogen leaf levels in some varieties of pecans exceeding 3.50 percent may result in early defoliation. Nitrogen leaf levels greater than 4.50 to 5 percent retard fruit set in greenhouse tomato. High N levels (>3.50 percent) in forage crops such as fescue is thought to be related to the incidence of grass tetany. Small changes in N content for some crops can result in large effects on yield, plant growth, and the quality of forage and fruit. Therefore, it is important that the N level be maintained within the prescribed limits of the sufficiency range by the proper use of N fertilizer (Bryson and Mills, 2015).

CONCLUSION

The results indicated that all the parameters examined in this study were significantly affected by different doses of N. Across N application rates, the highest values of most parameters except plant height and N content were obtained from 250 kg ha^{-1} and the lowest values from control. N application at all levels increased leaf dry yield at harvest by 99 to 505% in acid soil and 69 to 438% 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 N content proportionately increased with the increase of N level. The highest N content was obtained from its highest application. The minimum amount of N for maximum leaf biomass production in the plants grown in acid and non-calcareous soils was estimated to be ca 273 and 257 kg ha^{-1}, respectively. The critical N 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 1.43 and 1.50% in the plants grown in acid and non-calcareous soils, respectively.

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