EFFECT OF NITROGEN AND NAPTHELIC ACETIC ACID ON GROWTH, YIELD AND NUTRIENT CONTENT OF STEVIA (*Stevia rebaudiana* Bertoni)

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DHAKA-1207, BANGLADESH

JUNE 2022

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A Thesis

Submitted to the Department of Agricultural Chemistry,

Sher-e-Bangla Agricultural University, Dhaka-1207,

In partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURAL CHEMISTRY

SEMESTER: JANUARY-JUNE, 2022

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CERTIFICATE

This is to certify that the thesis entitled **"EFFECT OF NITROGEN AND NAPTHELIC ACETIC ACID ON GROWTH, YIELD AND NUTRIENT CONTENT OF STEVIA (*Stevia rebaudiana* Bertoni)"** submitted to the Department of Agricultural Chemistry, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE (M.S.)** in **AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bonafide research work carried out by **MD. SOHAG HOSSAIN**, Registration No. **20-11149** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

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Dedicated To
My Beloved Parents

ACKNOWLEDGEMENTS

The author seems it a privilege to express his enormous sense of gratitude to the almighty Allah for there ever ending blessings for the successful completion of the research work.

The author wishes to express his gratitude and best regards to his respected Supervisor, ***Dr. Mohammed Ariful Islam,*** *Professor, Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka, for his continuous direction, constructive criticism, encouragement, and valuable suggestions in carrying out the research work and preparation of this thesis.*

I would like to express my heartiest respect and profound appreciation to my co-supervisor, ***Dr. Md. Shariful Islam,*** *Senior Scientific Officer (SSO), Physiology and Sugar Chemistry Division, Bangladesh Sugarcrop Research Institute (BSRI), Bangladesh for his valuable assistance, cordial inspiration, cooperation's and constructive suggestion to conduct the research work as well as preparation of the thesis.*

The author feels to express his heartfelt thanks to the honorable Chairman, ***Professor Dr. Mohammed Ariful Islam****, Department of Agricultural Chemistry along with all other teachers and staff members of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka, for their co-operation during the period of the study.*

The author feels proud to express his deepest and endless gratitude to all of his course mates and friends to cooperate and help his during taking data from the field and preparation of the thesis. The author wishes to extend his special thanks to Mst. Ruba Akter and his room mates, classmates, and friends for their keen help as well as heartiest co-operation and encouragement.

The author expresses his heartfelt thanks to his beloved parents, younger sister, Brother, and all other family members for their prayers, encouragement, constant inspiration, and moral support for his higher study. May Almighty bless and protect them all.

The Author

EFFECT OF NITROGEN AND NAPTHELIC ACETIC ACID ON GROWTH, YIELD AND NUTRIENT CONTENT OF STEVIA (*Stevia rebaudiana* Bertoni)

ABSTRACT

The experiment was conducted to study the effect of nitrogen and napthelic acetic acid on growth, yeild and nutrient content of stevia. The trail was laid out in Randomized Complete Block Design (RCBD) with three replications. The treatments (nirogen) used were N_1: control, N_2: 105 kg ha^{-1}, N_3: 140 kg ha^{-1}, N_4: 175 kg ha^{-1}, N_5: 210 kg ha^{-1}. The applied napthelic acitic acids (NAA) were H_1: control, H_2: 50 ppm, H_3: 100 ppm and H_4: 150 ppm. Data were collected on plant height (cm), number of branch $plant^{-1}$, number of leaves $plant^{-1}$, leaf area $plant^{-1}$ (cm^2), fresh weight $plant^{-1}$ (g), dry weight $plant^{-1}$ (g), fresh leaf yield $plant^{-1}$ (g), dry leaf yield $plant^{-1}$ (g), fresh leaf yield ha^{-1} (kg), dry leaf yield ha^{-1} (kg), N(%), P (%), K (%), S (%), Ca (%), Mg (%), and Zn (µg g^{-1}) of stevia leaf along with initial and post harvest soil analysis. Significant variations were observed in plant height, number of leaves $plant^{-1}$, leaf area $plant^{-1}$ (cm^2) number of primary and secondary branch $plant^{-1}$ in different level of nitrogen application. Significant the highest plant height, number of leaves $plant^{-1}$, leaf area $plant^{-1}$ (cm^2) number of primary and secondary branch $plant^{-1}$ was observed in N_3 treatmnet and the lowest was observed in N_1 (control) treatment at 21 DAT to 147 DAT, respectively. Applying 100 ppm NAA was the significant effect in increasing height in H_3 (100 ppm NAA) at 21 DAT to 147 DAT, respectively and the lowest plant height, number of leaves $plant^{-1}$, leaf area $plant^{-1}$ (cm^2) number of primary and secondary branch $plant^{-1}$ was observed in control (NAA) treatment. The highest plant height, number of leaves $plant^{-1}$, leaf area $plant^{-1}$ (cm^2) number of primary and secondary branch $plant^{-1}$ heights was found in N_3H_3 treatment and the lowest was found in N_1H_1 (with no nitrogen application × with no NAA application) at all growth stages. The N, K, Mg, and Zn content of stevia leaf was significantly affected by different levels of nitrogen and NAA. The highest N, K, Mg, and Zn content was observed when the plot was treated with nitrogen140 kg ha^{-1} × 100 ppm NAA (N_3H_3) and the lowest N, K, Mg and Zn content was recorded in the control treatment. Significantly highest fresh weight $plant^{-1}$, fresh weight ha^{-1}, dry weight $plant^{-1}$, dry weight ha^{-1} was observed in N_3 treatment and lowest was observed in N_1 treatment in stevia plant. The intraction effect of nitrogen and NAA in most of the combination should significantly the highest fresh weight $plant^{-1}$, fresh weight ha^{-1}, dry weight $plant^{-1}$, dry weight ha^{-1}, fresh leaf yield ha^{-1} and dry leaf yield ha^{-1} was observed in N_3H_3 treatment and lowest was observed in N_1H_1 treatment. The intraction effect of nitrogen and NAA in most of the combination should significantly the highest fresh weight $plant^{-1}$, fresh weight ha^{-1}, dry weight $plant^{-1}$, dry weight ha^{-1}, fresh leaf yield ha^{-1} and dry leaf yield ha^{-1} was observed in N_3H_3 and lowest was observed in N_1H_1 treatment. Thus, the application of nitrogen and napthelic acitic acid had positive impact on leaf production resulted in higher yield economic stevia. From the result it can be recommended that nitrogen 140 kg ha^{-1} and NAA 100 ppm is might be suitable for commercial field cultivation of stevia production in Bangladesh.

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ABBREVIATIONS AND ACRONYMS

Cu	=	Copper
N	=	Nitrogen
P	=	Phosphorus
K	=	Potassium
%	=	Percentage
AEZ	=	Agro-ecological Zone
BSRI	=	Bangladesh Sugarcane Research Institute
Contd.	=	Continued
CRD	=	Completely Randomized Design
etc.	=	Etcetera
S	=	Sulphur
g	=	Gram
FDA	=	Food And Drug Administration
L	=	Liter
Max	=	Maximum
Min	=	Minimum
ml	=	Mili Litre
M.S.	=	Master of Science
No.	=	Number
SAU	=	Sher-e-Bangla Agricultural University
SD	=	Standard Deviation
^{o}C	=	Degree Celsius
t ha^{-1}	=	Ton per hectare
i.e.	=	id est = that is
Mg	=	Magnesium
mg	=	Milligram
kg	=	Kilogram
e.g.	=	Exempli gratia = for example
et al.	=	Et alia = and others
pH	=	Negative logarithm of hydrogen ion (H^{+}) concentration
Fig.	=	Figure
AAS	=	Atomic Absorption Spectrometer
*	=	5 % level of probability
WHO	=	World Health Organization
Ca	=	Calcium
Zn	=	Zinc
B	=	Boron
OM	=	Organic Matter
H	=	Hormone
N	=	Nitrogen
NAA	=	Napthelic Acetic Acid

H_2O	=	water
FAO	=	Food and Agricultural Organization

CHAPTER I

INTRODUCTION

Stevia (*Stevia rebaudiana* Bert.) belongs to the family Compositae is sweet herb and caloric free natural sweetener and originated in the Paraguay. Dr. M. S. Bertoni officially discovered the sweet herb stevia in early 20th century (1905). Stevia now successfully grown in Paraguay, Maxico, Central American, Japan, China, Malaysia, South Korea and recently started in different parts of India especially in south India. In Europe it is also reported to be cultivated in Spain, Belgium and United Kingdom. The first reports of commercial cultivation in Paraguay were in 1964 (Katayama *et al.,* 1976). Stevia leaves and stevioside are safe for human consumption (Kinghorn and Soejarto, 1985; Kinghorn, 1992). There have been no reports to date of adverse effects from the use of Stevia products by humans (Brandle and Rosa, 1992). The dry leaves of this plant are 30 times sweeter than sugar, with zero calories. Whereas pure extract stevioside is non-caloric and 300 times sweeter than sugar (Bhosle, 2004). Stevioside, a sweet compound contained in leaves of stevia, has recently been attracting much attention as an alternative sweetener (Crammer and Ikan, 1987). It is now commonly used as a natural sweetener in beverages and foods (Ishi *et al.,* 1987), being preferred over other non-caloric sucrose substances as it is heat-stable, some what resistant to acid hydrolysis, and non-fermentable (Kinghorn and Soaejarto, 1991). Literature survey revels stevia's versatile application in treatment of obesity, weight loss, dental health, high blood pressure, oral health, carbohydrates cravings, skin toning and healing, tobacco and alcohol cravings and antihyperglycemic effects of stevioside in diabetic subjects (Gregersen *et al.,* 2004). The global market size and business of medicinal plant materials including stevia and health care products based on this herbs comes to around 62 billon US $ and is likely to cross the 1 trillion mark by 2020 and 5 trillion by 2050 (Patra and Khanuja, 2005). Stevioside content varies with the dry weight of the leaves depending growing condition (Nepovim*et al.,* 1998 and Guens, 2003). Hence, it is necessary to improve agro techniques for increasing the biomass yield and stevioside of stevia. Influence of nitrogen and NapthelicAcitic Acid (NAA) on growth, yield and nutrient uptake of stevia was reported by Chalapthi *et al.,* 1997, Chalapthi *et al.,* 1999 and Zaman *et al.,* 2015 but no such comparative study of agro technique so far been reported till. Stevia needs an optimum temperature of 20 – 28°C for good growth, in addition to intensive illumination, the day length of more than 13 hours, clay sand soil

rich in organic fertilizers and intensive damping (Mohammad Ghawas et al,2009). Malaysia can provide the plant with all these conditions expects for the day length, which leads to early flowering the day length of Malaysia can reach a maximum of only 12 hours the problem can reduce the yield and glycosides content of considered to increase/** glycoside content can be such as by improving plant vegetative growth. The vegetative growth can be increased by application of high nutrient especially nitrogen to the soil at the vegetative stage. Nitrogen is a macronutrient and very important to the plant because it leads to longer vegetative growth and greater leaf expansion. Deficiency of nitrogen will reduce vegetative growth causing reduced leaf production, which ultimately reduces the marketable part of stevia.

Previous works on nutrient requirements of stevia were conducted in other countries. However, the nutrient requirement of stevia under local conditions has not been systematically investigated and no specific recommendation can be made. Thus, specific recommendation for nutrient especially nitrogen requirement should be developed under local condition. Protein and carbohydrate contents in dry weight basis of Stevia leaves ranges from 10.0 to 18.0 and 52 to 64.06, respectively (Srivastava *et al*., 2016; Maira Segura-Campos *et al*., 2014).

Plant growth regulators (PGRs) are being used as an aid to enhance yield of different crops (Nickell, 1982; Sarkar *et al.,* 2002; Sarker *et al.,* 2009; Bakhsh *et al.,* 2011). Lee (1990) examined the foliar application of NAA has also found to increase plant height, number of leaves plant^{-1}, Fruit size with consequent enhancement in seed yield in different crops and are being advised to use PGRs to get higher production. However, the research on examining the combination effects of PGRs and lime for better pulse yield is still in initial stage. The productivity of pulses is very low as compared to cereals, which have been selected for high grain yield under high input conditions (Narasimhan *et al.* 2010). The role of plant growth regulators in crop growth and development is indispensable. The major areas where growth regulators have successfully played their role in crops include propagation, seed and bud dormancy, growth control and regulation of flowering, fruiting etc. Further, they are also known to help in yield and quality improvement. The enhanced application of nitrogen has been reported to reduce the production of flowering in sugarcane.

Although stevia is an important crop, very little effort has been given to its influence of plant growth regulators and nitrogen on regulation of stevia. Only few researches on urea and NAA

are available on the influence of nitrogen and NAA on regulation of production of stevia cultivars in Bangladesh.

Therefore keeping this in mind the present investigation was performed to fulfill the following objectives -

1. To study the effect exogenous application of nitrogen on growth, yield and nutrient of stevia.
2. To study effect of exogenous application of NAA on growth, yield and nutrient content of stevia.
3. To find out the combined effects of nitrogen and NAA on stevia leaf production.

CHAPTER II

REVIEW OF LITERATURE

Bangladesh is an agro-based country. Most of the farmers of the country are involved in rice production rather than nutrient rich or medicinal plant. Noor-E-Ferdous, 2010 reported that stevia is a new crop in Bangladesh Sugarcane Research Institute (BSRI), Ishurdi, Pabna first introduced in 2001 from Thailand. It is a calorie free natural sweetener and the sweetness is due to presence of sweetening agent called Stevioside. The principal importance of stevia is due to the possibility of substituting it for saccharine. It is used as a natural sweetener, diabetes, for high blood pressure etc. The world wide prevalence of diabetes is increasing at such a rapid pace that the World Health Organization (WHO) has identified diabetes as an epidemic condition (Friedman, 2002). Bangladesh ranks 10th among the diabetic populations of 40 countries in 2000 but its incidence so much that in 2030 it will occupy 7th position (Karim *et al.,* 1986). Stevia can provide the taste of sweet to the diabetes patients without increasing the glucose level.

This crop is not cultivated by farmers due to lake of knowledge on cultivation procedure in Bangladesh soil condition. So different cultivation method are important to find the establish suitable cultivation method. An attempt is made to enumerate some of the potential works done on stevia cultivation elsewhere of the world including Bangladesh.

2.1 Position of Stevia in plant kingdom

Taxonomic position from kingdom to Species (http://website[1], 2010)

Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Asterales
Family	Compositae
Genus	*Stevia*
Species	*Stevia rebaudiana* (Bertoni)

2.2 Origin of stevia

Stevia rebaudiana Bert. is one of 154 members of the genus Stevia and one of only two that produce sweet steviol glycosides (Robinson, 1930; Soejarto *et al.*,1982; Soejarto *et al.*,1983). It is native to the valley of the Rio Monday in highlands of Paraguay, between 25 and 26 degrees south latitude, where it grows in sandy soils near streams (Katayama *et al.*, 1976).

2.3 Characteristics of stevia

Stevia is a member of the Compositae family. It is a small shrubby perennial growing up to 65 cm tall, with sessile, oppositely arranged lanceolate to oblancoelate leaves, serrated above the middle. Trichome structures on the leaf surface are of two distinct sizes, one large (4-5 µm), one small (2.5 µm) (Shaffert and Chetobar, 1994b). The flowers are small (7-15 mm), white and arranged in an irregular cyme. The seed is an achene with a feathery pappus (Robinson, 1930).

Stevia is an obligate short day plant with a critical day length of about 13 h. Extensive variability within populations for day length sensitivity has been reported (Valio and Rocha, 1966; Zaidan *et al.*, 1980). Plants can initiate flowering after a minimum of four true leaves have been produced (Carneiro, 1990). Sumida reported the results from a complete diallel cross with 8 parents and found that the amount of selfing ranged between 0 and 0.5%, while outcrossing ranged from 0.7 to 68.7%, indicating that some form of self-incompatibility system is operating (cited in Katayama et al. 1976). The reproductive anatomy of the male and female gametophytes is typical for angiosperms (Shaffert and Chetobar, 1992, 1994a). Stevia is diploid and has 11 chromosome pairs, which is characteristic for most of the South American members of the genus (Frederico *et al.*, 1996).

2.4 Crop production of stevia

Stevia plants can be propagated from cuttings or seed. Since germination rates are poor and seedlings very slow to establish it is best grown as an annual or perrenial transplanted crop. Clonal propagation is practical for small scale production, but is probably not economically viable for large scale stevia production where labor costs are high. The cost of producing vegetatively propagated transplants in Canada is high, So low cost transplants produced from seed is the only viable method on which to base stevia production in Canada. Only production as an annual is possible in most regions of Canada. The discussion of crop production in this review will therefore be limited to seed-based propagation of an annual transplanted crop.

In the temperate latitudes of the Northern hemisphere and South Western Ontario in Canada more specifically, the production cycle for annual stevia begins with the 6-7 week old plants grown from seed, in cells, in heated greenhouses. Seedlings are transplanted to the field in mid to late May. Fertilizer is banded along with the transplants. The crop is irrigated as required. Stevia is slow to establish under Canadian conditions and growth is sluggish until mid July. Most of the leaf yield is accumulated from July until mid to late September. The whole plant is harvested just above ground level, elevated into wagons and then dried. Following drying, the leaves are separated from the stems using a thesher. The leaves are then stored ready for processing (http:// website[2], 2010).

2.5 Cultural practices of stevia

Planting densities ranging from 40,000 to 400,000 plants ha^{-1} have been tried in experiments conducted in Japan (Katayama *et al.,* 1976). Leaf yield increased with increasing density up to 83,000 and 111,000 plants ha^{-1} for the first year of production. The concentration of stevioside in the leaves of stevia increases when the plants are grown under long days (Metvier and Viana, 1979). Since glycoside synthesis is reduced at or just before flowering, delaying flowering with long days allows more time for glycoside accumulation. It follows that stevia production would be best situated in a long day environment where vegetative period is longer and steviol glycoside yields will be higher.

Fertility requirements for stevia grown as an annual crop are moderate. Results from Japan demonstrate that, at the point of maximum dry matter accumulation, stevia plants consist of 1.4% N, 0.3% P, and 2.4% K (Katayama *et al.,* 1976). In Ontario total biomass production of 7500 kg ha^{-1} are possible and of that total, 26% would be roots, 35% stems, and 39% leaves (R. Beyaert pers. comm.). Based on the composition observed by Katayama, 1976 such biomass would require approximately 105 kg N, 23 kg P and 180 kg K from both soil and fertilizer. The actual rates of application will vary according to soil type and production environment, and need to be optimized for each specific situation.

Two fungal diseases, *Septoria steviae* and *Sclerotinia sclerotiorum*, have been reported in stevia grown in Canada (Lovering and Reeleder, 1996; Chang *et al.,* 1997). *Septoria* disease was characterized by depressed, angular, shiny olive gray lesions, sometimes surrounded by a chlorotic halo, that rapidly coalesce. *Sclerotinia* disease was characterized by brown lesions on the stem, near the soil line, followed by wilting and eventually by the complete collapse of

affected individuals. No means of controlling these diseases have yet been published. Since stevia is very slow to establish and does not compete well with weeds, herbicides or other means will be essential to control weed growth to produce ample yield and a clean crop. The herbicide trifluralin appears to be well tolerated by stevia (Katamaya, 1979).

Stevia is harvested just prior to flowering when steviol glycoside content in the leaves is at its maximum (Sumida, 1980, Xiang, 1983). Following harvest the whole plant is dried and the leaves separated from the stems for further processing (Murai, 1988). The stems have very low concentrations of sweet glycosides and are removed to minimize processing costs (Brandle and Rosa, 1992). Drying stevia under artificial conditions is affected by a number of factors including loading rate, temperature, and ambient air conditions (Van Hooren and Lester, 1992). The effect of drying conditions on glycoside levels or processing quality of the leaves has not been investigated.

2.6 Tissue culture of Stevia

Mitra and Pal, 2007 found that higher proliferation of shoots and multiplication was obtained on Murashige and Skoog's basal medium (MS) supplemented with 1.0 mg L^{-1} Indolacetic acid (IAA) plus 10.0 mg L^{-1} Kinetin and 30.0 mg L^{-1} adenine sulphate.

Himanshu *et al.,* 2006 reported that MS basal medium supplemented with 13.5 µM benzyladenine + 8.0 µM NAA showed best performance compared to 13.3 µM benzyladenine alone with respect to bud breaking in S. *rebaudiana*, while combination of 13.3 µM benzyladenine + 61.8 micro M AdSO4 and 13.5 µM benzyladenine + 8.0 µM NAA in MS basal medium showed enhanced shoot multiplication. NAA at 16.1 µM + 10.7 µM benzyladenine showed significant enhancement in the induction of rooting compared to 16.1 µM IAA alone. The best callusing was observed in the media containing 10.7 µM NAA + 8.8 µM benzyladenine.

Hossain *et al.,* 2005 showed that higher number of shoot and micro cuttings were found in the MS medium supplemented with 3 mg L^{-1} BA and 2.0 mg L^{-1} BA respectively. Higher number of root per shoot was obtained in MS medium supplemented with 1.0 mg L^{-1} NAA followed by 1.5 and 0.5 mg L^{-1} NAA, respectively.

The most effective explants for large scale production of the plants appeared to be micro-cuttings with apical or axial buds. They were successfully cultivated on the hormone- free medium, and this allowed producing the plants by several thousands for 3-4 months (Salas, 2001).

Dhir and Dhir, 2004 stated that multiple shoots were induced from shoot tips cultured on Murashige and Skoog (MS) supplemented with 0-3.0 mg L^{-1} of Benzyladenine (BA). Green true shoots with fully developed leaves were observed in almost 70% of initial cultures. Roots were induced in 30d old shoots, transferred to MS medium individually supplemented with IAA or IBA (1-4 mg L^{-1}).

Sivaram and Mukundan, 2003 regenerated shoots from shoot apex, nodal, and leaf explants of *S. rebaudiana* Bertoni by culturing them on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 6-benzyladenine (BA) 8.87 μM and indole-3-acetic acid (IAA) 5.71 μM. Rooting of the *in vitro*-derived shoots could be achieved following subculture onto auxin-containing medium. A survival rate of 70% was recorded at the hardening phase on the substrate cocopeat. The presence of the sweet diterpene glycosides, viz. stevioside and rebaudioside, was confirmed in the *in vitro*-derived tissues of Stevia using HPTLC techniques. Callus cultured on agar-solidified MS medium supplemented with BA (8.87 μ*M*) and indole-3-butyric acid (9.80 μ*M*) showed the highest sweetener content.

Morini *et al.,* 2003 reported that four genotypes of Stevia were tested on MS culture medium, comparing the effects of two Cytokinins: Kinetin and 6-Benzylaminopurine. Shoot rooting response was evaluated by IBA at 0.5 mg L^{-1} and 0.1 mg L^{-1}.

Salas, 2001 found that the most effective explants for large scale production of the plants appeared to be micro-cuttings with apical or axial buds. They were successfully cultivated on the hormone- free medium, and this allowed producing the plants by several thousands for 3-4 months.

Acuna *et al.,* 1997cultured *in vitro* nodal segments of 6-week-old seedlings on MS medium with 50% macroelement content and in the presence of 0.1 ppm NAA. At the transfer of plants from *in vitro* into *in vivo* conditions nodal segments were dipped in a 5% IAA solution to promote rooting. Treated plants were grown for 1 month in a greenhouse and then planted into the field. The most effective preparation for increasing the concentration of stevioside in leaves was Humiforte (synthetic amino acids, N, P, K and trace elements) in combination with Aminol (amino acids and N) but Melatran (lactic and anthranilic acids) gave the highest biomass yields.

Sritongkum, 1995 developed the procedures for micropropagation from primary leaf and shoot tip of *S. rebaudiana* Bertoni. The optimal conditions for callus induction from primary leaf were observed on MS medium supplemented with 2.0 mg L^{-1} NAA and 2.0 mg L^{-1} BA. The optimal conditions for primary leaf callus regeneration was achieved on MS medium containing (Nitsch, 1969) vitamins supplemented with 2.0 mg L^{-1} NAA and 2.0 mg L^{-1} BA. Root formation was occurred on MS medium supplemented with 0.1 mg L^{-1} NAA. The efficiency of adventitious shoot formation could be increased a number of 60 shoots per callus within 3 months. Shoot multiplication from shoot tips were observed on MS medium supplemented with 12.0 mg L^{-1} kinetin. The optimal condition for root induction was occurred both in MS medium supplemented with 0.01 mg L^{-1} NAA and MS medium without plant growth regulator. Plantlets could be transplanted to potting soil.

Puite, 1992 developed protocols for regeneration of *Stevia rebaudiana* via somatic embryogenesis. It is important as this technique can be used in the clonal propagation of this plant, or as explant material for protoplast isolation and regeneration.

Shoot primordia, which were able to propagate vegetatively with a very high rate and to redifferentiate easily to new plants, were induced from shoot tips of S*tevia rebaudiana* Bertoni on Gamborg B5 medium containing 6-benzylaminopurine (BAP) and alpha-naphthalic acetic acid (NAA) under light. The propagation of the shoot primordia of S*tevia rebaudiana*is rapid, and they are highly stable in chromosome number and karyotype. The shoot primordia can propagate at a high rate for a long time without differentiation. At any time, the shoot primordia readily developed into plantlets with shoots and roots within 2 or 3 weeks in static culture on B5 medium containing 0.02 mg L^{-1} BAP and 2% sucrose. The plantlets were transplanted to sterilized soil to grow to normal adult plants (Miyagawa *et al.*, 1986).

Yield of sweetening compounds present in leaf tissue can vary according to method of propagation (Tamura *et al.*, 1984), day-length (Metivier and Viana, 1979) and agronomic practices (Shock, 1982).

2.7 Seed production and quality of stevia

Given stevia's daylength requirements, seed production in the Northern hemisphere would be best situated between 20 and 30EN latitude. The crop could be transplanted in February or March and seed collected in late summer. Flowering under these conditions should occur between 54-104 d following transplanting, depending on the daylength sensitivity of the cultivars

used for seed production (Katayama *et al.,* 1979). One-thousand seed weights for stevia seed usually range between 0.15 and 0.30 g and, depending on plant density, seed yields of up to 8.1 kg ha^{-1} are possible (Carniero, 1990). Seed germination is often poor and rates less than 50% are common (Miyazaki and Wantenabe, 1974). Given the aforementioned conditions, seed produced on one ha could be enough to supply transplants for up to 200 ha of leaf production. Seed viability and yield are affected by growing conditions during pollination and seed filling. Excessive rainfall during pollination can affect both seed yield and germination (Carneiro, 1990, Shuping and Shizhen, 1995). Seed is best stored at 0 EC, but even under low temperature conditions germination will still decline 50% over three years (Shuping and Shizhen, 1995). Sealing of storage containers or using lower temperatures did not prevent the decrease in germination over time.

2.8 Cultivar development of stevia

A variety of plant breeding procedures have been used to improve leaf yield and rebaudioside A concentration in the leaves. Based on cultivar descriptions from Japan, China and Korea and our own work, it appears that sufficient genetic variability exists to make significant genetic gains in leaf yield, rebaudioside A content and the ratio of rebaudioside A to stevioside (Brandle and Rosa, 1992; Lee *et al.,* 1979 and 1982; Shizhen, 1995; Shyu *et al.,*1994 and Morita, 1987). Brandle and Rosa, (1992) found that the heritability of stevioside content to be high (83%), based on calculations from a group of half-sib families. Heritabilities for leaf yield (75%) and leaf to stem ratio (83%) were also substantial indicating that selection would be effective. Total sweet glycoside concentration in some lines from China was reported to be as high as 20.5%, and a rebaudioside A to stevioside ratio of 9:1 was disclosed in the Japanese patent literature (Shizhen, 1995 and Morita, 1987). Two breeding methods reported by the latter authors were: phenotypic mass selection and, recurrent selection for phenotype where selected plants are intercrossed before another round of selection. Some cultivars such as the high rebaudioside A selection from Japan, and Suweon 2 and 11 from Korea are based on the selection of single plant and because of self-incompatibility they can only be reproduced vegetatively, which limits their utility.

Nakamura and Tamura, 1985 studied a population of 300 random individuals and found that total glycoside concentrations at the seedling and harvest stages were not correlated suggesting that

early selection for total glycosides would not be effective. However, the proportion of individual glycosides relative to the total was correlated between seedlings and mature plants making early selection for glycoside composition possible. The authors also observed a wide range of variation in the four main glycosides and found that dulcoside A and stevioside, and rebaudioside A and C were positively correlated with each other. Stevioside and rebaudioside A and dulcoside and rebaudioside C were negatively correlated with each other. These correlations can be partially explained by the biosynthetic relationships between the individual glycosides because stevioside is the substrate for the synthesis of rebaudioside A plants high in rebaudioside A will probably be low in stevioside (Shibata *et al.*,1991).

2.9 Chemical properties of stevia

The sweet diterpene glycosides of stevia have been the subject of a number of reviews (Kinghorn and Soejarto, 1985, Crammer and Ikan, 1986, and Hanson and De Oliveira, 1993). Although interest in the chemistry of the sweet principles dates from very early in the century, significant progress towards chemical characterization was not made until 1931, with the isolation of stevioside (Bridel and Lavieille, 1931a). Treatment of this substance with the digestive juice of a snail yielded three moles of glucose and one mole of steviol, while acid hydrolysis gave isosteviol (Bridel and Lavieille, 1931b). Stevia is a naturally derived, high potency sweetener that can be up to 250-300 times sweeter than sucrose, or table sugar. It is similar in sweetness intensity to many of the artificial sweeteners currently on the market (U.S. Food and Drug Administration, 2015). Isosteviol was also obtained when steviol was heated in dilute sulfuric acid. Subsequent studies have led to the isolation of seven other sweet glycosides of steviol. Typical proportions, on a dry weight basis, for the four major glycosides found in the leaves of wild stevia plants is 0.3% dulcoside, 0.6% rebaudioside C, 3.8% rebaudioside A and 9.1% stevioside.

2.10 Structure of steviol, isosteviol and stevioside

The structure, stereochemistry and absolute configuration of steviol and isosteviol were established, through a series of chemical reactions and correlations over 20 years after the pioneering work of Bridel and Lavieille (Mosettig and Nes, 1955; Dolder *et al.,* 1960; Djerassi *et al.,* 1961 and Mosettig*et al.,* 1963). Structures of these and other diterpenes and diterpene glucosides are presented in Plate 1. Concurrent studies on the parent glycoside indicated that one

D-glucopyranose residue, hydrolyzed under alkaline conditions yielding steviolbioside, was attached to a carboxyl group (Wood *et al.*, 1955) while the other two were components of a sophorosyl group (Vis and Fletcher, 1956) bound to the aglycone through a $-glycosidic linkage (Yamasaki *et al.*, 1976). Support for the proposed stereochemistry was achieved by the synthetic transformation of steviol into stevioside (Ogawa *et al.*, 1980).

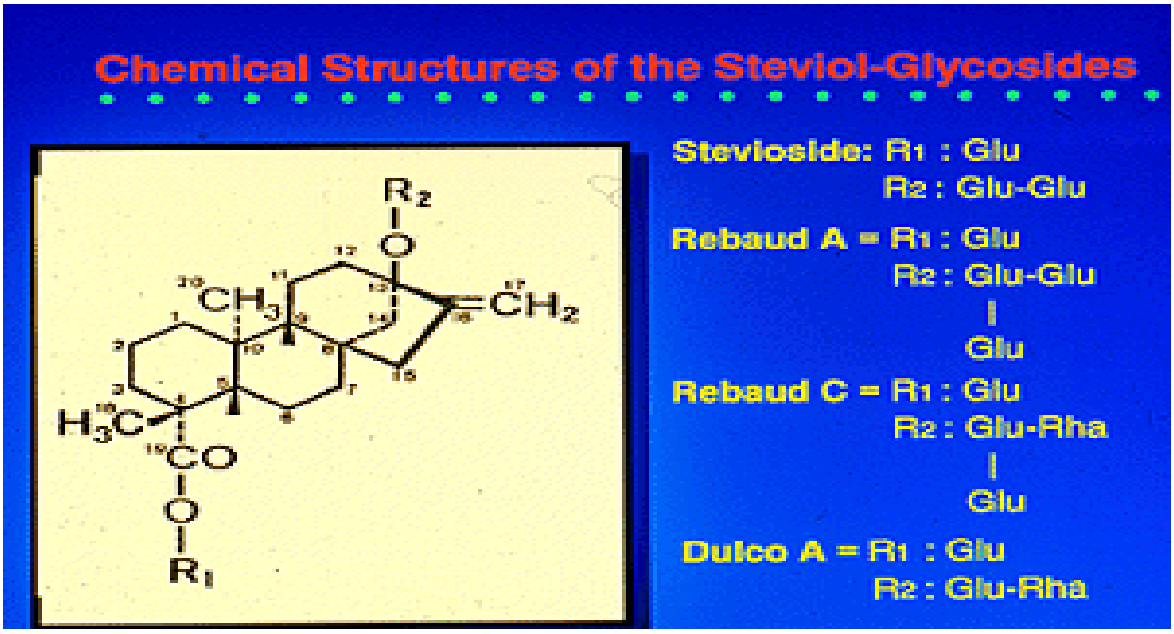


Plate 1. Structural formula of Steviol – Glycosides

Earlier, several approaches to the *in vitro* synthesis of steviol had been reported (Cook and Knox, 1970; Nakahara *et al.*, 1971; Mori *et al.*,1972; Ziegler and Kloek, 1977). Recently, spectroscopic data concerning stevioside and steviolbioside were published (Van Calsteren*et al.*, 1993).

2.11 Functional and sensory properties of steviol glycoside sweeteners

Of the four major sweet diterpene glycoside sweeteners present in stevia leaves only two, stevioside and rebaudioside A, have had their physical and sensory properties well characterized. Stevioside and rebaudioside A were tested for stability in carbonated beverages and found to be both heat and pH stable (Chang and Cook, 1983). However, rebaudioside A was subject to

degradation upon long term exposure to sunlight. Kinghorn and Soejarto, 1985also cite numerous Japanese studies that demonstrate that stevioside is very stable.

Phillips, 1989 has summarized the early sensory research. Stevioside was between 110 and 270 times sweeter than sucrose, rebaudioside A between 150 and 320, and rebaudioside C between 40 and 60. Dulcoside A was 30 times sweeter than sucrose. Rebaudioside A was the least astringent, the least bitter, had the least persistant aftertaste and was judged to have the most favourable sensory attributes of the four major steviol glycosides (Phillips, 1989, Tanaka, 1997). Dubois and Stephanson, (1984) have also confirmed that rebaudioside A is less bitter than stevioside and demonstrated that the bitter notes in stevioside and rebaudioside A are an inherent property of the compounds and not necessarily the result of impurities in whole plant extracts. Relative to other high potency sweeteners such as apsartame, bitterness tends to increase with concentration for both stevioside and rebaudioside A (Schiffman *et al.,* 1994). Both stevioside and rebaudioside A are synergistic in mixtures with other high potency sweeteners such as aspartame and are good candidates for inclusion in blends (Schiffman *et al.,* 1995). Although specialty applications may exist for the other glycosides, increasing levels of rebaudioside A in stevia leaves is a clear objective for breeding work.

2.12 Sweetness and metabolism of stevia

Although there are more than 180 species of the Stevia plant, only *stevia rebaudiana*gives the sweetest essence due to the fact that these leaves accumulate eight sweet diterpene glycosides (Soejarto*et al*., 1983).

On the whole plant level, steviol glycosides tend to accumulate in tissues as they age, so that older lower leaves have more sweetener that younger upper leaves. Since chloroplasts are important in precursor synthesis, those tissues devoid of chlorophyll, like roots and lower stems contain no or trace amounts of glycosides. Once flowering is initiated glycosides concentrations in the leaves begin to decline. Distribution of glycosides within a stevia plant (http:// website[3], 2010) is presented in Plate 2.

Stevioside, the main sweet component in the leaves of Stevia rebaudiana (Bertoni) Bertoni tastes about 300 times sweeter than sucrose (0.4% solution) (Geuns, 2003). Other compounds present but in lower concentration are: steviolbioside 2, rebaudioside A 4, B 5, C 6, D 7, E 8, F 9 and dulcoside A 10 (Kennelly, 2002 and Starrat*et al*., 2002).

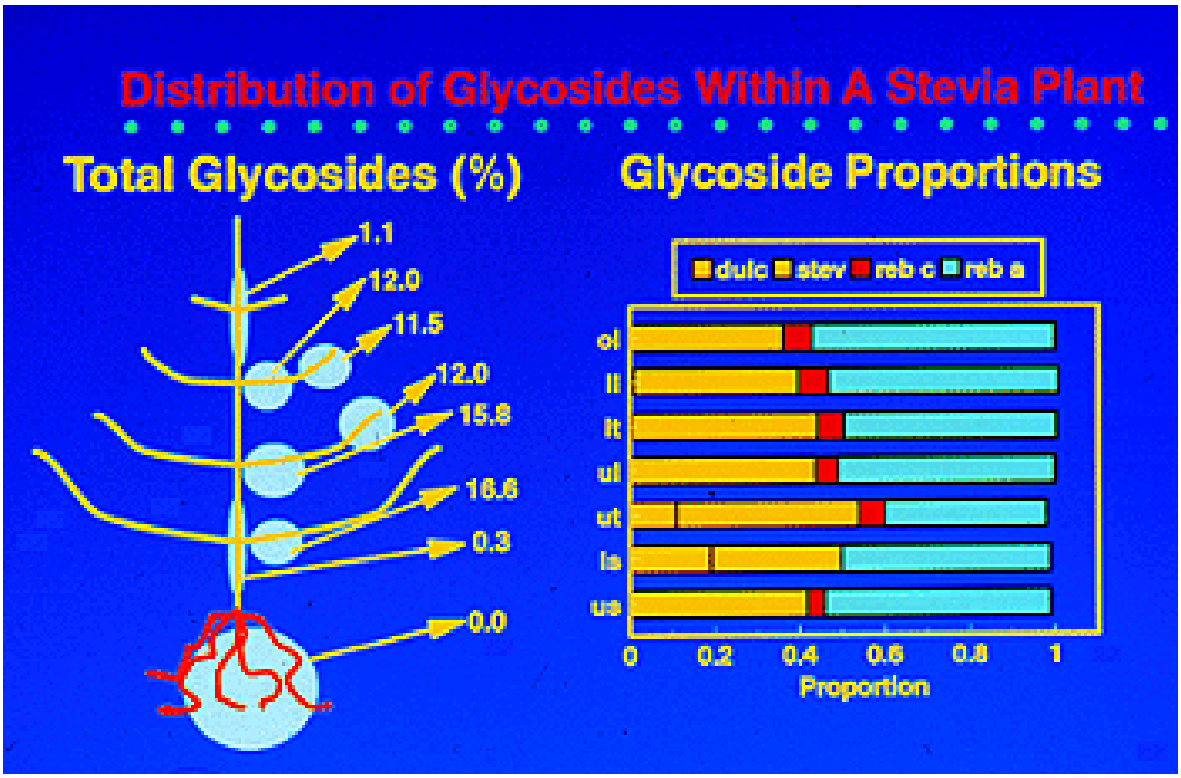


Plate 2. Distribution of Glycosides within a Stevia plant

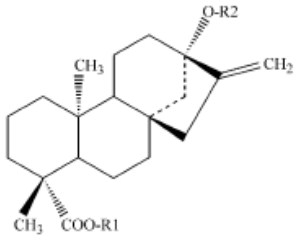
An extract of one or more these compounds may be up to 300 times sweeter than sugar (Duke, 1993). Total sweet glycoside concentration in some lines from China was reported to be as high as 20.5% and rebaudioside-A to stevioside ratio of 9**:**1 was disclosedin the Japanese patent literature (Shizhen 1995 and Morita, 1987).

Stevia has virtual no calories. It dissolves easily in water and mixes well with all other sweeteners. It was used in delicious homemade ice cream that was low in carbohydrates (Atkins, 1994).

Unlike many low- calorie sweeteners, stevioside is stable at high (100^{o}c) temperatures and over a range of pH values (pH 3-9) (Kinghorn and Soejarto, 1985).

Chang and Cook in 1983 tested stability of pure stevioside and rebaudioside A in carbonated phosphoric and citric acidified beverages during long-term storage chemically, microbiologically, and organoleptically. Thin-layer chromatography and high-pressure liquid chromatography were used to follow the chemical degradation of these Stevia sweeteners. Some

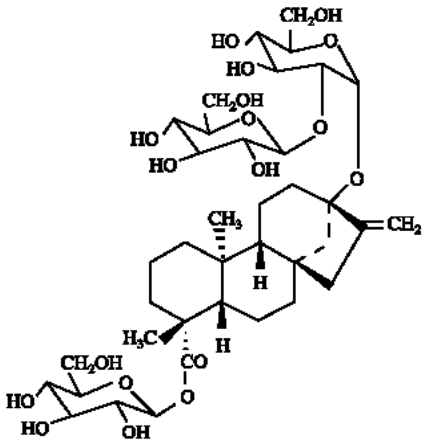
degradation of both sweeteners was observed after 2 months of storage at 37°C; however, there were no significant changes at room temperature or below following 5 months of storage of stevioside, or 3 months of storage of rebaudioside A. Exposure to 1 week of sunlight did not affect stevioside but resulted in approximately 20% loss of rebaudioside A. Heating at 60°C for 6 days resulted in 0-6% loss of the sweeteners.



	Compound name	R1	R2
1	steviol	H	H
2	steviolbioside	H	β-Glc-β-Glc(2→1)
3	stevioside	β-Glc	β-Glc-β-Glc(2→1)
4	rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
5	rebaudioside B	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
6	rebaudioside C (dulcoside B)	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
7	rebaudioside D	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
8	rebaudioside E	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)
9	rebaudioside F	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
10	dulcoside A	β-Glc	β-Glc-α-Rha(2→1)

Stevioside is a noncaloric natural sweetener isolated from the leaves of *Stevia rebaudiana*. It is about 300 times sweeter than sucrose (Bridel and Lavielle, 1931). Therefore, it is a popular sugar substitute in a variety of foods and often used as a food supplement in Japan, China, South Korea, and Taiwan. Stevioside can be degraded to steviol, an aglycone, by the intestinal microflora from various animal species, including man (Hutapea*et al*., 1997 and Koyama *et al*., 2003b). Stevioside is a large neutral molecule with both polar and hydrophobic regions, whereas steviol contains a hydrophobic ring and one negative charge on the carboxylic group. In addition

to its sweetening properties, stevioside has been shown to have potential therapeutic value as a contraceptive (Melis, 1999) and an antihypertensive agent (Chan *et al*., 1998; Jeppesen *et al*., 2002, 2003). Continued consumption of stevioside extract for 3 months reduced blood pressure in hypertensive patients (Chan *et al*., 2000). Both stevioside and steviol alter glucose metabolism (Suanarunsawat and Chaiyabutr, 1997) and glucose absorption (Toskulkao*et al*., 1995). They have also been proposed to have a potential role as antihyperglycemic agents by stimulating insulin secretion from pancreatic beta cells (Jeppesen *et al*., 2000, 2002, 2003). It was correctly concluded that steviol is the only metabolite in faeces and is not further metabolized (Hutapea*et al*., 1997; Koyama *et al*., 2001, 2003a; Gardana*et al*., in press and Geuns*et al*., 2003, in a press).



Stevioside ($C_{38}H_{60}O_{18}$)

2.13. Safety of stevia

It has been shown that oral stevioside is not taken up by the human body or the uptake is extremely low (Yamamoto *et al*., 1985; Bracht *et al*., 1985; Koyama *et al*., 2003b and Geuns*et al*., in press a) and none of the digestive enzymes from the gastro-intestinal tract of different animals and man are able to degrade stevioside into steviol, the aglycone of stevioside (Wingard *et al*., 1980; Hutapea*et al*., 1997 and Koyama *et al*., 2001, 2003a).

Suttajit (1993) found that stevioside and steviol are neither mutagenic nor clastogenic *in vitro* at the limited doses; however, *in vivo* genotoxic tests and long-term effects of stevioside and steviol are yet to be investigated.

The sweet compounds pass through the digestive process without chemically breaking down, making Stevia safe for those who need to control their blood sugar level (Strauss, 1995).Stevioside and rebaudioside A are both non- carcinogenic (Das *et al*., 1992).

Yodyinguad and Bunyawong (1991) concludedthat stevioside at a dose as high as 2.5 g kg^{-1} body wt day^{-1} affects neithergrowth nor reproduction in hamsters.

Recent clinical studies have shown it can increase glucose tolerance and decrease blood sugar levels. Of the two sweeteners (aspartame and Stevia), Stevia wins hands down for safety (Whitaker, 1994).

Toskulkao*et al.,* 1997 investigated the acute toxicity of stevioside and steviol (a product of enzymatic hydrolysis of stevioside) in three animal species including rat, mouse and hamster. The susceptibility to stevioside and steviol acute toxicity in both sexes of these animal species was compared. The animals were treated intragastrically with stevioside or steviol and general signs and symptoms were observed. The numbers of dead animals were recorded within a period of 14 days after administration for estimation of LD_{50}. Stevioside at a dose as high as 15 g kg^{-1} BW was not lethal to mice, rats or hamsters. Hamsters were found to be more susceptible to steviol than rats or mice. LD_{50} values of steviol in hamsters were 5.20 and 6.10 g kg^{-1} BW for males and females, respectively. In rats and mice, LD_{50} values of steviol were higher than 15 g kg^{-1} BW in both sexes. Histopathological examination in the kidney of hamsters induced by steviol revealed severe degeneration of the proximal tubular cells. These structural alterations

were correlated with the increases in serum blood urea nitrogen (BUN) and creatinine. Therefore, the possible cause of death induced by steviol might be due to acute renal failure.

In studies of acute toxicity, a LD_{50} of 8.2 g kg^{-1} for a refined stevioside extract and an acceptable daily Stevioside intake of 7.9 mg kg^{-1} was suggested by Xili*et al.,* 1992.

Geuns, 2003concluded that Stevia and stevioside are safe when used as a sweetener. It is suited for both diabetics, and PKU patients, as well as for obese persons intending to lose weight by avoiding sugar supplements in the diet. No allergic reactions to it seem to exist.

Literature related to safety of Stevia sweeteners concluded that Stevia leaves and stevioside are safe for human consumption (Kinghorn and Soejarto, 1985 and Kinghorn, 1992). There have been no reports to date of adverse effects from the use of Stevia products by humans (Brandle and Rosa, 1992).

Though Stevia got its attention as sweetening agent other medicinal were reported. According to World Health Organization (WHO) findings it regulates blood pressure, fights cavities, induces pancreas to produce more insulin, and act as bactericidal agent (Bhosle, 2004).

2.14 Stevia is a new crop in Bangladesh.

Khan *et al.,* (2010) reported that stevia is a new crop in Bangladesh. It has been introduced by Bangladesh Sugarcane Research Institute. Stevia yield significantly influence some factors like time of planting, cultivation methods, dose of fertilizer, spacing, tillage, pruning, irrigation, ratooning, insects and diseases. The authors observed drying of the soft green leaf material is completed immediately after harvesting utilizing a drying wagon and estimated 100 kg of green weight is dried down to 25 kg dry weight. Drying stevia under artificial conditions is affected by weather conditions. Dry leaves stored in plastic container with air tied condition.

Diabetic is a serious problem throughout the world. In our country about 90 lakhs peoples are suffering from this serious disease which is about 5.9% of total population. But the most alarming message is numbers of children aged from 8 to 20 years old are also suffering from this serious disease. Under this situation stevia can help these large number of people and in addition to this stevia can contribute to our national economy.

Roy (2009) observed that all of the growth and yield contributing characters of stevia including leaf dry weight $plant^{-1}$ and leaf dry weight ha^{-1} were significantly influenced due to combined NPK fertilizers, FYM and their interaction. In the case of combined NPK fertilizers stevia

showed higher values for all characters including leaf dry weight plant^{-1} and leaf dry weight ha^{-1} with combined NPK fertilizers @ 1.14g Urea, 0.58g TSP and 1.39g MP i.e.; 0.52 N, 0.12g P and 0.69g K pot^{-1}, NPK nutrients @ 105.3, 24.29 and 135.3 kg ha^{-1}. Farm yard manure (FYM) 202.68g FYM i.e.; 1.03g N, 0.85g P and 1.30g K pot^{-1}, NPK nutrients @ 204, 168 and 256 kg ha^{-1} produced the highest values for all characters including leaf dry weight plant^{-1} and leaf dry weight ha^{-1}. The highest values for all characters including leaf dry weight plant^{-1} and leaf dry weight ha^{-1} due to interaction of combined NPK fertilizer @ 1.14g Urea, 0.58g TSP and 1.39g MP i.e.; 0.52g N, 0.12g P and 0.69g K pot^{-1} NPK nutrients @ 105.3, 24.29 and 135.3 kg ha^{-1}) × FYM @ 202.68g FYM i.e.; 1.03g N, 0.85g P and 1.30g K pot^{-1}, NPK nutrients @ 204, 168 and 256 kg ha^{-1}) i.e.; combinedly 1.56g N, 0.97g P and 1.98g K pot^{-1}, NPK nutrients @ 307.5, 192.29 and 391.3 kg ha^{-1} to obtain the highest leaf dry weight plant^{-1} and the highest leaf dry weight ha^{-1} and the highest leaf dry weight ha^{-1} respectively ensuring good health of the soil environment.

Hossain *et al.,* (2009) reportedthat poor seeds production and low germination became cultivation barrier of stevia. Therefore, quality seeds production and easy propagation become the best option for research. An experiment was conducted to produce seeds using spacing 45 cm × 45 cm at the BSRI farm in March 01, 2007. Shoots and stem cuttings with 3 to 4 nodes were used to vegetative propagation. The average plant height and number of branch per plant were 1.17 m and 10.42 respectively. Seeds production was possible followed by successful germination. Shoots and stem cuttings were successfully established in the field for cultivation. Dry leaves yield per plant and per hectare were 38.63g and 1433.43 kg ha^{-1}, respectively. The results indicate the possibility of seed production and easy propagation of stevia for successful cultivation.

Nasrin (2008) reported that the values of growth, leaf yield and yield attributes of stevia increased with increase in N level. The highest plant height, branch and leaf number, leaf length and breadth, leaf fresh and dry weight were observed from the plant which was fertilized by 250 Kg N ha^{-1} and the lowest from control. The chlorophyll and protein contents of stevia leaves were significantly influenced by the application of different levels of N and the highest values were obtained from the plant fertilized with 200 kg N ha^{-1}. Highest values of all the nutrient contents and their uptake were obtained from N_{200} treatment except Mn, Cu and Na contents as well as their uptake which were highest in plants treated with 250 kg N ha^{-1}. Significant and

positive correlations were observed among different physical and chemical parameters of stevia viz. plant height and leaf number, branch number and leaf number, N and S, Zn and B, Zn and Cu and B and Cu contents. The overall results thus suggest that stevia can be grown in non calcareous soil fertilized with 200 kg N ha^{-1} for higher leaf yield and nutrient contents for its large scale production in Bangladesh.

2.15 The principal advantages of Stevia are the following
(http:// website[4], 2010)

- It is a completely natural non-synthetic product.
- It has got ZERO Sugar. Stevioside (the sweetener) contains absolutely no calories.
- The leaves can be used in their natural state.
- Thanks to its enormous sweetening power, only small quantities need to be used.
- The plant is non-toxic.
- The leaves as well as the pure stevioside extract can be cooked.
- No aftertaste or bitterness.
- Stable when heated up to 200 degrees.
- Non fermentative.
- Flavour enhancing.
- Clinically tested and frequently used by humans without negative effect.
- Ideal, non-addictive sweetener for children.

2.16 Effect of nitrogen on yield and yield components

Esra Ucar *et al* 2018 reported that the efficiency of different nitrogen doses (0, 50, 100, 150, and 200 kg ha^{-1}) on growth, yield, and quality of stevia (*Stevia rebaudiana* Bert.) was investigated in 2011–2013. The study was conducted in Antalya located in the Mediterranean Region of Turkey. Terra rossa type soil (LVx, FAO) characteristics of the experimental field were clay loam, with high amounts of lime (33,9%) and slightly alkaline (pH 7.7). The experiment was carried out in randomized block design with four replications. All the results were summarized as mean of three years. The highest fresh and dry biomass yields (26.75 t ha^{-1} and 7.5 ha^{-1}, respectively) were obtained from 150 kg ha^{-1} N dose and followed by 100 kg ha¡1 N dose (26.29 t ha^{-1} and

7.24 ha^{-1} , respectively). Whereas the highest fresh and dry leaf yields (13.27 t ha^{-1} and 3.82 t ha^{-1} , respectively) were realized in 100 kg ha^{-1} N dose. Actually, all nitrogen doses gave higher biomass and leaf yields compared to the control. On the hand, major steviol glycosides (stevioside and rebaudioside A) in the leaf were not influenced by nitrogen levels. In conclusion, 100 kg ha¡1 N dose was found to be suitable for cultivation of stevia under field conditions.

Razzaque *et al.* (2017) observed that increasing nitrogen level in nutrient stress soil increased growth and dry matter production up to 60 kg N ha^{-1} irrespective of genotype and thereafter decreased. Among the mungbean genotype IPSA 12 showed maximum leaf area, dry matter production and seed yield (14.22 g $plant^{-1}$) in nutrient stress soil. The lowest seed yield (7.33 g $plant^{-1}$) was recorded in ACC12890053 under control condition.

Hossen et al. (2015) stated that longest pod (7.96 cm), maximum pods $plant^{-1}$ (10.45), maximum seeds pod–1 (9.70), higher weight of 100–seed (4.52 g), higher weight of seed (5.73 g $plant^{-1}$) and greater seed yield (1.85 t ha^{-1}) were also obtained in 45 kg N ha^{-1} compare other N levels. The BARI mung–6 × 45 kg N ha^{-1} for seed yield was found under the regional condition of Patuakhali (AEZ-13).8

Amin et al. (2015) investigated that root dry weight increased with combined application of N and K fertilizers. Flooded plants treated with 14 kg N ha^{-1} + 25 kg K ha-1 produced the highest TDM and seed yield, though the yield was statistically similar to that obtained when the levels of N and K were applied separately, as well as with that of 1% urea + 25 kg K ha^{-1}.

Mainul *et al.* (2014) observed maximum plant height (40.52 cm), number of leaves (19.14), number of branches (10.09), average dry weight/plant (7.35 g), number of pods/plant (15.90),number of seeds/pod (4.49), 1000-seed weight (42.56 g), seed yield (1.06 t/ha), stover yield (2.08 t/ha), N content in seed (3.60), P content in seed (0.48) and K content in seed (1.26) were found in N3 which was statistically similar with N2 whereas minimum from N0. Hossain et al. (2014) found that Mungbean variety namely BARI Mung-6 performed better in respect to growth and yield (seed and stover) as compared to BARI Mung-5 with the application of nitrogen (50 kg ha^{-1}) and inoculums Bradyrhizobium (1.5 kg ha^{-1}).

Kumar and Tomar (2013) investigated that the growth and yield attributes increased with the decrease in plant density and with the increase in the levels of nitrogen and phosphorus while

plant height was positively increased with the increase in plant density and levels of nitrogen and phosphorus. Interaction effect revealed that decreasing plant density and increasing levels of nitrogen and phosphorus increased dry matter accumulation and grain yield significantly. The maximum dry matter (34.4 g/plant) and grain yield (2.07 t ha^{-1}) were recorded in 333 × 103 plants ha^{-1} plant density with 20 kg N and 60 kg P2O5 ha^{-1}. Nigamananda and Elamathi (2007) conducted an experiment during 2005-06 to evaluate the effect of N application time as basal and as DAP (diammonium phosphate) or urea spray and plant growth regulator (NAA at 40 ppm) on the yield and yield components of greengram. Results showed that 2% foliar spray as DAP and NAA, applied at 35 DAS resulted in the highest values for number of pods $plant^{-1}$ (38.3), seeds pod-1 , test weight, flower number, fertility coefficient, grain yield (9.66 q ha^{-1}).9 Azadi et al. (2013) observed that different nitrogen levels influenced different growth and yield attributes of mungbean such as plant height, seed yield, stem diameter, number of node and 75 kg N ha^{-1} showed higher values than the other N doses (50, 100 and 150 kg N ha^{-1}). Achakzai et al. (2012) found that different Nitrogen levels influenced most of the growth attributes of the mungbean. Maximum days to flowering, number of branches $plant^{-1}$, number of leaves $plant^{-1}$, plant height, number of branches $plant^{-1}$, leaf area and grain yield recorded for plants subjected to highest dose of applied N fertilizer at 100 kg ha^{-1}.

Sultana *et al*. (2009) reported that application of 20 kg N ha^{-1} as basal dose and 20 kg N ha^{-1} with one weeding at vegetative stage showed significantly higher values of all growth parameters like leaf area, shoot dry weight, number of branches, pods plant-1 and seed yield. Asaduzzaman et al. (2008) Application of 30 kg N ha^{-1} as basal with one irrigation at flower initiation stage (35 DAS) significantly improved dry matter accumulation. This greater dry matter production eventually partitioned to pods per plant, seeds per plant and 1000-seed weight which is get her resulted with maximum seed yield per plant (5.53 g) or per hectare (1.65 t). A functional positive relationship was observed in with pods per plant and seeds per plant. Sultana et al. (2008) reported that the highest grain yield was obtained in 30 kg N ha^{-1} due to improvement of yield components. The lowest grain yield was obtained in the no nitrogen i.e. control treatment. Asaduzzaman (2006) found that plant height and number of leaves per plant of mungbean was significantly increased by the application of nitrogen fertilizer at 30 kg ha^{-1}.

Sultana (2006) noticed that plant height of mungbean showed superiority at 30 kg N ha^{-1} followed by 40 kg N ha^{-1}. Nitrogen fertilizer significantly influenced plant height at all growth stages of mungbean. At 20, 35, 50, 65 DAS and 10 harvest the maximum heights were observed in the plants treated with 30 kg N ha^{-1}. Mungbean genotypes require additional N for better pod development although it is capable to fix atmospheric N through rhizobium species living in root nodules (Anjumet al., 2006).

Oad and Buriro (2005) conducted a field experiment to determine the effect of different NPK levels (0-0-0, 10-20-20, 10-30-30, 10-30-40 and 10-40-40 kg/ha) on the growth and yield of mungbean cv. AEM 96 in Tandojam, Pakistan, during the spring season of 2004. The different NPK levels significantly affected the crop parameters. The 10-30-30 kg NPK/ha was the best treatment, recording plant height of 56.3. germination of 90.5%. satisfactory plant population of 162.0. prolonged days taken to maturity of 55.5. long pods of 5.02 crn, seed weight of 10.5 g, seed index of 3.5 g and the highest seed yield of 1205.2 kg/ha. There was no significant change in the crop parameters beyond this level. Ghosh (2004) used different levels of nitrogen and indicated that number of branches $plant^{-1}$ of mungbean was gradually increased with increasing N level at 25 kg N ha^{-1}.

Nadeem et al. (2004) studied the response of mungbean cv. NM-98 to seed inoculation and different levels of fertilizer (0-0, 15-30, 30-60 and 45-90 kg NP2O5 ha^{-1}) under field conditions. Application of fertilizer significantly increased the yield and the maximum seed yield was obtained when 30 kg N ha^{-1} was applied along with 60 kg P2O5 ha^{-1}. Masud (2003) observed that highest plant height of mungbean with the application of 30 kg N ha^{-1} while Ghosh (2004) at 25 kg N ha^{-1}. Mozumder et al. (2003) also stated that application of 40 kg N ha^{-1} gave the highest seed yield of mungbean.11 Malik et al. (2003) conducted an experiment to determine the effect of varying levels of nitrogen (0, 25 and 50 kg ha^{-1}) and phosphorus (0, 50, 75, and 100 kg ha^{-1}) on the yield and quality of mungbean cv. NM-98. Growth and yield components were significantly affected by varying levels of nitrogen and phosphorus. A fertilizer combination of 25 kg N + 75 kg ha^{-1} resulted with maximum seed yield (1112.96 kg ha^{-1}). Rajander et al. (2003) investigated the effects of N (0, 10, 20 and 30 kg ha^{-1}) and P (0, 20, 40 and 60 kg ha^{-1}) fertilizer rates on mungbeangenotypes MH 85- 111 and T44. They observed grain yield increased with increasing N rates up to 20 kg ha^{-1}.

Mahboob and Asghar (2002) studied the effect of seed inoculation at different nitrogen levels on the yield and yield components of mungbean at the agronomic research station, Farooqabad in Pakistan during the year of 2000 and 2001. They revealed that with the application of NPK at the rate of 50-50-0 kg ha^{-1} significantly affected the 1000 grain weight. Rudreshhappa and Halikatti (2002) explained the effect of N levels (0, 12.5 and 25 kg) on growth, yield and nutrient uptake of green gram in paddy fallows. Application of 12.5 kg N ha^{-1} was recorded to produce significantly higher seed yield. Further increase in N doses (25 kg ha^{-1}) did not significantly increase the yield. Srinivas et al. (2002) examined the effect of nitrogen (0, 20, 40 and 60 kg ha^{-1}) and P (0, 25, 50 and 75 kg ha^{-1}) on the growth and seed yield of mungbean. They observed that the number of pods per plant was increased with the increasing rates of N up to 40 kg ha^{-1} followed by a decrease with further increase in N.

Pathak et al. (2001) evaluated the effect of N levels (0, 10, 20 and 30 kg ha^{-1}) on growth and yield of mungbean under rainfed condition during the summer of 1999 and found that application of 20 kg N ha^{-1} yielded poorer than 30 kg N ha^{-1}. 12 Ashraf (2001) found that number of pods per plant, seeds per pod, 1000-seed weight were significantly affected by the application of nitrogen from 20 to 50 kg ha-1. Tariq et al. (2001) found that application of 30: 30.8: 58.10 kg ha-1 N-P-K enhanced production of pods $plant^{-1}$, 1000-seed weight and gain the highest grain yield (876.32 kg ha^{-1}) of mungbean. Hamid (1999) revealed the effects of foliar application of nitrogen on mungbean cv. Mubarik. In both pot and field trials he showed 10 kg N ha^{-1} increased the number of pods $plant^{-1}$. Mandal and Sikdar (1999) laid out a greenhouse pot experiment where mungbean (BARI Mung-5) grown on saline soil and given 0, 50 or 100 kg N ha-1 and 0, 75 or 150 kg P ha^{-1}. Growth and yield increased significantly with N application while P significantly increased the setting of pods and seeds. Root growth was significantly improved by both individual and combined application of these two fertilizers. Mozumder (1998) studied the effect of five N levels (0, 20, 40, 60 and 80 kg N ha^{-1}) and two varieties of summer mungbean, BINA Mung-2 and Kanti, found that N exerted negative effect on the harvest index. In an experiment with the foliar application of nutrients on the growth and yield of mungbean cv. Kowmy-1.

Abd-El-Latif et al. (1998) revealed that application of urea increase the number of branches $plant^{-1}$ on mungbean plant. Akhtaruzzaman (1998) conducted a field experiment on mungbean where plant height increased almost linearly up to 40 kg N ha^{-1} although response of 30 and 40

kg N ha^{-1} was identical. Karle and Power (1998) examined the effect of varying levels of N and P fertilizers on summer mungbean. They reported that mungbean produced higher seed yield with the application of 15 kg N ha^{-1} and 40 kg P2O5 ha-^{1}. 13 Provorov et al. (1998) observed the effect of seed inoculation of mungbean with strain CIAMI 901 of Bradyrhizobium and found that the seed yield was increased by 39.2% and 1000 seed weight 16%. These results were equivalent to applying 120 kg N ha^{-1}. Best results obtained with inoculations + 60 kg N ha^{-1}. Patra and Bhattacharyya (1997) observed that the highest seed yield and yield components were obtained by applied urea at the rate of 25 kg N ha^{-1}. In a field experiment conducted by Satyanarayanammaet al. (1996), five mungbean cultivars were sprayed with 2% urea at pre-flowering, flowering, pod development or at all the combinations or at combination of two of three growth stages. They reported that spraying urea at flowering and pod development stages produced the highest seed yield. Kaneria and Patel (1995) conducted a field experiment on mungbean cv. K 581 using 0 or 20 kg N ha^{-1} levels. They found that application of 20 kg N ha^{-1} significantly increased the seed yield. Bachchhavet al. (1994) who found that application of 30 kg N ha^{-1} resulted in highest seed yield of mungbean. Quah and Jafar (1994) noted that 1000 seed weight of mungbean increased significantly with 40 kg N ha^{-1}. Santos (1993) carried out an experiment on mungbean cv. Berken grown in pots in podzolic soil with 7 levls of N (0,25,50,100,200,400,500 $kgha^{-1}$), applied as NH4NO3 and noted that application of N up to 200kg ha^{-1} increased the total dry matter, higher rates decreased it. Patel et al. (1993) studied that, in summer season on clayey soil application of 0, 10, 20 and 30 kg N ha^{-1} significantly increased the number of pods plant-1. 14 Ardeshana et al. (1993) conducted a field experiment on response of mungbean to nitrogen. Seed yield increased with the application of nitrogen fertilizer up to 20 kg N ha^{-1} in combination with phosphorus fertilizer up to 40 kg P2O5 ha^{-1}. Gopala et al. (1993) found that the response of mungbean cultivars (PusaBaishakhi, LGG 407, LGG 410 and MS 267) to a uniform dose of 20 kg N ha^{-1} and found that plant height, net assimilation rate (NAR), crop growth rate (CGR), relative growth rate (RGR) were increased at 20 kg N ha^{-1}. Chowdhury and Rosario (1992) studied the effect of 0, 30, 60 or 90 kg N ha^{-1} levels on the rate of growth and yield performance of mungbean at los Banos, Philippines in 1988. They observed that N above the rate of 30 kg N ha^{-1} reduced the dry matter yield. Tank et al. (1992) reported that mungbean fertilized with 20 kg N ha^{-1} along with 75 kg P2O5 ha^{-1} significantly increased the number of pods per plant. Agbeninet al. (1991) carried out a field

experiment under glass house condition and found that nitrogen application significantly increased the dry matter yield of mungbean. Suhartatik (1991) also reported that NPK fertilizers significantly increased the plant height of mungbean. Sarkar and Banik (1991) conducted a field experiment to evaluate the effect of varying rates of N on mungbean. Results revealed that application of 10 kg N ha^{-1} resulted in the appreciable improvement in different yield attributes along with number of pods per plant and 1000 seed weight over control. Result also showed that application of N along with P significantly increased the seed yield of mungbean. The maximum seed yield was obtained with the combination of 20 kg N and 60 kg P2O5 ha^{-1}. Leelavathi et al. (1991) reported that different levels of N showed significant difference in seed yield of mungbean up to a certain level.15 Different varieties of mungbean differed significantly with respect to plant height was reported by Thakuria and Shaharia (1990). Increase in plant height of mungbean at higher nitrogen levels may be ascribed to increase of N in chlorophyll which increased photosynthesis and enhanced meristematic activity of plant (Sawwaret al., 1989). Hamid (1988) conducted a field experiment to investigate the effect of nitrogen and carbon on the growth and yield performance of mungbean (Vigna radiate L. wilczek). He found that the plant height of mungbean cv. to be increased with nitrogen at 40 kg ha^{-1}. Pongkao and Inthong (1988) applied N at the rate of 0-60 kg ha^{-1} on mungbean and reported that application of 15 kg N ha^{-1} was found to be superior giving 23% higher seed yield over the control. Mahmoud and Gad (1988) observed that application of N increased the stover yield up to a certain level under different row spacing of mungbean. Samiullah et al. (1987) recorded that number of seeds pod^{-1} were the highest with 10 kg N + 75 kg P2O5 + 60 kg K2O in summer mungbean. Patel and Parmer (1986) observed that increasing N application to rainfed mungbean (Vigna radiata cv. Gujrat-1) from 0-45 kg ha^{-1} increase average seed yield from 0.83 to 0.94 t ha^{-1} and also increased protein content, plant height, number of branches $plant^{-1}$, pods $plant^{-1}$, seeds $plant^{-1}$ and 1000 seed weight. Patel et al. (1984) showed that increased in the dose of nitrogen from 20 to 40 kg ha^{-1} at flowering improved grain yield from 39 to 89 percent over control. It is interesting to note that half dose of 20 kg ha^{-1} of nitrogen applied at sowing and remaining at the time of flowering gave higher yield than the application of 40 kg N ha^{-1} as basal in mungbean. It was also found that application of 30 kg N ha^{-1} along with 40 kg P2O5 ha^{-1} significantly increased the number of pods per plant.16 Raju and Verma (1984) conducted a field experiment on response of mungbean var. Pusabaishaki to varying levels of nitrogen (15, 30, 45 and 60 kg N ha^{-1}) in the

presence and absence of seed inoculation with Rhizobium. They found that maximum dry matter weight per plant was obtained by the application of 60 kg N ha^{-1} inoculated with Rhizobium.They also reported that application of 15-60 kg N ha^{-1} significantly increased seed yields of mungbean. Trung and Yoshida (1983) conducted a field trial on mungbean in nutrient-rich soil, involving 0-100 ppm N as treatments. They observed that maximum plant height at all the stages of plant growth were obtained by the application of 25 ppm N. In an experiment, Yein et al. (1981) applied nitrogen and phosphorus fertilizers to mungbean and reported that combined application of nitrogen and phosphorus fertilizers increased the number of pods per plant. The rate of nitrogen and phosphorus was 50 kg and 75 kg per hectare, respectively.

2.17 Effect of naphthalene acetic acid (NAA) on the morphlogical and physiological characteristics

2.17.1 Plant height

Bakhsh *et al.* (2011) studied the effect of NAA on transplanted coarse rice and concluded that plant height was found maxiumum at 90 ml followed by 60 and 120 ml ha^{-1} over control. Adam and Jahan (2011) reported that application of NAA at 200 ppm produced the highest plant height in BRRI dhan 29 and BRRI dhan 50.

Samuszzaman (2004) conducted an experiment to investigate the effect of NAA and GABA (a mixture of GA_3 and ABA) on growth and yield contributing characteristics of groundnut and found that plant height was increased significantly with the application of 100 mg L^{-1} and 200 mg L^{-1} NAA. Abro *et al.* (2004) reported that application of Planofix (Naphthalene Acetic Acid) significantly increased the plant height in cotton.

Mondal (2003) carried out an experiment in the farm of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh to investigate the effect of NAA and IBA on growth, yield and yield contributing characters of groundnut var. Binachinabadam-1 and found that application of 80 ppm NAA significantly increased plant height. Deotale*et al.* (1998) studied the effect of GA_3 and NAA on growth parameters of soybean and obtained the highest plant height with 100 mg L^{-1} NAA. Kelaiya*et al.* (1991) conducted an experiment with four growth regulators such as, CCC (chlormequat), NAA, was found to be most effective increasing plant height.

2.17.2 Number of leaves $plant^{-1}$

Conducting an experiment on the effect of NAA and GABA on growth and yield contributing characters. Samsuzzaman (2004) found that application of 100 mg L^{-1} and 200 mg L^{-1} NAA significantly increased the number of leaves $plant^{-1}$ in groundnut.

Das and Prasad (2003) conducted a study on sandy clay loam soil in New Delhi, India, during summer season. The treatment comprised of three mungbean cultivars and two levels (20 ppm and 40 ppm) of NAA significantly increased the number of leaves.

Mahla *et al.* (1999) reported that spraying 20 ppm NAA on blackgram had greater effect in increasing the number of leaves and brances. Deotale*et al.* (1998) studied the effect of GA_3 and NAA on growth parameter of soybean and obtained highest number of leaves plant $^{-1}$ 100 mg L^{-1} NAA. Singh *et al.* (1983) observed that two foliar spray of 100 ppm NAA to groundnut at 40-50 days after sowing increased the number of leaves $plant^{-1}$. Reddy and Shah (1984) observed that application of NAA at a rate of 50ppm to groundnut significantly increased the number of leaves.

2.17.3 Leaf area $plant^{-1}$

Mondal (2003) reported that leaf area index was significantly increased with the application of 80 ppm NAA on groundnut. Deotale*et al.* (1998) studied the effect of GA_3 and NAA on growth parameters of soybean and obtained the highest leaf area with 100 mg L^{-1} NAA. Leaf area index is a measure of leafiness and photosynthetic surface area of a crop. depends of leaf growth, number of leaves, mode of branching and leaf senescence (Khan, 1981),. Kelaiya*et al.* (1991) worked with four growth regulators such as CCC (chlormequate), NAA, GA_3 and Tercentennial along with water sprayed at 25, 50 and 75 DAS on ground nut and found that among the four growth regulators, NAA was most effective to increase leaf area index.

2.17.4 Dry weight $plant^{-1}$

Azad (2002) conducted a pot culture experiment in Bangladesh Institute of Nuclear Agriculture (BINA) and found that application of 50 ppm NAA significantly increased the dry root weight $plant^{-1}$. Wang and Deng (1992) observed that spraying with 100 and 150 ppm NAA at tillering stage significantly increased dry root weight. NAA also promoted new root growth.

2.17.5 Yield

Udensi *et al.* (2013) stated that plants rasied from pigeon pea seeds soaked in 100 and 150 mg L^{-1}paclobtazol + NAA excellently increased the yield. Bakhsh *et al.* (2011) studied the effect of NAA on transplanted coarse rice and concluded that seed yield was found maximum at 90 ml followed by 60 and 120 ml ha^{-1} over control. Aslam *et al.* (2010) conducted a study on the effect of NAA application on Chickpea applied at 200 ml ha^{-1} in three split doses at 45, 90 and 135 DAS and observed that the yield was increased 13.98% over control.

Imam *et al.* (2010) observed that the interaction of plant growth regulator (NAA) and phosphorus level had significantly beneficial effect on the yield attributes of rice. Reddy *et al.* (2009) reported that application of NAA increased the yield and yield components of rice. Abro *et al.* (2004) reported that the application of Planofix (Naphthalic Acetic Acid) significantly increased the volume of boll and yield in cotton. Das and Prasad (2003) conducted a study on sandy clay loam soil in New Delhi, India, during summer season. The treatments comprised of three mungbean cultivars and two levels of NAA (20 ppm and 40 ppm) sprayed at 30 DAS and at flowering stages. Both the concentrations of NAA significantly increased the grain yield in summer mungbean.

Alam *et al.* (2002) stated that application of 20 ppm NAA enhanced the straw and grain yield of three cultivars of wheat. Khanzada *et al.* (2002) stated the application of NAA on growth parameters of soybean and obtained the highest seed yield with 100 mg L^{-1} NAA. Shukla *et al.* (1997) concluded that a double spray of growth regulators increased the yield by 17.7% in soybean over the control.

Kalita *et al.* (1995) carried out an experiment with NAA on Vigna radiata cv AAU- 34 and found that the treatment combination of 3% P_2O_5 + 100 ppm NAA produced highest number of pods $plant^{-1}$ and seeds pod^{-1}. Upadhyay *et al.* (1993) sprayed 0, 10, 20 or 30 ppm NAA at bud initiation and pod formation stages of chickpea (*Cicer arietinum*). The highest seed yield of 2.35 t ha^{-1} was resulted from the treatment of 20 ppm NAA. Nawalagatti*et al.* (1991) reported that spraying of 10 or 20 ppm Planofix (NAA) on groundnut ev. DH 3-30 at 45 DAS increased seed yield from 1.76-2.22 t ha^{-1} was resulted from the treatment. Mahla *et al.* (1999) showed that *Vigna mungo* sprayed with 2 ppm Mixtato and 20 ppm NAA increased nodule, yield components

and seed yield. Growth, yield components and seed yield were the best with joint application of two growth regulators. Tripathy *et al.* (1994) reported that yields of groundnuts cv. IGGV-88079, TSG-JL-24 and TG-24 were 1.56, 1.66 and 1.82 t ha^{-1} respectively. Average yield (1.62 t ha^{-1}) was resulted without application of growth regulators while 1.83, 1.77 and 1.72 t ha^{-1} were found respectively with Planofix (NAA) applied at 30 and 60 days after sowing.

Foysal Kabir *et al.* 2016 reported thatplant growth regulator plays an important role of crops yield especially in mungbean. The experiment consists of four levels of NAA viz., 0, 20, 40 and 60 ppm and three different spacing *viz*., 20 cm × 10 cm, 30 cm × 10 cm and 40 cm × 10 cm. The results indicated significant variations in number of pod $plant^{-1}$, pod length, number of seed pods 1, 1000 seeds weight, seed yield, stover yield, biological yield and harvest index due to plant growth regulator (NAA) and/or row spacing. The maximum 1000 seeds weights, seed yield and harvest index were found when mungbean was sown with row spacing in 30 cm × 10 cm in combination with 40 ppm NAA. Therefore, yield of mungbean can be improved by applying NAA and row spacing.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted during March 2021 to September 2021 at the experimental farm of the Bangladesh Sugarcrop Research Institute (BSRI), Regional Sugarcrop Research Station, Thakurgaon. Materials required and methods followed in the experiment during the study period was presented in this chapter.

3.1 Description of experimental site

3.1.1 Location: Geographically the experimental field is located at 25^0 38' N latitude and 88^0 41' E longitude at a height of 34.5 m above the mean sea level. The experiment was conducted during March 2021 to September 2021 at the experimental farm of the Bangladesh Sugarcrop Research Institute (BSRI), Regional Sugarcrop Research Station, Thakurgaon.

3.1.2 Soil and land: The experiment was laid out in farm field soil having good internal drainage. The Agroecological Zone belongs to the AEZ No.1, Old Himalayan Piedmont Plain. The soil is sandy loam, a member of hyperthermic Aeric Haplaquept under the order Inceptisol having only few horizons, developed under acquired moisture regime and variable temperature conditions, Agro ecological Appraisal of Bangladesh, (UNDP and FAO, 1988). According to Fertilizer Recommendation Guide (2018) general characteristics of the soil and chemical characteristics of initial composite soil sample (0-15 cm depth), which were collected on February 2021 for initial status and teste.

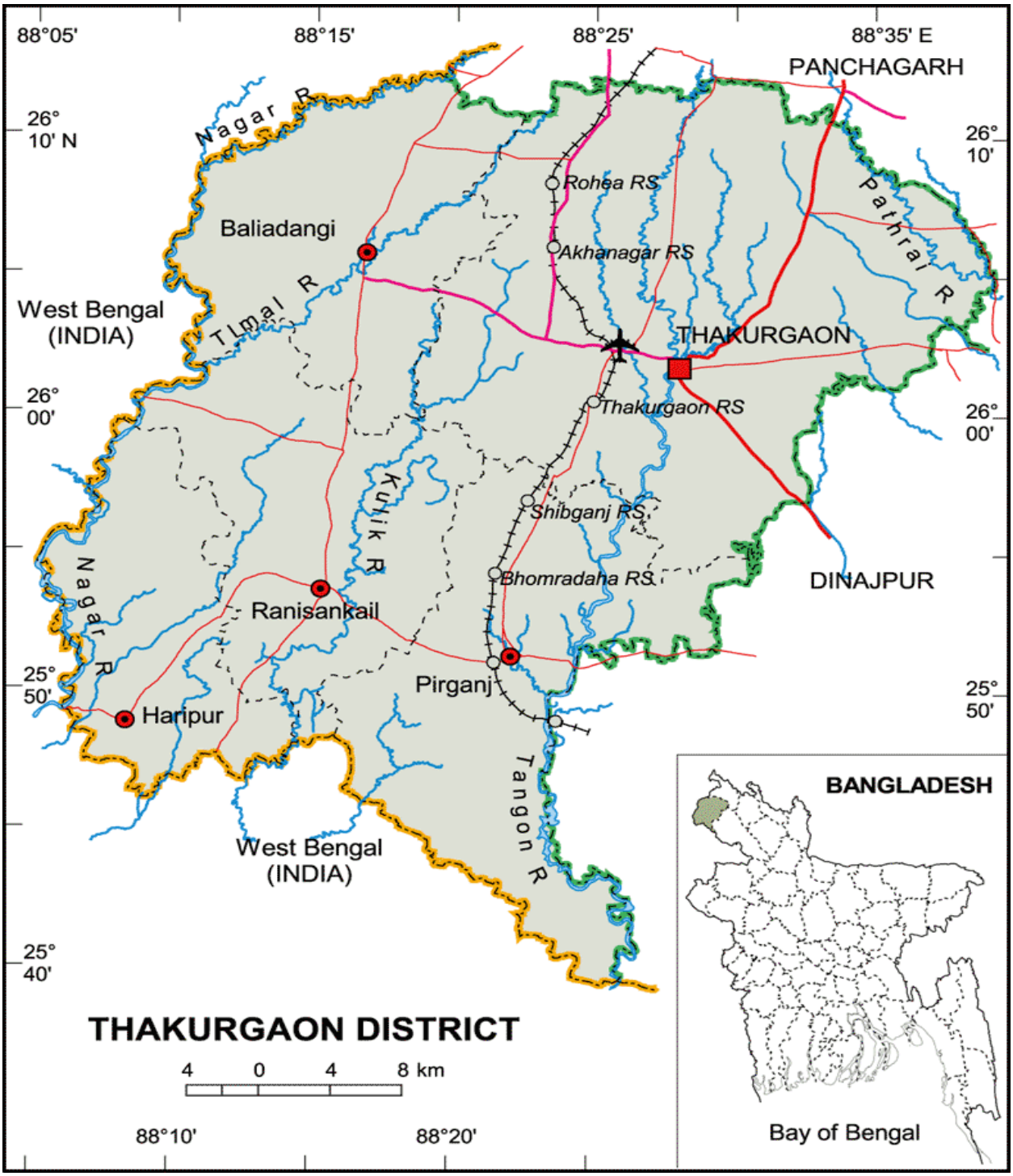


Figure 3.1 Map of Thakurgaon district showing the research conducting area

3.1.3 Climate and weather: The maximum, minimum and mean air temperature (ºC), relative humidity (%), total rainfall (mm), sunshine (hour's month^{-1}) and mean monthly Pan evaporation (mm) during the experimental period are shown in Table 3.3. The minimum temperature ranged from 17.60 to 26.60^{0}C, while the maximum temperature ranged from 31.70 to 34.40^{0}C with the mean temperature range from 24.65 to 29.99^{0}C. The maximum rainfall occurred in August, 2021 and while minimum rainfall was recorded in April, 2021 and no rainfall in March, 2021. Higher mean humidity was recorded in the month of August, 2021 followed by July, 2021 and September, 2021 and lowest in April 2021. Highest sunshine hour month^{-1} (7.48 hrs) was recorded in the month of April, 2021, while the lowest in (3.6 hrs) was in the month of August and September, 2021. Highest mean monthly Pan Evaporation (4.73 mm) was recorded in the month of April, 2021 and the lowest in (2.81 mm) was in the month of August, 2021.

Table 3.1 Climate Morphological, Physical and chemical characteristics of soil

Morphological description of soil

AEZ	AEZ 1 (Old himaqlayan piedmont Plain)
General soil type	Calcareous Brown Flood Plain
Parent material	Ganges River Alluvium
Soil series	Sara
Soil type	Sandy loam
Land type	High land, medium high land
Drainage	Moderate

Physical and chemical properties of the experimental site

Constituents	Value
Physical characteristics	
% Sand (2 – 0.05 mm)	60.0
% Silt (0.05 – 0.002 mm)	27.0
% Clay (< 0.002 mm)	13.0
Textural class	Sandy loam
Chemical characteristics	
pH (Soil: Water = 1: 2.5)	5.65
Organic carbon (%)	1.10
Total N (%)	0.07
Available P (mg kg^{-1})	37.56
Available S (mg kg^{-1})	18.42
Exchangeable K (meq 100 g^{-1} soil)	0.21
Available Zn (mg kg^{-1})	1.93
Available S (mg kg^{-1})	18.42
Available B (mg kg^{-1})	0.15
Available Mg (mg kg^{-1})	0.48

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Table 3.2 Meteorological data of the experimental period (March to September 2021) at BSRI, Farm, Thakurgaon.

Month	Year	** Air temperature (°C)			** Relative Humidity (%)	* Total Rainfall month^{-1} (mm)	Sunshine hours month^{-1}	Mean monthly Pan evaporation (mm)
		Maximum	Minimum	Average				
March	2021	31.7	17.6	24.65	70.4	00	7.42	4,22
April	2021	34.4	20.8	27.6	67.25	19.2	7.48	4.73
May	2021	32.1	23.4	27.75	78.67	337.6	4.87	3.78
June	2021	33.1	25.5	29.8	81.83	246.4	4.91	3.73
July	2021	33.3	26.6	29.95	82.88	181	4.5	3.08
August	2021	33.20	26.56	29.88	86.83	407	3.6	2.81
September	2021	33.96	26.02	29.99	82.23	144	4.07	3.72

* Monthly total

** Monthly average

Source: Bangladesh Sugarcrop Research Institute (BSRI), Ishurdi, Pabna.

3.2 Test crop

The test crop was stevia. The stevia seedlings (Figure 3.2) were collected from Bangladesh Stevia and Food Industries Limited, Dhaka and Bangladesh Sugarcane Research Institute, Regional Sugarcrop Research Station, Thakurgaon, Bangladesh. The seedlings were healthy and vigorous in growth 10-12 cm in height 30 days old.

Figure 3.2 Stevia seedling

3.3 Land preparation

3.3.1 Field planting

The land was opened by a tractor drawn disc plough and final preparation was made by ploughing and cross-ploughing with a tractor plough and harrow followed by laddering. The layout of the field was made 7 march 2021 after final land preparation.

Total TSP were applied (50 kg ha^{-1}) as basal dose during final soil preparation. The basal dose of urea (1/3 rd) was applied as side dressing at 30 days after transplanting. The rest amount of urea and MOP were applied (40 kg ha^{-1}) as top dressing in two equal splits at 60 and 90 DAT.

3.4 Experimental design

The experiment was laid out in a Randomized Complete Block Design (RCBD), factor A: (Nitrogen) N_1: control, N_2: 105 kg ha-1, N_3: 140 kg ha-1, N_4: 175 kg ha-1, N_5: 210 kg ha-1. factor and B: (NAA) H_1: control, H_2: 50 ppm, H_3: 100 ppm and H_4: 150 ppm. The layout of the experiment was prepared for distributing the treatment combinations in each plot of each block. There were 60 plots in total. The unit plot size was 2m×2m. Row to row distance 40 cm, plot to plot distance 50 cm and plant to plant distance 40cm.

3.5 Application of nitrogen

All plants require nitrogen for healthy growth and reproduction. More importantly, plants use nitrogen for photosynthesis. While native plants are better adapted to their surroundings and oftentimes less affected by nitrogen deficiency. Five levels of nitrogen are applied in the form of urea. These are respectively N_1: control, N_2: 105 kg ha^{-1}, N_3: 140 kg ha^{-1}, N_4: 175 kg ha^{-1}, N_5: 210 kg ha^{-1}. The basal dose of nitrogen (1/3 rd) was applied as side dressing at 30 days after transplanting. The rest amount of nitrogen were applied as top dressing in two equal splits at 60 and 90 DAT.

3.6 Application of NAA

Napthelic Acitic Acid spray was sprayed using the concentration of H_1: control, H_2: 50 ppm, H_3: 100 ppm and H_4: 150 ppm at monthly intervals from 15 days after transplanting up to 90 days. The crop leaves was harvested after 6 months of transplanting.

3.7 Intercultural operations

Intercultural operations like weeding, irrigation drainage etc. was done as and when necessary considering the present situation of the field. On an average weeding was done 15–20 days interval and flooding irrigation was applied at 7–10 days intervals considering rainfall. Insects and pest infestation of stevia plant in this period was trace.

3.8 Gap filling

Dead settlings were replaced by fresh settlings within 15 days after transplanting.

3.9 Harvest

The crop harvested 147 days after transplanting when it attained maturity. The vegetative part of the plant especially laves were plucked carefully and washed briefly to removed soils and other foreign materials. The fresh leaves were than weighted as plant^{-1}.

3.10 Collection of experimental data

Data were recorded on the following parameters

1. Plant height (cm)
2. Number of leaves plant^{-1}
3. Leaf area plant^{-1} (cm)
4. Number of primary branch plant^{-1}
5. Number of secondary branch plant^{-1}
6. Fresh weight plant^{-1} (g)
7. Dry weight plant^{-1} (g)
8. Fresh leaf yield plant^{-1} (g)
9. Dry leaf yield plant^{-1} (g)
10. Stevia Leaves (N, P, K, S, Ca, Mg%, and Zn µg g^{-1})
11. Initial and post hervest soil

3.11 Procedure of data collection

3.11.1 Plant height (cm)

Plant height was measured from the base of the plant (ground level) to the tip of the upper most leaf and was expressed in cm. It was done just before harvesting.

3.11.2 Number of leaves plant^{-1}

Number of leaves of each plant was counted by hand counting and recorded it.

3.11.3 Leaf area plant^{-1} (cm)$^{-2}$

Leaf area of all separated leaves from each plant was measured with the help of leaf area meter at the physiology and sugar chemistry laboratory, BSRI, Ishurdi, Pabna. It was performed soon after harvesting to avoid curling of the leaves.

3.11.4 Number of branch plant^{-1}

The number of branches of each plant was counted by hand counting and recorded it. It was performed at the time of height measurement.

3.12. Analysis of soil sample

3.12.1 Preparation of soil sample

The collected soil a sample were composite and was air-dried, ground and sieved through a 2-mm sieve and analyzed for soil texture, soil pH, organic carbon, CEC, total N, available S, P, exchangeable K and Zn.

3.12.2 Particle size analysis

Particle size analysis of the collected soils was done by hydrometer method (Black, 1965) and textural classes were identified by plotting the values for % sand, % silt and % clay to the "Marshall's Triangular Coordinate" following the USDA system (Black, 1965).

3.12.3 Soil pH

Soil pH was measured using a glass electrode pH meter (WTW pH 522) at a soil-water ratio of 1:2.5 as described by Ghosh *et al.*, 1983. Twenty grams of air dried soil was taken in a plastic container and 50 ml of distilled water was added to it. The suspension was stirred well several times and allowed to stand for about an hour. Then the electrode was immersed into the partly settled soil suspension and pH was measured.

3.12.4 Organic carbon

Organic carbon in soil was determined volumetrically by wet oxidation method of Walkley and Black (1975). The organic matter content was calculated by multiplying the percent organic carbon by 1.73 (Van Bemmelen factor).

3.12.5 Cation exchange capacity

Cation exchange capacity of the soil was determined by sodium saturation method. The sample was saturated with 1 N NaOAc solution followed by replacing Na^+ from the saturated samples

by 1 N NaOAc at pH 7.0. The amount of Na^+ in the solution was then determined by flame photometer (Gruba and Mulder, 2015).

3.12.6 Available phosphorus

Available soil P was extracted with 0.5 M $NaHCO_3$ at a pH 8.5. The P in the extract was determined by $SnCl_2$ method. The intensity of blue color of molybdophosphate blue complex was measured with the help of spectrophotometer (Supertonic® GENESYS ^{TM} 5 336001 CAT) set at 660 nm (Olsen *et al.*, 1954).

3.12.7 Exchangeable potassium content

Exchangeable K was extracted with 1 N NH_4OAc solution. Then K was determined directly with the help of flame emission spectrophotometer (Jenway PFP 7) using specific standard.

3.12.8 Available sulphur

Sulphur was determined by turbidimetric method with the help of a spectrophotometer (Wolf, 1982). $CaCl_2$ solution (0.15%) was used as soil extractant. Twenty gram soil was taken in a 250 ml conical flask and 40 ml $CaCl_2$ solution was added. After 30 minutes shaking, the contents were filtered through filtered through filter paper. About 10 ml extract was taken in tube, 1 ml acid seed solution was added and 0.5 g $BaCl_2$ $2H_2O$ was added and mixed thoroughly. The intensity of colors were read in a spectrophotometer at 420 nm wave length after 20 minutes.

3.13 Plant analysis

The collected plant sample from each plot pot^{-1} was dried in an at 60^0C for about 48 hours and they were ground to pass through a 20-mesh sieve in a grinding mill. The prepared sampled were then put into paper bags and kept in desiccators until use.

3.13.1 Nitrogen determination

The estimation of N was done by Micro- kjeldahl method (Bremner and Mulvaney, 1982), which depends on the fact that organic N, when digested with concentrated sulphuric acid was converted into ammonium sulphate. Ammonia liberated by making the solution alkaline was distilled into a known volume of standard boric acid, which is then back titrated.

Reagents

- Mixed indicator: 0.099 g of bromocresol green and 0.065 g of methyl red was dissolved in 100 ml of ethanol (rectified spirit).
- Boric acid (H_3BO_3) indicator solution: 20g of boric acid was dissolved to make to volume 1 L with distilled water

- 40% 1N sodium hydroxide (NaOH) solution: 40 g of NaOH was dissolved in distilled water to make the volume 1 L.
- 0.1N Concentrate sulphuric acid (H_2SO_4)
- Catalyst mixture: $CuSO_4.5H_2O$: K_2SO_4: Se = 1: 5: 0.05

Procedures

The method consists of the following steps:

- Digestion of the sample
- Distillation and
- Titration

Portion of 0.5 g oven dried grind sample was wrapped in a piece of qualitative filter paper and dropped as a package into an 500 mL kjeldahl flask in presence of 5 g potassium sulphate, 1 g copper sulphate and 15 mL concentrated H_2SO_4 and 2 glass beads in the digestion tube. The sample mixture was heated at 390^0C for an hour. After the completion of digestion the flask was cooled at room temperature and added about 25 mL of distilled water. Then the flask was swirled to bring any insoluble material into the solution and it was made volume to 100 mL. For performing distillation 10 mL of the digested solution was taken in a distillation unit with 10 mL of 40% NaOH. The distillate was collected in 25 mL 2% boric acid containing mixed indicator to adjust the pH at 5.0 and was titrated against 0.1N sulphuric acid. A blank was simultaneously to avoid the N either already present in chemicals or atmospheric nitrogen absorbed during digestion. The percentage was calculated by the following formula with the help of titration value:

$\% N = (T-B) \times 0.014 \times 100 / S$

Where,

T = Sample titration (ml) value of standard H_2SO_4

B = Blank titration (ml) value of standard H_2SO_4

N = Strength of H_2SO_4S = Sample weight (g)

3.13.2 Preparation of leaf sample:

Exactly 1.0 g of finely ground leaves were taken into a 250 ml conical flask and 10 ml of di-acid mixture (HNO_3:$HCIO_4$ = 2:1) was added to it. Then it was placed on an electric hot plate for heating at 180-200^0C until the solid particles disappeared and white fumes were evolved from

the flask. Then it was cooled at room temperature, washed with distilled water and filtered into 100 ml volumetric flask through filter paper Whatman No. 1 making the volume up to the mark with distilled water following wet oxidation method as described by Jackson (1973). The solution was used for the analysis of P, K, Ca, Mg, Zn, B, Cu and Na.

3.13.3 Phosphorus content

Phosphorus was determined colorimetrically by stannous chloride method. Stannous chloride ($SnCl_2$. H_2O) was used as a reducing agent to form molybdophosphoric blue complex with sulphomolybdate. Exactly 10 ml aliquot was in a 50 ml volumetric flask followed by the addition of 10 ml of sulphomolybdic acid and and 2 ml of stannous chloride solution. The volume was made up to the mark with distilled water and was shaken thoroughly. Finally the intensity of blue color was measured with the help of spectrophotometer (Supertonic® GENESYS ^{TM} 5 336001 CAT) at 660 nm within 15 minutes after the addition of stannous chloride reagent (Jackson, 1973).

3.13.4 Potassium content

Potassium content of the leaf sample was determined by flame photometer and the intensity of light emitted by potassium at 768 nm wave lengths measured by Jackson (1973).

3.13.5 Sulphur content

Sulphur content was determined turbidimetrically as $BaSO_4$ from leaf sample with the help of a spectrophotometer (Supertonic® GENESYS ^{TM} 5 336001 CAT). Turbidity was developed by using barium chloride ($BaCl_2$. $2H_2O$) and the solution was transferred to a spectrophotometer tube. The reading was taken in spectrophotometer at 420nm incident light within 2 to 8 minutes as described by Black (1965).

3.13.6 Calcium and Magnesium contents

Five ml leaf sample was transferred into 50 ml volumetric flask using a pipette and 5 ml of $LaCl_3$ solution was added. The volume was made up to the mark with distilled water and was shaken thoroughly. Then the contents of Ca and Mg were measured by Atomic Absorption Spectrometer (AAS).

3.13.7 Zinc content

From the leaf extract, Zn content was directly analyzed by atomic absorption spectrophotometer.

3.14 Statistical analysis

Analysis of variance was done following the Completely Randomized Design (CRD) with the help of computer package program M-STAT developed by Russell (1986). The mean differences of the treatments adjusted by Least Significant Difference (LSD) test.

CHAPTER IV

RESULTS AND DISCUSSION

The results of the studies on nitrogen and napthelic acitic acid on growth, yeild and nutrient content of steviaand its interaction effects have been presented experiment according to the following headings and sub headings. This chapter contains results and discussion on the basis of data presented in tables and figures. Results were presented on each data of growth, yield and nutrient element content parameters.

4.1 Plant height

Plant height increased gradually with advancement of the growth stage (21 DAT to 147 DAT) of the stevia plants (Table 4.1 and Appendix I). Significant variations (P<0.05) were observed in plant height in different level of nitrogen application. The highest plant heights (24.87, 37.59, 49.33, 62.89, 72.16, 85.10 and 96.44 cm) were observed applying N_3 (nitrogen140 kg ha^{-1}) at 21 DAT to 147 DAT, respectively, The lowest plant height was observed in 23.62, 35.86, 47.60, 60.22, 68.41, 81.11 and 94.23cm in N_1 (control) treatment at 21 DAT and 147 DAT, respectively.The results was in agreement with the findings of Hawke (2003) who observed that the mature stevia plant height 65-180 cm when cultivated in field condition. Zaman *et al.,* (2015b) found that significantly influenced grown in stevia at different soil type of Bangladesh and reported that stevia plant height varies from 75.33 to 91.33 cm. Lokesh *et al.,* 2018 reported that the plant height was significantly influenced by nitrogen levels. At harvest, those plants applied with 120 Kg N ha^{-1} recorded maximum plant height (53.59 cm) compared to lower nitrogen level 60 Kg N ha^{-1}. But these levels of nitrogen hinder the growth and plant height decreased in stevia. Similar results were reported by Chung *et al*., 1992 in tomato. The positive influence of nitrogen on plant height might be due to the fact that nitrogen is required for cell division and cell elongation which triggers the growth of meristamatic tissue and the efficient utilization of this by the plants manifested in production of taller plants. Similar observations were also made by Raghuraja (1992) in Gailardia pulchella. The results are in agreement with the findings of Laura *et al*., (2011) in french basil

Table 4.1 Effect of N and NAA on plant height of stevia and their interaction

Treatments	Plant height (cm)						
Nitrogen	21DAT	42 DAT	63DAT	84DAT	105DAT	126DAT	147DAT
N1	23.62ab	35.86ab	47.60ab	60.22ab	68.41ab	81.11 b	94.23ab
N2	23.88ab	36.76a	48.31ab	61.48ab	69.34ab	82.11ab	94.98ab
N3	24.87a	37.59a	49.33a	62.89a	72.16a	85.10a	96.44a
N4	23.99ab	36.80a	48.13ab	62.18a	69.86ab	82.87ab	95.31a
N5	23.65ab	36.65a	47.80ab	60.91ab	68.85ab	81.45ab	94.54ab
LSD (0.05)	2.266	2.58	3.014	3.707	3.789	3.71	3.45
NAA	-	-	-	-	-	-	-
H1	20.49c	33.97b	43.33c	56.94c	64.59c	75.22c	91.97b
H2	23.35b	36.98a	47.43b	60.48b	69.85b	82.44b	95.26a
H3	26.89a	38.92a	52.14a	65.510a	73.83a	89.14a	97.71a
H4	25.27ab	37.05a	50.04ab	63.18ab	70.62ab	83.31b	95.46a
LSD (0.05)	2.027	2.309	2.696	3.315	3.389	3.32	3.09
Interaction	-	-	-	-	-	-	-
N1H1	20.18c	34.12c	42.80d	55.34e	63.45d	74.38h	91.45b
N1H2	22.87a-c	36.76ab	46.65b-d	58.34b-e	68.29b-d	80.23e-h	94.56ab
N1H3	26.76 a	38.67ab	51.28ab	64.63a-c	72.38a-c	88.29a-d	96.28ab
N1H4	24.65a-c	36.87ab	49.67a-c	62.57a-e	69.52a-d	81.52c-h	94.66ab
N2H1	20.43c	34.54bc	43.83cd	57.35c-e	64.32 d	74.40h	91.50b
N2H2	23.12a-c	36.87ab	47.78a-d	60.47a-e	69.39a-d	82.46b-g	95.26ab
N2H3	26.87a	38.72ab	51.90ab	65.21ab	73.78a-c	88.82a-c	97.84ab
N2H4	25.10ab	36.92ab	49.72a-c	62.87a-d	69.88a-d	82.74b-g	95.32ab
N3H1	21.12bc	35.43a-c	44.35cd	58.32b-e	66.71cd	77.37f-g	93.28ab
N3H2	24.65a-c	37.56ab	48.17a-d	62.37a-e	72.39a-c	85.35a-e	96.47ab
N3H3	27.15a	38.78a	53.45a	66.54a	75.87a	90.47a	98.80a
N3H4	26.56a	37.60ab	51.35ab	64.31a-c	73.67a-c	87.22a-e	97.24ab
N4H1	20.54c	34.56bc	42.85d	57.46c-e	64.37 d	75.55gh	92.16ab
N4H2	23.18a-c	36.94ab	47.80a-d	61.95a-e	70.36a-d	83.25a-f	95.34ab
N4H3	26.88a	38.74ab	52.10ab	66.03a	74.34ab	89.38ab	98.35ab
N4H4	25.34ab	36.96ab	49.76a-c	63.19a-d	70.38a-d	83.30a-f	95.41ab
N5H1	20.19c	34.20bc	42.82d	56.23de	64.10 d	74.39h	91.46b
N5H2	22.92a-c	36.79ab	46.73b-d	59.29a-e	68.83a-d	80.89d-h	94.70ab
N5H3	26.80a	38.70ab	51.95ab	65.14ab	72.80a-c	88.75a-c	97.32ab
N5H4	24.70a-c	36.90ab	49.68a-c	62.97a-d	69.65a-d	81.77c-h	94.69ab
LSD (0.05)	4.533	5.163	6.028	7.415	7.579	7.426	6.912

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.
N1: Control, N2: 105 kg ha-1, N3: 140 kg ha-1, N4: 175 kg ha-1, N5: 210 kg ha-1, H1: control, H2: 50 ppm, H3: 100 ppm and H4: 150 ppm

Plant height was significantly influence by the application of different concentration of NAA at all growth stage of stevia (Table 4.1 and Appendix I). Applying 100 ppm NAA had the significant effect in increasing height at 21 DAT, 42 DAT, 63 DAT, 84 DAT, 105 DAT, 126 DAT and 147 DAT (26.89, 38.92, 52.14, 65.51, 73.83, 89.14 and 91.97 cm) in H_3(100 ppm NAA) at 21DAT to 147 DAT, respectively and the lowest plant height was observed in control (NAA). Pahare and Das 2020 reported that Alpha- Napthelic Acetic Acid (NAA) 25 ppm greatly enhanced the plant height in Vinca rosea cv. *Catharanthus caramel*.

The intraction effect of nitrogen and different concentration of NAA were also ststisticaly significant at all growth stages (Table 4.1 and Appendix I). The highest plant heights (27.15, 38.78, 53.45, 66.54, 75.87, 90.47 and 98.80 cm) were found N_3H_3 (nitrogen 140 kg ha^{-1} × NAA 100 ppm) treatment. The lowest plant heights (20.18, 34.12, 42.80, 55.34, 63.45, 74.38, and 91.45 cm) were foundin N_1H_1(with no nitrogen application / control × with no NAA application / control) at all growth stages. Noor-E-Ferdous *et al.*, 2021 reported that mature stevia plant height 112.31 cm when cultivated in field condition. Gupta *et al.*, 2022 reported that plant height had positive intraction effect on of nitrogen 130 kg ha^{-1} and NAA 150 ppm in summer onion.

4.2 Number of leaves plant^{-1}

Number of leaves plant^{-1} differed significantly among the different level of nitrogen anddifferent concentration of NAA application at 21 DAT to 147 DAT (Table 4.2 and Appendix II). Nitrogen140 kg ha^{-1} (N_3) had the highest number of leaves plant^{-1}*i.e.*18.87, 45.96, 223.67, 508.86, 777.67, 965.26 and 1106.20, respectively which were statistically different among other level of nitrogen application. Significantly the lowest number of leaves plant^{-1} was observed in N_1 (control) treatment. It was reportrd that (17.46, 42.75, 210.37, 497.20, 763.69, 910.65 and 1091.10) number of leaves plant^{-1}at 21 DAT 147 DAT, respectively (Table 4.2 and Appendix II). Noor-E-Ferdous *et al.*, 2021 observed that number of leaf plant^{-1} (1215.32) was recorded in field condition.

Number of leaves plant^{-1} was significantly influence by the application of different level of NAA applicationat all growth stage of stevia (Table 4.2 and Appendix II). The concentration of 100 ppm NAA produced significantly the highest number of leaves (19.82, 47.03, 233.07, 522.21, 817.83, 1000.60 and 1144.10) was obtained in H_3 (NAA 100 ppm) treatmentat 21DAT to 147 DAT, respectively. Significantythe lowest number of leaves plant^{-1} was observed in H_1 (control)

treatment. Table 4.2 reported that the lowest number of leaves plant^{-1}was observed in (16.83, 40.24, 194.34, 471.22, 689.88, 879.60 and 1052.70) at 21 DAT to 147 DAT, respectively.

Table 4.2 Effect of N and NAA on number of leaves plant^{-1} of stevia and their interaction

Treatments	Number of leaves plant^{-1}						
Nitrogen	21DAT	42 DAT	63DAT	84DAT	105DAT	126DAT	147DAT
N1	17.46c	42.75c	210.37b	497.20c	763.69c	910.65b	1091.10c
N2	17.91bc	43.64bc	214.14b	502.55b	772.37ab	911.43b	1094.70bc
N3	18.87a	45.96a	223.67a	508.86a	777.67a	965.26a	1106.20a
N4	18.18b	44.49ab	216.73ab	504.88b	774.56a	916.54b	1098.60b
N5	17.53bc	42.95c	212.46b	498.54c	767.07bc	905.98b	1092.00c
LSD (0.05)	0.664	1.523	7.706	3.467	5.750	11.382	6.475
NAA	-	-	-	-	-	-	-
H1	16.83c	40.24c	194.34c	471.22c	689.88d	879.60c	1052.70d
H2	17.67b	43.83b	215.22b	506.96b	779.25c	901.80b	1090.90c
H3	19.82a	47.03a	233.07a	522.21a	817.83a	1000.60a	1144.10a
H4	17.63b	44.73b	219.28b	509.24b	797.34b	905.90b	1098.50b
LSD (0.05)	0.594	1.362	6.893	3.101	5.143	10.180	5.791
Interaction	-	-	-	-	-	-	-
N1H1	16.45e	38.89h	191.78g	465.67k	681.28h	866.14g	1048.79g
N1H2	16.97de	42.28e-g	205.47d-g	500.43h	766.78e	881.24d-g	1082.36f
N1H3	19.38ab	46.65a-c	231.67a-c	519.35bc	815.15a	998.86a	1141.29a
N1H4	17.04de	43.18d-g	212.57de	503.36gh	791.54b-d	896.35cd	1091.92d-f
N2H1	16.8de	40.18gh	193.48g	471.41jk	691.55f-h	869.23fg	1051.53g
N2H2	17.29c-e	43.17d-g	212.29de	508.23e-g	782.36d	886.71d-g	1087.64d-f
N2H3	19.84a	47.11ab	232.48a-c	521.38a-c	817.28a	998.97a	1143.19a
N2H4	17.66c-e	44.12b-e	218.33b-d	509.16d-g	798.31b	890.82d-f	1096.53c-e
N3H1	17.28c-e	42.59e-g	197.35e-g	478.92i	697.56f	917.82c	1061.34g
N3H2	19.35ab	46.82ab	230.52a-c	514.63c-e	791.46b-d	964.23b	1107.45bc
N3H3	20.46a	47.60a	235.57a	526.34a	820.35a	1006.34a	1145.46a
N3H4	18.39bc	46.84ab	231.26a-c	515.57cd	801.32b	972.64b	1110.46b
N4H1	17.02de	40.84f-h	196.63fg	473.22ij	693.42fg	876.35d-g	1052.25g
N4H2	17.86cd	44.27b-e	217.36cd	510.51d-f	785.41cd	892.46de	1093.39d-f
N4H3	20.02a	47.13ab	233.47ab	523.48ab	819.24a	999.82a	1148.74a
N4H4	17.82cd	45.75a-d	219.44b-d	512.33d-f	800.19b	897.53cd	1100.13b-d
N5H1	16.55de	38.72h	192.46g	466.89jk	685.57gh	868.28fg	1049.36g
N5H2	16.91de	42.63e-g	210.45d-f	500.98h	770.23e	884.52d-g	1083.64ef
N5H3	19.40ab	46.68a-c	232.17a-c	520.52a	817.13a	998.87a	1141.71a
N5H4	17.26c-e	43.77c-f	214.78f-h	505.76bc	795.34bc	872.24e-g	1093.35d-f
LSD (0.05)	1.328	3.047	15.414	6.934	11.500	22.763	12.950

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.
N1: Control, N2: 105 kg ha-1, N3: 140 kg ha-1, N4: 175 kg ha-1, N5: 210 kg ha-1, H1: control, H2: 50 ppm, H3: 100 ppm and H4: 150 ppm

The intraction effect of nitrogen and NAA in most of the combination should significantly the highest number of leaves at all growth stages (Table 4.2 and Appendix II). From the Table 4.2, it was found that the highest number of leaves plant^{-1} (20.46, 47.60, 235.57, 526.34, 820.35, 1006.34 and 1145.46) at 21 DAT to 147 DAT, respectivelywas found in N_3H_3 treatment (nitrogen 140 kg ha^{-1} × NAA 100ppm). Significantly the lowest number of leavesplant^{-1} was observed in N_1H_1 (control) treatment. It was found in number of leavesplant^{-1} (16.45, 38.89, 191.78, 465.67, 681.28, 866.14 and 1048.79) at 21 DAT to 147 DAT, respectively.

4.3 Leaf area plant^{-1} of stevia

Effects of different level of nitrogen application was significantly in leaf area plant $^{-1}$ of stevia at 21 DAT to 147 DAT (Table 4.3 and Appendix III). The highest leaf area plant $^{-1}$(59.64, 774.26, 1151.40, 2512.40, 3237.70, 3861.20 and 4227.60 cm^2) was observed in N_3 (140 kg ha^{-1}) at 21 DAT to 147 DAT, respectively. The lowest leaf area plant^{-1} (54.32, 743.30, 1088.20, 2353.10, 3045.60, 3717.10 and 4038.50 cm^2) was observed in N_1 (control) treatment at 21 DAT 147 DAT, respectively.

Leaf area plant^{-1} was significantly influence by the application of different of NAA at all growth stage of stevia (Table 4.3 and Appendix III). The concentration of 100 ppm NAA produced the highest leaf area plant^{-1}(59.27, 811.30, 1209.60, 2594.10, 3285.40, 3901.30 and 4273.50cm^2) was found in H_3 (NAA 100 ppm) at 21 DAT to 147 DAT, respectively. Significantly the lowest leaf area plant^{-1} (53.69, 677.65, 1016.40, 2239.40, 2944.90, 3645.50 and 3988.60 cm^2) was found in H_1 (control) treatmentat 21 DAT to 147 DAT, respectively.

A significant variation was found leaf area plant^{-1} at 21 DAT to 147 DAT by the interaction effect of nitrogen and different concentration of NAA (Table 4.3 and Appendix III). The highest leaf area plant (62.65, 821.76, 1231.57, 2610.33, 3336.42, 3941.64 and 4451.96 cm^2) was found N_3H_3 treatment (nitrogen 140 kg ha^{-1}) × NAA 100 ppm). The lowest leaf areaplant^{-1}(52.37, 672.81, 1010.36, 2153.37, 2871.91, 3617.21 and 3978.21 cm^{-2}) was found in N_1H_1treatment at all growth stages. Zaman *et al.,* 2015b reported that the area of total leaves plant-1 was significantly affected by different soil types. Maximum leaf area (2010 cm^2 plant^{-1}) was measured from the plant grown in non-calcareous soil which was statistically identical with the leaf area of the plants grown in acid (1865 cm2 plant^{-1}) and calcareous (1555 cm2 plant-1) soils.

Noor-E-Ferdoud *et al.*, 2021 that the highest number of leaf was recorded in field cultivation (1215.32) and the lowest number of leaf pot cultivation (659.73).

Table 4.3 Effect of N and NAA on Leaf area plant^{-1} of stevia and their interaction

Treatments	Leaf area plant^{-1} (cm^{-2})						
Nitrogen	21DAT	42 DAT	63DAT	84DAT	105DAT	126DAT	147DAT
N1	54.32c	743.30b	1088.20c	2353.10e	3045.60d	3717.10d	4038.50d
N2	56.01b	766.10a	1100.50b	2444.50c	3093.20c	3739.80c	4135.30c
N3	59.64a	774.26a	1151.40a	2512.40a	3237.70a	3861.20a	4227.60a
N4	56.73b	768.73a	1106.10b	2464.70b	3146.30b	3750.30b	4180.40b
N5	55.01c	745.93b	1091.30c	2375.80d	3042.10d	3720.50d	4050.10d
LSD (0.05)	0.996	9.048	8.674	11.357	7.882	5.213	21.910
NAA	-	-	-	-	-	-	-
H1	53.69c	677.65d	1016.40d	2239.40d	2944.90d	3645.50d	3988.60d
H2	55.79b	767.75c	1097.00c	2432.30c	3071.60c	3736.30c	4111.90c
H3	59.27a	811.30a	1209.60a	2594.10a	3285.40a	3901.30a	4273.50a
H4	56.61b	781.96b	1107.10b	2454.70b	3150.10b	3747.90b	4131.60b
LSD (0.05)	0.891	8.093	7.759	10.158	7.050	4.663	19.597
Interaction	-	-	-	-	-	-	-
N1H1	52.37i	672.81h	1010.36h	2153.37j	2871.91k	3617.21k	3978.21h
N1H2	53.92f-i	738.31g	1067.46g	2318.81h	2981.29i	3680.52h	4016.62gh
N1H3	56.28de	800.27b-e	1192.38cd	2567.93cd	3247.88d	3883.23d	4124.37f
N1H4	54.72e-h	761.82f	1082.72fg	2372.44g	3081.44f	3687.62h	4034.81g
N2H1	53.28g-i	678.91h	1016.28h	2276.82i	2918.42j	3628.91j	3982.81h
N2H2	55.27e-g	782.55e	1082.27fg	2426.19f	3051.27g	3710.29g	4142.75ef
N2H3	59.24bc	812.45ab	1211.24b	2602.82ab	3275.35c	3891.42b-d	4236.59c
N2H4	56.27de	790.51c-e	1092.36f	2472.19e	3127.57e	3728.62ef	4178.92de
N3H1	55.28ef	682.33h	1023.26h	2314.36h	3127.88e	3719.55fg	4014.66gh
N3H2	59.92bc	792.11c-e	1172.28e	2561.72d	3241.65d	3884.90d	4217.28cd
N3H3	62.65a	821.76a	1231.57a	2610.33a	3336.42a	3941.64a	4451.96a
N3H4	60.71ab	800.83b-d	1178.37de	2563.29d	3244.71d	3898.53bc	4226.54c
N4H1	54.72e-h	680.26h	1019.31h	2282.10i	2921.26j	3641.72i	3987.11h
N4H2	55.65ef	784.43de	1092.53f	2483.26e	3072.28f	3723.28f	4162.25ef
N4H3	60.26b	816.28ab	1214.29ab	2603.28ab	3310.35b	3901.22b	4392.10b
N4H4	56.29de	793.95c-e	1098.35f	2490.17e	3281.30c	3734.81e	4180.24de
N5H1	52.82hi	673.94h	1012.57h	2170.18j	2884.93k	3620.35jk	3980.23h
N5H2	54.19f-i	741.36g	1070.42g	2371.52g	3011.35h	3682.62h	4020.52gh
N5H3	57.96cd	805.76a-c	1198.44bc	2586.26bc	3256.82d	3888.94cd	4162.47ef
N5H4	55.05e-g	762.68f	1083.58fg	2375.17g	3015.36h	3689.91h	4037.25g
LSD (0.05)	1.993	18.097	17.350	22.714	15.765	10.428	43.819

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.
N1: Control, N2: 105 kg ha-1, N3: 140 kg ha-1, N4: 175 kg ha-1, N5: 210 kg ha-1, H1: control, H2: 50 ppm, H3: 100 ppm and H4: 150 ppm

4.4 Number of primary branches plant^{-1} of stevia

Significantly difference was observed on number of primary branchplant^{-1}of stevia among different level of nitrogen application (Table 4.4 and Appendix IV). It was observed that highest number of primary branch plant^{-1}(4.57, 6.20, 8.01, 9.11, 10.13, 11.66 and 13.02) was found in N_3 (nitrogen 140 kg ha^{-1}) at 21 DAT to 147 DAT, respectively. The lowest number of primary branch (4.40, 5.73, 7.64, 8.65, 9.74, 11.00 and 12.77) was observed in N_1 (control) treatment at 21 DAT 147 DAT, respectively. Lokesh *et al.,* 2018 reported that the production of number of branches per plant increased significantly with the optimum level of nitrogen dose. Among different levels of nitrogen, 120 Kg ha^{-1} had the highest (15.81) number of branches per plant. The production of more number of branches might be due to the fact that in the initial stages of growth the number of branches produced per plant was minimum and as the growth proceeds, production of more branches occurs. This could be discribed to the availability of optimum quantity of nitrogen during vegetative growth and split application influenced the availability of nitrogen.

Effects of different level of NAA application was significantly in number of primary branch plant^{-1} of stevia at 21 DAT to 147 DAT (Table 4.3 and Appendix IV). The concentration of 100 ppm NAA produced the highest number of primary branch (4.99, 6.63, 8.53, 9.26, 10.52, 12.27 and 13.15) was observed in H_3 treatment at 21 DAT to 147 DAT, respectively. The lowest number of primary branch plant^{-1} (4.00, 5.45, 6.96, 8.38, 9.31, 10.44 and 12.55) was found in H_1 (control) treatment (without NAA) at 21DAT to 147 DAT, respectively.

Number of primary branches plant^{-1} of stevia was significant different in different level nitrogen and NAA application at all growth stages (Table 4.4 and Appendix IV). The highest number of primary branch plant^{-1} (5.10, 6.78, 8.56, 9.34, 10.65, 12.37 and 13.2) was found N_3H_3 treatment (nitrogen 140 kg ha^{-1} × NAA 100 ppm). The lowest number of primary branch (3.97, 5.22, 6.88, 8.16, 9.11, 10.22 and 12.40) was found in N_1H_1 treatment at 21 DAT to 147 DAT, respectively. Zaman *et al.,* 2015b reported that different types of soil significantly influenced the number of branches of stevia plant and maximum number of branches plant^{-1} was counted from the plant grown in non-calcareous soil (9.33) which was identical with the branch number of the plant

grown in acid soil (8.67) but statistically different from all those plants grown in other soils of the study.

Table 4.4 Effect of N and NAA on number of primary branches of stevia and their interaction

Treatments	Number of primary branches plant^{-1}						
Nitrogen	21DAT	42 DAT	63DAT	84DAT	105DAT	126DAT	147DAT
N1	4.41b	5.73b	7.64ab	8.65b	9.74ab	11.00ab	12.77ab
N2	4.47ab	5.84b	7.82ab	8.81ab	9.83ab	11.37ab	12.86ab
N3	4.57a	6.20a	8.01a	9.11a	10.13a	11.66a	13.02a
N4	4.46ab	5.84b	7.84ab	8.84ab	9.87ab	11.41a	12.87ab
N5	4.43b	5.79b	7.66ab	8.69ab	9.78ab	11.04ab	12.79ab
LSD (0.05)	0.141	0.200	0.261	0.419	0.354	1.146	0.896
NAA	-	-	-	-	-	-	-
H1	4.01c	5.45c	6.96c	8.38c	9.32b	10.44b	12.55ab
H2	4.42b	5.71b	7.79b	8.80b	9.77b	11.19b	12.85ab
H3	4.99a	6.63a	8.53a	9.26a	10.52a	12.27a	13.15a
H4	4.46b	5.75b	7.88b	8.84b	9.87b	11.28ab	12.90ab
LSD (0.05)	0.126	0.179	0.413	0.374	0.585	1.025	0.801
Interaction	-	-	-	-	-	-	-
N1H1	3.97d	5.22e	6.88f	8.16d	9.11d	10.22ab	12.40ab
N1H2	4.32bc	5.57de	7.53b-f	8.64a-d	9.67a-d	10.76ab	12.76ab
N1H3	4.94a	6.52a-c	8.51a	9.12a-c	10.40a-d	12.17a	13.11a
N1H4	4.40b	5.62de	7.65a-f	8.70a-d	9.79a-d	10.86ab	12.83ab
N2H1	4.00d	5.50de	6.97ef	8.34cd	9.20cd	10.45ab	12.56ab
N2H2	4.45b	5.68d	7.85a-e	8.78a-d	9.75a-d	11.34a	12.85ab
N2H3	4.98a	6.60ab	8.53a	9.30a	10.52ab	12.28a	13.15a
N2H4	4.47b	5.61de	7.92a-c	8.82a-d	9.84a-d	11.43a	12.88ab
N3H1	4.06cd	5.70d	7.10cd-f	8.85a-d	9.86a-d	10.76ab	12.79ab
N3H2	4.54b	6.12c	8.16ab	9.12a-c	9.98a-d	11.73a	13.03a
N3H3	5.10a	6.78a	8.56a	9.34a	10.65a	12.37a	13.21a
N3H4	4.60b	6.23bc	8.23ab	9.14a-c	10.05a-d	11.80a	13.07a
N4H1	4.01d	5.46de	6.99d-f	8.40b-d	9.25b-d	10.50ab	12.58ab
N4H2	4.42b	5.61de	7.90a-d	8.80a-d	9.78a-d	11.38a	12.86ab
N4H3	5.00a	6.65a	8.53a	9.32a	10.59a	12.30a	13.17a
N4H4	4.43b	5.64d	7.94a-c	8.84a-d	9.88a-d	11.46a	12.90ab
N5H1	3.99d	5.37de	6.90f	8.19d	9.16cd	10.28ab	12.43ab
N5H2	4.35b	5.58de	7.55b-f	8.67a-d	9.68a-d	10.78ab	12.77ab
N5H3	4.97a	6.60ab	8.52a	9.22ab	10.46a-c	12.23a	13.12a
N5H4	4.42b	5.63d	7.68a-f	8.71ab-d	9.82a-d	10.89ab	12.86ab
LSD (0.05)	0.282	0.401	0.923	0.838	1.308	2.292	1.793

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.
N1: Control, N2: 105 kg ha-1, N3: 140 kg ha-1, N4: 175 kg ha-1, N5: 210 kg ha-1, H1: control,

H2: 50 ppm, H3: 100 ppm and H4: 150 ppm

4.5 Number of secondary branches plant^{-1} of stevia

Number of secondary branches plant^{-1} of stevia was significantly increased gradually with advancement of the growth stage at 21, 42, 63, 84, 105, 126 and 147 DAT, respectively with different level of nitrogen application (Table 4.5 and Appendix V). The highest number of secondary branch plant^{-1} (11.15, 13.73, 17.09, 26.74, 30.53, 33.50 and 37.77) was found in N_3(140 kg ha^{-1}) at 21 DAT to 147 DAT, respectively. The lowest number of primary branch plant^{-1} (9.72, 13.10, 16.77, 25.99, 29.54, 32.91 and 36.84) was observed in N_1 (control) treatment and observed in at 21, 42, 63, 84, 105, 126 and 147 days after transplanting, respectively. Noor-E-Ferdous *et al.*, 2021 reported that the highest secondary branches (12.92, 13.45, 24.28, 26.91, 28.48 and 31.83) at 42, 63, 84, 105, 126 and 147 DAT, respectively was observed in field planting compaired to pot planting in stevia.

Number of secondary branchplant^{-1} was significantly influence by the application of different of NAA at all growth stage of stevia (Table 4.5 and Appendix V). The concentration of 100 ppm NAA produced the highest number ofsecondary branch (11.54, 14.35, 17.47, 27.08, 30.57, 34.26 and 38.90) was observed in H_3treatment at 21 DAT to 147 DAT, respectively. The lowest number of secondary branch (9.26, 12.52, 16.36, 25.64, 28.87, 32.16 and 35.28) was reported that H_1 (control) with no NAA applicationat 21, 42, 63, 84, 105, 126 and 147 days after transplanting, respectively.

The intraction effect of nitrogen and NAA in most of the combination should significantly the highest number of secondary branch at all growth stages (Table 4.5 and Appendix V). The highest number of secondary branch plant^{-1} (11.87, 14.94, 17.75, 27.34, 31.76, 34.64 and 39.12) was found in N_3H_3 (nitrogen 140 kg ha^{-1} × NAA100 ppm). The lowest number of secondary branch (8.90, 12.52, 16.36, 25.64, 28.87, 32.16 and 35.28) was observed in N_1H_1 treatment at all growth stages.

The formation of secondary and tertiary branches is directly related to total dry weight yield. According to Pal *et al.* (2015), the growth of lateral branches in stevia is associated with the development of the root system. This author reported that more developed root systems

synthesize larger amounts of cytokines, which are phytohormones responsible for stimulating lateral branch formation. Changes in the nutritional balance of stevia G8 and decreased photoperiods impair dry weight yield, so plants have reduced root system development and lower cytokine production (Carvalho *et al.*, 1998).

Table 4.5 Effect of N and NAA on number of secondary branches of stevia and their interaction

Treatments	Number of secondary branches						
Nitrogen	21DAT	42 DAT	63DAT	84DAT	105DAT	126DAT	147DAT
N1	9.72b	13.10b	16.78b	25.99b	29.54b	32.92ab	36.84b
N2	9.88b	13.21ab	16.89a	26.14b	29.79b	33.05a	36.99b
N3	11.15a	13.73a	17.09a	26.74a	30.53a	33.50a	37.78a
N4	9.96b	13.27ab	16.93a	26.18b	29.67b	33.09a	37.04ab
N5	9.83b	13.13b	16.79a	26.03b	29.17b	32.96ab	36.89b
LSD (0.05)	0.381	0.531	0.121	0.535	0.626	0.765	0.762
NAA	-	-	-	-	-	-	-
H1	9.26c	12.59c	16.45c	25.78b	28.99c	32.27c	35.54c
H2	9.72b	13.07b	16.78bc	25.98b	29.67b	32.90bc	36.85b
H3	11.54a	14.36a	17.47a	27.08a	30.57a	34.26a	38.90a
H4	9.91b	13.13b	16.88b	26.04b	29.74b	32.98b	37.15b
LSD (0.05)	0.341	0.475	0.377	0.478	0.560	0.684	0.681
Interaction	-	-	-	-	-	-	-
N1H1	8.90c	12.52g	16.36e	25.64f	28.87d	32.16d	35.28f
N1H2	9.27c	12.87e-g	16.67c-e	25.73ef	29.38cd	32.67cd	36.64c-f
N1H3	11.17ab	14.08a-d	17.29a-d	26.84a-d	30.47bc	34.10a-c	38.72ab
N1H4	9.54c	12.94e-g	16.78b-e	25.78d-f	29.45cd	32.73b-c	36.75c-f
N2H1	8.95c	12.58fg	16.44e	25.68ef	28.96d	32.24d	35.40d-f
N2H2	9.43c	12.97e-g	16.80b-e	25.85c-f	29.47cd	32.86b-d	36.81c-e
N2H3	11.53a	14.28ab	17.48a-c	27.14ab	31.17ab	34.16a-c	38.91a
N2H4	9.63c	13.01e-g	16.86b-e	25.92c-f	29.57cd	32.93b-d	36.86cd
N3H1	10.42b	12.74e-g	16.60de	26.22b-f	29.18d	32.46d	36.26c-f
N3H2	11.15ab	13.59b-f	16.96a-e	26.69ab-f	30.58a-c	33.38a-d	37.27bc
N3H3	11.87a	14.94a	17.75a	27.34a	31.76a	34.64a	39.12a
N3H4	11.17ab	13.66b-e	17.08a-e	26.74a-e	30.61a-c	33.52a-d	38.46ab
N4H1	9.12c	12.60e-g	16.48de	25.72ef	29.06d	32.28d	35.45d-f
N4H2	9.48c	13.04d-g	16.82b-e	25.91c-f	29.50cd	32.90b-d	36.84cd
N4H3	11.61a	14.36ab	17.57ab	27.16ab	30.55a-c	34.21ab	38.97a
N4H4	9.64c	13.10c-g	16.88b-e	25.96c-f	29.60cd	32.97b-d	36.89cd
N5H1	8.93c	12.55fg	16.38e	25.66f	28.90d	32.21d	35.31ef
N5H2	9.30c	12.91e-g	16.69c-e	25.76ef	29.41cd	32.70b-d	36.67c-f
N5H3	11.55a	14.13a-c	17.30a-d	26.92a-c	28.92d	34.19a-c	38.80a
N5H4	9.56c	12.95e-g	16.81b-e	25.79d-f	29.46cd	32.76b-d	36.78c-f
LSD (0.05)	0.763	1.063	0.843	1.070	1.252	1.530	1.524

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.
N1: Control, N2: 105 kg ha-1, N3: 140 kg ha-1, N4: 175 kg ha-1, N5: 210 kg ha-1, H1: control, H2: 50 ppm, H3: 100 ppm and H4: 150 ppm

4.6 Nutrient content in stevia leaf

4.6.1 Nitrogen

Nitrogen (N) content of stevia was significantly influenced by the application of different levels of nitrogen (Table 4.6 and Appendix VI). The highest N content (3.04%) was observed in (N_3) when the plot was treated with nitrogen140 kg ha^{-1}. The lowest N content (2.74%) was recorded in control (N_1) plot which was significantly inferior to all treatments (Table 4.6 and Appendix VI). Present findings agree with Katayama *et al.,*1976 who also obtained stevia plants consist of 1.4% N. Noor-E-Ferdous *et al.,* 2021 observed that the N content in stevia plant ranged from 1.62 to1.71%. The results indicated that N content of stevia was also significantly affected by different levels of NAA. Significantly the highest N content (3.15%) was observed when the plot was treated with 100 ppm NAA (H_3). The lowest N content (2.63%) was recorded in H_1 treatment. Nitrogen content of stevia was significantly affected by different levels of nitrogen and NAA (Table 4.6and Appendix VI). The highest N content (3.47%) was observed when the plot was treated with nitrogen140 kg ha^{-1}× 100 ppm NAA (N_3H_3). The lowest N content of stevia leaf (2.56%) was recorded in the control plot which was significantly inferior to all treatments. Zaman *et al.,* 2015a reported that different levels of foliar application of urea significantly affected the N content and uptake by stevia leaf at harvest. N content of the leaf was increased with the increased levels of urea irrespective of soils used. N uptake did not follow the trend of N content of stevia leaves. N uptake as expected increased with the increase in foliar application of urea up to 2.0g and then decreased with further addition (U2.5 and U3.0) irrespective of soils used. Both N content and uptake by stevia was higher in acid soil than non- calcareous soil. 46 Response of stevia to foliar application of prilled urea Khan *et al.* (2009) reported that foliar application of urea significantly increased the N uptake by wheat. The foliar spray of 4% urea solution was found to be most effective dose for N uptake by wheat.

4.6.2 Phosphorus

Table 4.6 and Appendix VI shows that phosphorus (P) content of stevia was non significant influenced by the application of different levels of nitrogen. The content of phosphorus in varied from 0.16 to 0.18%. The highest P content (0.18%) was observed when the plot was treated with nitrogen140 kg ha^{-1} (N_3). The lowest Pcontent (0.16%) was recorded in control (N_1) plot which was significantly inferior to all treatments (Table 4.6 and Appendix VI). The results indicated that P content of stevia was also non significant affected by different levels of NAA. The phosphorus was ranged in 0.16 to 0.18%. The highest P content (0.18) was observed when the plot was treated with 100 ppm NAA application (H_3). The lowest P content (0.16%) was recorded in H_1 treatment. Phosphorus content of stevia was non-significant affected by different levels of nitrogen and NAA (Table 4.6 and Appendix VI). The phosphorus content was ranged in 0.15 to 0.19%. The highest P content (0.18%) was observed when the plot was treated with (N_3H_3) nitrogen 140 kg ha^{-1} × 100 ppm NAA level. The lowest P content (0.158%) was recorded in the (N_1H_1) treatment which was non-significant.

4.6.3 Potassium

Significantly the highest K content (0.18%) was observed in N_3(nitrogen140 kg ha^{-1}) treatment andthe lowest K content (0.16%) was recorded in control (N_1) treatment (Table 4.6 and Appendix VI). The results indicated that K content of stevia was also significantly affected by different levels of NAA. Significantly the highest K content (0.18%) was observed when the plot was treated with 100 ppm NAA (H_3) and the lowest N content (0.16%) was recorded in H_1 treatment (Table 4.6 and Appendix VI). Potassium content of stevia was significantly affected by different levels of nitrogen and NAA (Table 4.6 and Appendix VI). The highest K content (0.19%) was observed in (N_3H_3) treatment (nitrogen140 kg ha^{-1} × 100 ppm NAA) and the lowest K content (0.15%) was recorded in (N_1H_1) treatment.

4.6.4 Sulphur

It appears from the data presented in (Table 4.6 and Appendix VI) that sulphurcontent ranged in 0.27 to 0.28% among the different treatment. Sulphur (S) content of stevia was non significant effect influenced by the application of different levels of nitrogen. The results indicated that S

content of stevia was also non- significant affected by different levels of NAA and ranged in 0.26-0.28 (Table 4.6 and Appendix VI). Nitrogen content of stevia was insignificant affected by different levels of nitrogen and NAA (Table 4.6 and Appendix VI). The sulphurcontent was ranged in 0.26 to 0.30% (Table 4.6 and Appendix VI).

4.6.5 Calcium

Table 4.6 and Appendix VI showed that the calcium (Ca) content of stevia was ranged from 1.47 to 1.60% and showed insignificant but the different levels of nitrogen application. The results indicated that Ca content of stevia was not affected by different levels of NAA. The content of calcium in varied from 1.36 to 1.69%. Calcium content of stevia was non-significant affected by different levels of nitrogen and NAA and content of Ca in ranged from 1.31 to 1.72% (Table 4.6 and Appendix VI).

4.6.6 Magnesium

Significantly the highest Mg content (0.14%) was observed when the plot was treated with nitrogen140 kg ha^{-1} (N_3). The lowest magnesium (Mg) content (0.13%) was recorded in control (N_1) plot which was significantly inferior to all treatments (Table 4.6 and Appendix VI). The results indicated that Mg content of stevia was also significantly affected by different levels of NAA. The content of magnesium in varied from 0.12 to 0.15%. The highest Mg content (0.15%) was observed when the plot was treated with 100 ppm NAA (H_3). The lowest Mg content (0.12%) was recorded in H_1 treatment. Magnesium content of stevia was significantly affected by different levels of nitrogen and NAA (Table 4.6 and Appendix VI). The highest Mg content (0.15%) was observed (N_3H_3) treatment (nitrogen140 kg ha^{-1} × 100 ppm NAA) and the lowest Mg content (0.12%) was recorded in(N_3H_3) control treatment.

4.6.7 Zinc

The zinc (Zn) content of stevia have been presented in the (Table 4.6 and Appendix VI). It was significantly effect by the application of different levels of nitrogen. The highest Zn content (71.66%) in was observed in (N_3) treatment (nitrogen140 kg ha^{-1}). The lowest Zn content (56.16%) was recorded in control (N_1) treatments. The results indicated that Zn content of stevia was also significantly affected by different levels of NAA. Significantly the highest Zinc content (76.93%) was observed when the plot was treated with 100 ppm NAA (H_3). The lowest Zinc

content (47.66%) was recorded in H_1 treatment. Zinc content of stevia was significantly affected by different levels of nitrogen and NAA (Table 4.6 and Appendix VI). The highest Zn content (85.18%) was obtainedin (N_3H_3) while nitrogen140 kg ha^{-1} × 100 ppm NAA applications and the lowest (42.48%) was recorted in control (N_1H_1). The results are in agreement with the findings of Nasrin (2008) who reported that the Zn content in stevia leaf (100.46 µg g^{-1}). Noor-E-Ferdous *et al.*, (2021) observed that Zn content in stevia leaf ranged from 55.52 to 62.87%.

Table 4.6 Effect of N and NAA on N, P, K, S, Ca, Mg and Zn of stevia leaf and their interaction

Treatments	N%	P%	K%	S%	Ca%	Mg%	Zn µg g^{-1}
Nitrogen	-	-	-	-	-	-	-
N1	2.74a	0.16	0.16b	0.27	1.47	0.13b	56.16d
N2	2.81ab	0.17	0.16ab	0.28	1.51	0.14b	60.21c
N3	3.04a	0.18	0.18a	0.28	1.60	0.14a	71.66a
N4	2.85ab	0.17	0.17ab	0.28	1.53	0.14ab	62.95b
N5	2.76ab	0.17	0.17b	0.28	1.49	0.13b	57.74d
LSD (0.05)	0.300	NS	0.012	NS	NS	7.186	2.470
NAA	-	-	-	-	-	-	-
H1	2.63b	0.16	0.16c	0.26	1.36	0.12c	47.66d
H2	2.77b	0.17	0.16bc	0.27	1.50	0.14b	58.77c
H3	3.15a	0.18	0.19a	0.29	1.69	0.15a	76.93a
H4	2.81b	0.17	0.17b	0.28	1.54	0.14ab	63.63b
LSD (0.05)	0.269	NS	0.010	NS	NS	6.428	2.209
Interaction	-	-	-	-	-	-	-
N1H1	2.56c	0.16	0.15g	0.26	1.31	0.12g	42.48l
N1H2	2.69bc	0.16	0.16e-g	0.27	1.45	0.13c-f	53.57hi
N1H3	2.98a-c	0.17	0.18a-e	0.29	1.66	0.14a-d	71.36d
N1H4	2.72bc	0.16	0.16c-g	0.28	1.48	0.14b-e	57.24f-h
N2H1	2.61bc	0.16	0.16fg	0.27	1.35	0.12fg	47.42jk
N2H2	2.74bc	0.17	0.16c-g	0.28	1.48	0.14b-d	56.33f-h
N2H3	3.10a-c	0.18	0.19a-c	0.30	1.69	0.14a-c	76.38bc
N2H4	2.78bc	0.17	0.17a-g	0.28	1.52	0.14a-d	60.74f
N3H1	2.75bc	0.17	0.17b-g	0.28	1.42	0.13d-g	55.62gh
N3H2	2.93a-c	0.18	0.18a-f	0.28	1.63	0.15a-c	71.46cd
N3H3	3.47a	0.18	0.19a	0.30	1.72	0.15a	85.18a
N3H4	3.01a-c	0.18	0.18a-e	0.29	1.66	0.15ab	74.38b-d
N4H1	2.64bc	0.16	0.16e-g	0.27	1.37	0.12e-g	49.19ij
N4H2	2.78bc	0.18	0.16b-g	0.28	1.50	0.14a-d	57.84f-h
N4H3	3.19ab	0.18	0.19ab	0.30	1.70	0.15ab	78.55b
N4H4	2.80bc	0.18	0.17a-g	0.28	1.55	0.14a-c	66.25e
N5H1	2.58c	0.16	0.16fg	0.27	1.33	0.12g	43.62kl
N5H2	2.70bc	0.16	0.16d-g	0.27	1.46	0.14b-e	54.66gh
N5H3	3.02a-c	0.17	0.18a-d	0.30	1.67	0.14a-c	73.18cd
N5H4	2.74bc	0.17	0.17b-g	0.29	1.51	0.14b-d	59.52fg

LSD (0.05)	0.601	NS	0.024	NS	NS	0.014	4.939

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.
N1: Control, N2: 105 kg ha-1, N3: 140 kg ha-1, N4: 175 kg ha-1, N5: 210 kg ha-1, H1: control, H2: 50 ppm, H3: 100 ppm and H4: 150 ppm

4.7 Fresh and dry weightof stevia plant

4.7.1. Fresh wt. plant^{-1} (g)

Effects of different level of nitrogen application were significant in fresh weight plant^{-1}of stevia (Table 4.7 and Appendix VII). The highest fresh weight plant^{-1} was observed in N_3(147.24 g) treatment and lowest was observed in N_1(134.12 g) treatment (Table 4.7 and Appendix VII). Significantly highest fresh weight plant^{-1} was observed in H_3 (152.91 g) treatmentand lowest was observed in H_1(122.04 g) treatment (Table 4.7and Appendix VII). The intraction effect of nitrogen and NAA in most of the combination should significantly the highestfresh weight plant^{-1} was observed in N_3H_3 (147.4 g) treatmentand lowest was observed in N_1H_1 (119.62 g) treatment (Table 4.7 and Appendix VII). Zaman *et al.,* 2015b reported that the highest leaf fresh weight (31.09 g) was obtained from the plant grown in non-calcareous soil which was identical with the fresh weight (29.07 g) of the plant grown in acid soil.

4.7.2. Fresh weight ha^{-1} (kg)

Fresh weightha^{-1}of stevia was significant different in different level nitrogen and NAA application (Table 4.7 and Appendix VII). From Table 4.7, it was observed that different level of nitrogen applicationwas signifificant varition infresh weightha^{-1}of stevia. The highest fresh weightha^{-1} was observed in N_3 (9202.80 kg) and lowest was observed in N_1(8380.70 kg). Fresh weightha^{-1} was significantly influence by the application of different concentration of NAA of stevia production (Table 4.7 and Appendix VII). Significantly highest fresh weightha^{-1}was observed in H_3(9556.70 kg) and lowest was observed in H_1(7627.60 kg) treatment (Table 4.7and Appendix VII). The interaction effect of Nitrogen and NAA in most of the combination should significantly the highestfresh wt. ha^{-1} was observed in N_3H_3 (10464.54 kg) and lowest was observed in N_1H_1 (7476.45 kg) treatment (Table 4.7 and Appendix VII).

4.7.3 Dry weight plant^{-1} (g)

Dry weight plant^{-1} differed significantly among the different level of nitrogen and different concentration of NAA application (Table 4.7 and Appendix VII). Significantly highest dry weight plant^{-1} was observed in N_3 (43.98 g) treatment and lowest was observed in N_1 (41.84 g) treatment (Table 4.7 and Appendix VII). Significantly highest dry weight plant^{-1} was

Table 4.7 Effect of N and NAA onfresh wt. plant^{-1}, fresh wt. ha^{-1}, dry wt. plant^{-1} and dry wt. ha^{-1}of stevia and their interaction

Treatment	Fresh wt. plant^{-1} (g)	Fresh wt. ha^{-1} (kg)	Dry wt. plant^{-1} (g)	Dry wt. ha^{-1} (kg)
Nitrogen	-	-	-	-
N1	134.12c	8382.70c	41.84ab	2615.00 c
N2	140.04b	8752.20b	42.79ab	2674.40 bc
N3	147.24a	9202.80a	43.98a	2748.90 a
N4	145.30a	9081.30a	43.23a	2701.90 ab
N5	138.32b	8644.90bc	42.21ab	2638.30 c
LSD (0.05)	3.524	267.28	3.999	60.185
NAA	-	-	-	-
H1	122.04d	7627.60d	39.73b	2483.30 c
H2	141.19c	8824.10c	42.66ab	2666.60b
H3	152.91a	9556.70a	45.65a	2853.00 a
H4	147.88b	9242.70b	43.19ab	2699.90 b
LSD (0.05)	3.152	239.06	3.577	53.831
Interaction	-	-	-	-
N1H1	119.62j	7476.45h	39.27b	2454.65k
N1H2	138.29i	8643.15g	41.26ab	2578.63ij
N1H3	140.11hi	8756.87fg	44.59ab	2786.98b-e
N1H4	138.47hi	8654.48g	42.23ab	2639.64g-i
N2H1	120.63j	7539.47h	39.84ab	2489.76jk
N2H2	141.85f-i	8865.65e-g	42.75ab	2672.12e-i
N2H3	149.71c-e	9356.76c-d	45.30ab	2831.33bc
N2H4	147.95c-f	9246.94c-f	43.27ab	2704.37d-h
N3H1	125.61j	7850.65h	40.02ab	2501.49jk
N3H2	143.15e-i	8946.87e-g	44.19ab	2761.83b-f
N3H3	167.43a	10464.54a	47.73a	2983.23a
N3H4	152.79b-d	9549.24b-d	43.98ab	2748.90b-g
N4H1	124.60j	7787.83h	39.96ab	2497.36jk
N4H2	142.03f-i	8876.79e-g	43.13ab	2695.61d-i
N4H3	159.70b	9981.60ab	45.89ab	2867.93ab
N4H4	154.86bc	9678.86bc	43.95ab	2746.85c-g
N5H1	119.74j	7483.56h	39.57b	2473.29jk

N5H2	140.61g-i	8787.96fg	41.99ab	2624.90hi
N5H3	147.58d-g	9223.87c-f	44.73ab	2795.56b-d
N5H4	145.35e-h	9084.17d-g	42.55ab	2659.57f-i
LSD (0.05)	7.0469	534.56	7.998	120.37

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.
N1: Control, N2: 105 kg ha-1, N3: 140 kg ha-1, N4: 175 kg ha-1, N5: 210 kg ha-1, H1: control, H2: 50 ppm, H3: 100 ppm and H4: 150 ppm

observed in H_3 (45.65 g) treatment and lowest was observed in H_1(39.74 g) treatment (Table 4.7and Appendix VII). The intraction effect of Nitrogen and NAA in most of the combination should significantly the highestdry wt. ha $^{-1}$ was observed in N_3H_3(43.98 g) treatment and lowest was observed in N_1H_1 (39.27g) treatment (Table 4.7 and Appendix VII).

Zaman *et al.,* 2015b reported that the highest leaf dry weight was obtained from the plant grown in non-calcareous soil (8.46g) which was identical with the dry weight of the plant grown in acid soil (7.90g).

4.7.4 Dry weight ha^{-1} (kg)

Significant variations (P<0.05) was observed indry weight ha^{-1} in different level of nitrogen application (Table 4.7 and appendix VII). Significantly highest dry weight ha^{-1} was observed in N_3(2748.90kg) treatment and lowest was observed in N_1(2615.00kg) treatment (Table 4.7 and Appendix VII). Dry weight ha^{-1} was significantly influence by the application of different concentration of NAA application of stevia (Table 4.7 and Appendix VII). The highest dry weight ha^{-1} plant was observed in H_3 (2853.00 kg) treatment and lowest was observed in H_1(2483.30 kg) treatment (Table 4.7and Appendix VII). The intraction effect of nitrogen and different concentration of NAA were also ststisticaly significant in dry weight ha^{-1}of stevia production (Table 4.7 and Appendix VII). The highest dry weight ha^{-1} (2983.23 kg) was observed in N_3H_3 (nitrogen 140 kg ha^{-1} × NAA 100 ppm) treatment and lowest dry weight ha^{-1} (2454.65kg) was observed in N_1H_1 (with no nitrogen application × with no NAA application) treatment.

4.8 Fresh and dry leaf yield of stevia plant

4.8.1 Fresh leaf yield plant^{-1} (g)

Fresh leaf yield plant^{-1} of stevia was significantly influenced by the application of different levels of nitrogen (Table 4.8and Appendix VII). The highest fresh leaf yieldplant^{-1} was observed in N_3(88.04 g) treatment and lowest was observed in N_1 (85.04) treatment. Effects of different level of NAA application were significant in fresh leaf yieldplant^{-1}of stevia (Table 4.8 and Appendix VII). The highest fresh leaf yieldplant^{-1}was observed in H_3 (92.21 g) treatment and lowest was observed in H_1 (78.87 g) treatment (Table 4.8 and appendix VII). The intraction effect of nitrogen and different concentration of NAA were also ststisticaly significant in fresh leaf yield plant^{-1}of stevia production (Table 4.8 and Appendix VII). Significantly highest fresh leaf yieldplant^{-1} was observed in N_3H_3 (93.60g) treatment and lowest was observed in N_1H_1 (77.23 g) treatment. Noor-E- Ferdous *et al.,* 2021 reported that the fresh leaf yield was produced in field cultivation 91.37 g plant-1.

4.8.2 Fresh leaf yield ha^{-1} (kg)

Effects of different level of nitrogen application were significant in fresh leaf yield ha^{-1}of stevia (Table 4.8 and Appendix VII). Significantly highest fresh leaf yield ha^{-1}was observed in N_3(5501.63 kg) treatment and lowest was observed in N_1(5335.12 kg) treatment. Fresh leaf yieldha^{-1}of stevia was significantly influenced by the application of different levels of NAA application (Table 4.8and Appendix VII).

Lokesh *et al.,* 2018 fresh leaf yield differed significantly due to nitrogen levels. Application of 120 Kg nitrogen per hectare resulted in production of maximum fresh leaf yield per plant (39.27g), per plot (1.88 Kg) and per ha (3.88 t). The increase in yield may be attributed to the fact that due to optimum levels of nitrogen, there would be improved growth of the plant, which leads to production of more number of leaves, branches and ultimately resulting in highest fresh leaf yield. This is in conformity with the results of Murayama *et al.,* 1980 in stevia who reported that the application of optimum doses nitrogen 100 Kg ha^{-1} produced better growth rate and dry leaf yield than the application of lower dose.

Significantly highest fresh leaf yield was observed in H_3 (5763.11 kg) treatment and lowest was observed in H_1 (4929.32 kg) treatment. The intraction effect of nitrogen and different concentration of NAA were also ststisticaly significant in fresh leaf yield ha^{-1}of stevia production (Table 4.8 and Appendix VII). Significantly highest fresh leaf yield ha^{-1}was observed in N_3H_3

(5849.76 kg) treatment (nitrogen 140 kg ha^{-1}× NAA 100 ppm) and lowest was observed in N_1H_1(4826.43 kg) control plot.

4.8.3 Dry leaf yield plant $^{-1}$(g)

Dry leaf yield $plant^{-1}$ of stevia was significantly influenced by the application of different levels of nitrogen (Table 4.8and Appendix VII). Significantly highest dry leaf yield $plant^{-1}$ was observed in N_3 (23.76 g) treatment and lowest was observed in N_1 (22.61 g) treatment (Table 4.8 and Appendix VII). Effects of different level of NAA application were significant in dry leaf yield $plant^{-1}$of stevia (Table 4.8 and Appendix VII). Significantly highest dry leaf yield $plant^{-1}$ was observed in H_3 (25.42 g) treatmentand lowest was observed in H_1 (20.44 g) treatment (Table 4.8 and Appendix VII). The intraction effect of Nitrogen and NAA in most of the combination should significantly the highestdry leaf yield was observed in N_3H_3 (25.65 g) treatment and lowest was observed in N_1H_1 (19.79 g) treatment (Table 4.8 and Appendix VII). Zaman *et al.*, 2015b reported that the highest leaf dry weight was obtained from the plant grown in non-calcareous soil (8.46 g) which was identical with the dry weight of the plant grown in acid soil (7.90 g). Mengesha *et al.,* 2014 reported that the dry weight of the leaves can vary from 15 to 35 g $plant^{-1}$.

4.8.4 Dry leaf yield ha^{-1} (kg)

Dry leaf yield ha^{-1} differed significantly among the different levels of nitrogen treatment (Table 4.8 and Appendix VII). From the Table 4.8, it was observed that N_3 (nitrogen140 kg ha^{-1}) treatment had the highest dry leaf yield ha^{-1} (1482.20 kg) and the lowest dry leaf yield ha^{-1} was observed in N_1 (1413.50 kg) treatment which was statistically different among other nitrogen level. Effects of different level of NAA application were significant in dry leaf yield $plant^{-1}$of stevia (Table 4.8 and Appendix VII). Significantly highest dry leaf yield was observed (1588.70 kg) in N_3 (nitrogen140 kg ha^{-1}) treatmentand lowest was observed in N_1 (1277.30kg) treatment (Table 4.8 and Appendix VII). The intraction effect of nitrogen and different concentration of NAA were also ststisticaly significant of stevia production (Table 4.8 and Appendix VII). The highestdry leaf yield was observed (1603.32 kg) in N_3H_3 (nitrogen 140 kg ha^{-1}× NAA 100 ppm) treatmentand lowest was observed (1236.91 kg) in N_1H_1 (with no nitrogen application × with no NAA application) which was statistically different among other treatment of stevia production

(Table 4.8 and Appendix VII). Mengesha *et al.*. 2014 observed that an estimated 6,000 kg ha^{-1} dried leaf yield can be obtained. Midmore and Rank (2002) reported that dry leaf yield 1,600 – 2,000 kg ha^{-1}. Noor-E- Ferdous *et al.*, 2020 observed that that dry leaf yield was obtained 1226.17 kg ha^{-1} in field condition. Stevia is a short-day plant and has a critical photoperiod of 12 to 13 h (Ceunen and Geuns, 2013). Long days increase the growth period of stevia, directly affecting growth rates and dry weight yield (Ceunen and Geuns, 2013).

Table 4.8 Effect of N and NAA onfresh leaf yield $plant^{-1}$, fresh leaf yield ha^{-1}, dry leaf yield $plant^{-1}$and dry leaf yield ha^{-1} of stevia and their interaction

Treatment	Fresh leaf yield $plant^{-1}$ (g)	Fresh leaf yield ha^{-1} (kg)	Dry leaf yield $plant^{-1}$ (g)	Dry leaf yield ha^{-1} (kg)
Nitrogen	-	-	-	-
N1	85.36ab	5335.12ab	22.61ab	1413.5c
N2	86.71ab	5418.61ab	23.00a	1437.6bc
N3	88.04a	5501.63a	23.76a	1485.2a
N4	87.14a	5446.52ab	23.22a	1451.5ab
N5	85.51ab	5344.34ab	22.75ab	1421.6bc
LSD (0.05)	4.1047	370.66	2.069	36.663
NAA	-	-	-	-
H1	78.87c	4929.32c	20.44b	1277.3d
H2	84.55b	5284.13b	22.19b	1386.2c
H3	92.21a	5763.11a	25.42a	1588.7a
H4	90.57a	5660.34a	24.24a	1515.3b
LSD (0.05)	3.671	331.53	1.851	32.792
Interaction	-	-	-	-
N1H1	77.23f	4826.43e	19.79d	1236.91i
N1H2	83.44d-f	5215.36a-e	21.67a-d	1354.94fg
N1H3	91.58a-d	5724.34a-c	25.19ab	1574.56ab
N1H4	89.19a-d	5574.39a-d	23.80a-d	1487.52cd
N2H1	79.48ef	4967.53de	20.28cd	1267.51hi
N2H2	84.31b-f	5269.54a-e	22.14a-d	1383.61ef
N2H3	91.99ab	5749.51a-c	25.44a	1589.76a
N2H4	91.04a-d	5687.87a-d	24.15a-c	1509.47b-d
N3H1	80.21ef	5013.47b-e	21.18b-d	1323.65f-h
N3H2	86.06a-e	5375.45a-e	22.99a-d	1436.87de
N3H3	93.60a	5849.76a	25.65a	1603.32a

N3H4	92.28ab	5767.61a	25.23ab	1576.97ab
N4H1	80.17ef	5010.58c-e	20.96cd	1310.16g-i
N4H2	85.19b-f	5324.51a-e	22.21a-d	1388.24ef
N4H3	92.07ab	5754.38ab	25.52a	1595.35a
N4H4	91.14a-d	5696.41a-d	24.20a-c	1512.37bc
N5H1	77.26f	4828.65e	19.97d	1248.38i
N5H2	83.77c-f	5235.56a-e	21.88a-d	1367.33e-g
N5H3	91.80a-c	5737.67a-c	25.29ab	1580.59ab
N5H4	89.20a-d	5575.32a-d	23.84a-d	1490.16cd
LSD (0.05)	8.209	741.33	4.139	73.326

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.
N1: Control, N2: 105 kg ha-1, N3: 140 kg ha-1, N4: 175 kg ha-1, N5: 210 kg ha-1, H1: control, H2: 50 ppm, H3: 100 ppm and H4: 150 ppm

4.9 Nutrient status of initial and post harvest soils

4.9.1 Initial soil

The changes in soil pH, organic carbon, total N, available P, exchangeable K, S, available Zn, B, and Mg status of the initial and post harvest soils due to different treatment combinations are presented in Table 4.9. Application of different levels of nitrogen and NAA, and changes pH, organic carbon, total N, available P, exchangeable K, S, available Zn, B, and Mg contents. The results in the present study revealed that pH, organic carbon, total N, available P, exchangeable K, S, available Zn, B, and Mg were built up in soils of plots when nitrogen and NAA were applied in different stevia cultivation compared to control plots.

4.9.2 pH

pH content of stevia was significantly influenced by the application of different levels of nitrogen (Table 4.9 and and Appendix VIII). The highest pH content (5.90%) in was observed when the plot was treated with nitrogen 210 kg ha^{-1}(N_5). The lowest pH content (5.20) was recorded in control (N_1) plot which was significantly inferior to all treatments (Table 4.9). The results indicated that pH content of stevia was also significantly affected by different levels of NAA. The highest pH content (5.61) was observed when the plot was treated with 150 ppmNAA (H_4). The lowest pH content (5.55) was recorded in H_1 treatment. pH content of stevia was significantly affected by different levels of nitrogen and NAA (Table 4.9). The highest pH

content (5.97) was observed when the plot was treated with nitrogen210 kg ha $^{-1}$× 50 ppm (N_5H_2). The lowest pH content (5.10) was recorded in control (N_1H_1) treatment.

4.9.3 Organic matter (OM)

It appears from the data presented in (Table 4.9 and Appendix VIII) that organic matter (OM) content of stevia was significantly influenced by the application of different levels of nitrogen. The highest OM content (2.12%) in was observed in N_5(nitrogen 210 kg ha^{-1}) treated plot and the lowest OM content (0.72%) was recorded in control (N_1) treatment. The results indicated that OM content of stevia was also significantly affected by different levels of NAA (Table 4.6 and Appendix VIII). The highest OM content (1.40%) was observed when the plot was treated with 50 ppm NAA (H_2). The lowest OM content (1.36%) was recorded in H_4 treatment. OM content of stevia was significantly affected by different levels of nitrogen and NAA (Table 4.9 and and Appendix VIII). The highest OM content (2.34%) was observedin (N_5H_1) when the plot was treated with nitrogen 210 kg ha $^{-1}$× control. The lowest OM content (0.62%) was recorded in the control × 100 ppm(N_1H_3) plot.

4.9.4 Nitrogen

The result indicated that nitrogen **(**N) content of stevia was significantly influenced by the application of different levels of nitrogen (Table 4.9 and Appendix VIII). The highest N content (0.35%) in was observed in (N_5) when the plot was treated with nitrogen 210 kg ha^{-1}. The lowest N content (0.07%) was recorded in (N_1) control treatement. The results indicated that N content of stevia was also significantly affected by different levels of NAA (Table 4.9and Appendix VIII). The highest N content (0.21%) was observed when the plot was treated with 150 ppm NAA (H_4). The lowest N content (1.19%) was recorded in H_1 treatment. N content of stevia was significantly affected by different levels of nitrogen and NAA (Table 4.9 and Appendix VIII). The highest N content (0.39%) was obtained in (N_5H_2) treatment (nitrogen 210 kg ha^{-1}× NAA 50 ppm) and the lowest N content (0.04%) was found in whithoutnitrogenand NAA application (N_1H_1).

4.9.5 Phosphorus

Phosphorus (P) content of stevia was significantly influenced by the application of different levels of nitrogen (Table 4.9and Appendix VIII). The highest P content (90.51 µg g^{-1}) in was observed when the plot was treated with nitrogen 210 kg ha^{-1}(N_5). The lowest P content (58.12

μg g^{-1}) was recorded in control (N_1) plot which was significantly inferior to all treatments. The results indicated that P content of stevia was also significantly affected by different levels of NAA (Table 4.9and Appendix VIII). The content of P in varied from 73.30to76.54 μg g^{-1}. The highest P content (76.54 μg g^{-1}) was observed when the plot was treated with 150 ppm NAA (H_4). The lowest P content (73.30 μg g^{-1}) was recorded in H_1 treatment. P content of stevia was significantly affected by different levels of nitrogen and NAA (Table 4.9 and Appendix VIII). The highest P content (92.79 μg g^{-1}) was observed when the plot was treated with nitrogen 210 kg ha^{-1} × NAA150 ppm (N_5H_4) while the lowest P content obtained in (N_1H_3) treatment (without nitrogen × NAA 100 ppm).

4.9.6 Potassium

An effect of different level of nitrogen application was significant in potassium (K) content of stevia production (Table 4.9 and Appendix VIII). The highest K content (0.46 meq/100g) in was observed when the plot was treated with nitrogen 210 kg ha^{-1} (N_5). The lowest K content (0.24meq/100g) was recorded in control (N_1) plot which was significantly inferior to all treatments. Potassium (K) content of stevia was significantly influenced by the application of different levels of different levels of NAA application (Table 4.9 and Appendix VIII). The highest K content (0.37meq/100g) was observed when the plot was treated with 150 ppm NAA (H_4). The lowest K content (0.33meq/100g) was recorded in H_1 treatment. Interaction effect of different levels of nitrogen and NAA application was significant (Table 4.9 and Appendix IX). The highest K content (0.48 meq/100g) was observed (N_5H_4) and N_5H_3 treatment, respectively. The lowest K content (0.22 meq/100g) was recorded in the (N_1H_1) treatment (without nitrogen × with no NAA) application in the experimental field.

4.9.7 Sulphur

Significantly the highest S content (14.93 μg g^{-1}) in was observed when the plot was treated with nitrogen 210 kg ha^{-1} (N_5) andthe lowest S content (3.27 μg g^{-1}) was recorded in control (N_1) plot which was significantly inferior to all treatments (Table 4.9and Appendix VIII). Sulphur (S) content of stevia was significantly influenced by the application of different levels of NAA application (Table 4.9and Appendix VIII). The highest S content (9.82%) was observed when the plot was treated with 150 ppm NAA (H_4). The lowest S content (8.96 μg g^{-1}) was recorded in H_1 treatment. S content of stevia was significantly affected by different levels of nitrogen and NAA (Table 4.9 and Appendix VIII). The highest S content (16.48μg g^{-1}) was observed when the plot

was treated with nitrogen 210 kg ha $^{-1}$× NAA 150 ppm (N_5H_4). The lowest S content (2.10 µg g^{-1}) was recorded in (N_5H_4) which was no nitrogen and no NAA application in the field.

4.9.8 Zinc

It appears from the data presented in (Table 4.9 and Appendix VIII) that Zinc (Zn) content of stevia was significantly influenced by the application of different levels of nitrogen. The highest Zn content (5.35 µg g^{-1}) in was observed when the plot was treated with nitrogen 210 kg ha^{-1}(N_5). The lowest Zn content (1.92 µg g^{-1}) was recorded in control (N_1) plot which was significantly inferior to all treatments (Table 4.9and Appendix IX). The results indicated that Zn content of stevia was also significantly affected by different levels of NAA. The content of Zn in varied from 3.46µg g^{-1} to 3.73 µg g^{-1}. The highest Zn content (3.73 µg g^{-1}) was observed when the plot was treated with 100 ppm NAA (H_3). The lowest Zn content (3.46 µg g^{-1}) was recorded in H_1 treatment. Zn content of stevia was significantly affected by different levels of nitrogen and NAA (Table 4.9and Appendix VIII). The highest Zn content (5.42 µg g^{-1}) was observed when the plot was treated with nitrogen 210 kg ha $^{-1}$× control (N_5H_1). The lowest Zn content (1.78 µg g^{-1}) was recorded in the control plot which was significantly inferior to all treatments.

4.9.9 Boron

The application of various nitrogen levels was significant effect in boron (B) content of stevia production was clearly seen in (Table 4.9 and Appendix VIII). The highest B content (2.46 µg g^{-1}) in was observed when the plot was treated with nitrogen 210 kg ha^{-1} (N_5). The lowest B content (1.55 µg g^{-1}) was recorded in control (N_1) plot which was significantly inferior to all treatments (Table 4.9and Appendix VIII). The results indicated that B content of stevia was also significantly affected by different levels of NAA. The content of B in varied from 1.92 µg g^{-1} to 2.04 µg g^{-1}. The highest B content (2.04µg g^{-1}) was observed when the plot was treated with 50 ppm NAA (H_2). The lowest B content (1.92%) was recorded in H_1 treatment. B content of stevia was significantly affected by different levels of nitrogen and NAA (Table 4.9and Appendix VIII). The highest B content (2.52 µg g^{-1}) was observed when the plot was treated with nitrogen 210 kg ha $^{-1}$× NAA 50 ppm (N_5H_2) and the lowest B content (1.48µg g^{-1}) was recorded in the control treatments.

4.9.10 Magnesium

Application of various nitrogen levels has a substantial impact on the magnesium (Mg) content of stevia (Table 4.9 and Appendix VIII). The highest Mg content (2.40 meq/100g) in was observed when the plot was treated with nitrogen 210 kg ha^{-1}(N_5). The lowest Mg content (0.44 meq/100g) was recorded in control (N_1) plot which was significantly inferior to all treatments (Table 4.9and Appendix VIII). The results indicated that Mg content of stevia was also significantly affected by different levels of NAA (Table 4.9 and Appendix VIII). The content of Mg in varied from 1.32 meq/100g to1.55 meq/100g. The highest Mg content (1.55 meq/100g) was observed when the plot was treated with 150 ppm NAA (H_4). The lowest Mg content (1.32 meq/100g) was recorded in H_1 treatment. Mg content of stevia was significantly affected by different levels of nitrogen and NAA (Table 4.9 and Appendix VIII). The highest Mgcontent (2.56 meq/100g) was recorded in N_5H_3 (nitrogen 210 kg ha^{-1} × NAA 100 ppm) treatment and lowest Mgcontent (0.36 meq/100g) was observed in N_1H_1(with no nitrogen and no NAA application) treatment.

Table 4.9 Effect of N and NAA on nutrient status of initial and post harvest soil changes of stevia production in soil properties and their interaction

Treatments	Nutrients								
	pH	OM (%)	N%	P µg g^{-1}	K meq/100g	S µg g^{-1}	Zn% µg g^{-1}	B µg g^{-1}	Mg meq/100g
Initial soil	5.63	1.14	0.07	70.78	0.21	13.25	4.89	1.95	1.46
Post harvest soil									
Nitrogen	-	-	-	-	-	-	-		
N1	5.20e	0.72e	0.07e	58.12e	0.24d	3.27e	1.92e	1.55d	0.44e
N2	5.45d	1.02d	0.13d	67.82d	0.28c	7.02d	2.58d	1.76c	1.03d
N3	5.63c	1.34c	0.18c	78.19c	0.34b	9.69c	3.54c	1.93c	1.38c
N4	5.78b	1.73b	0.26b	83.29b	0.44a	12.05b	4.74b	2.21b	1.88b
N5	5.90a	2.12a	0.35a	90.51a	0.46a	14.93a	5.35a	2.46a	2.40a
LSD (0.05)	0.098	0.172	0.042	1.989	0.021	1.813	0.177	0.196	0.133
NAA	-	-	-	-	-	-	-	-	-
H1	5.55b	1.40a	0.19ab	73.30b	0.33c	8.96a	3.46b	1.92b	1.32b
H2	5.61a	1.40a	0.19ab	76.00a	0.34bc	9.15a	3.64a	2.04a	1.36b
H3	5.59a	1.38a	0.19ab	76.50a	0.36ab	9.63a	3.73a	1.96a	1.48a
H4	5.61a	1.36ab	0.21a	76.54a	0.37a	9.82a	3.67a	2.00a	1.55a
LSD (0.05)	0.016	0.013	0.011	1.779	0.019	1.621	0.158	0.017	0.119
Interaction	-	-	-	-	-	-	-	-	-
N1H1	5.10m	0.69kl	0.04j	57.45mn	0.22 j	2.10n	1.78i	1.57h-j	0.43K
N1H2	5.24k-m	0.89j-l	0.09h-j	59.65lm	0.24ij	2.57mn	1.98hi	1.60g-j	0.39K
N1H3	5.15lm	0.62l	0.06ij	55.18n	0.23j	5.25k-n	1.80i	1.48j	0.36K
N1H4	5.32j-l	0.69kl	0.08h-j	60.19lm	0.25hij	3.14l-n	2.13g-i	1.54ij	0.58K

N2H1	5.37i-k	0.93j-l	0.10h-j	62.57l	0.28ghi	5.97j-m	2.56f	1.63g-j	0.89k
N2H2	5.48g-j	1.03i-k	0.15f-h	67.14k	0.26hij	8.29g-k	2.32f-h	1.89d-i	0.94jk
N2H3	5.56f-i	0.98jk	0.12g-j	72.47ij	0.28ghi	6.67i-l	2.98e	1.79e-j	1.18ij
N2H4	5.40h-k	1.12h-j	0.14f-i	69.10jk	0.29gh	7.16h-k	2.47fg	1.72f-j	1.10i-k
N3H1	5.65c-g	1.17h-j	0.22d-f	75.54hi	0.31fg	9.36e-j	3.10e	1.97c-g	1.22hi
N3H2	5.63d-g	1.45f-h	0.15f-h	79.02f-h	0.34ef	10.58d-h	3.86d	1.94d-h	1.45f-h
N3H3	5.59e-h	1.39gh	0.16f-h	81.23ef	0.35ef	8.74f-k	3.62d	1.90d-i	1.36g-i
N3H4	5.67c-g	1.34g-i	0.20e-g	76.98gh	0.37de	10.06d-i	3.56d	1.92d-i	1.49e-g
N4H1	5.83a-c	1.88b-e	0.29b-d	80.41e-g	0.41cd	12.89a-e	4.59c	2.04b-f	1.68d-f
N4H2	5.72b-f	1.60e-g	0.21d-f	86.53cd	0.43bc	11.26c-g	4.67c	2.26a-d	1.75de
N4H3	5.78a-e	1.78c-f	0.26c-e	82.57d-f	0.46ab	11.80c-g	4.92bc	2.17a-e	1.92cd
N4H4	5.80a-d	1.67d-g	0.29b-d	83.65de	0.47ab	12.26b-f	4.77c	2.36a-c	2.17bc
N5H1	5.84a-c	2.34a	0.31a-c	90.54ab	0.43bc	14.50a-c	5.27ab	2.41ab	2.39ab
N5H2	5.97a	2.03a-c	0.39a	87.68bc	0.45abc	13.05a-d	5.38a	2.52a	2.25b
N5H3	5.91ab	2.15ab	0.36ab	91.03ab	0.48a	15.69ab	5.33a	2.47a	2.56a
N5H4	5.89ab	1.97b-d	0.32a-c	92.79a	0.48a	16.48a	5.42a	2.45a	2.41ab
LSD (0.05)	0.197	0.344	0.084	3.978	0.042	3.626	0.355	0.393	0.266

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.
N1: Control, N2: 105 kg ha-1, N3: 140 kg ha-1, N4: 175 kg ha-1, N5: 210 kg ha-1, H1: control, H2: 50 ppm, H3: 100 ppm and H4: 150 ppm

CHAPTER V

SUMMARY AND CONCLUSION

A field experiment was conducted at Bangladesh Sugarcane Research Institute (BSRI), Regional Sugarcrop Research Station, Thakurgaon, Bangladesh from March to September, 2021 to investigate the effect of nitrogen and napthelic acitic acid on growth, yeild and nutrient content of stevia (*Stevia rebaudiana* Bertoni). The trail was laid out in Randomized Complete Block Design (RCBD) with three replications. The treatments (nirogen) used was N_1: control, N_2: 105 kg ha^{-1}, N_3: 140 kg ha^{-1}, N_4: 175 kg ha^{-1}, N_5: 210 kg ha^{-1}. The selected napthelic acitic acids (NAA) are H_1: control, H_2: 50 ppm, H_3: 100 ppm and H_4: 150 ppm. Plant height increased gradually with advancement of the growth stage (21 DAT to 147 DAT) of the stevia plants. Significant variations (P<0.05) were observed on plant height in different level of nitrogen application. The highest plant height was observed in N_3 (nitrogen140 kg ha^{-1}) treatmnet and the lowest plant height was observed in N_1 (control) treatment at 21 DAT to 147 DAT, respectively. Applying 100 ppm NAA was the significant effect in increasing height in H_3 (100 ppm NAA) at 21DAT to 147 DAT, respectively and the lowest plant height was observed in control (NAA). The highest plant heights was found in N_3H_3 (nitrogen 140 kg ha^{-1} × NAA 100 ppm) treatment and the lowest plant heights was found in N_1H_1 (with no nitrogen application × with no NAA application) at all growth stages. Nitrogen 140 kg ha^{-1} (N_3) had the highest number of leaves $plant^{-1}$ *i.e.* 18.87, 45.96, 223.67, 508.86, 777.67, 965.26 and 1106.20, respectively which were statistically different among other level of nitrogen application. Significantly the lowest number of leaves $plant^{-1}$ was observed in N_1 (control) treatment. It was reported that (17.46, 42.75, 210.37, 497.20, 763.69, 910.65 and 1091.10) number of leaves $plant^{-1}$ at 21 DAT 147 DAT, respectively. Significantly the highest number of leaves was obtained in H_3 (NAA 100 ppm) treatment and the lowest number of leaves $plant^{-1}$ was observed in H_1 (control) treatment. The interaction effect of nitrogen and NAA in most of the combination should significantly the highest number of leaves at all growth stages. It was found that the highest number of leaves $plant^{-1}$ (20.46, 47.60, 235.57, 526.34, 820.35, 1006.34 and 1145.46) at 21DAT to 147 DAT, respectively was found in N_3H_3 treatment (nitrogen 140 kg ha^{-1} × NAA 100 ppm). Significantly the lowest number of leaves $plant^{-1}$ was observed in N_1H_1 (control) treatment. It was found in number of leaves $plant^{-1}$ (16.45, 38.89, 191.78, 465.67, 681.28, 866.14 and 1048.79) at 21DAT

to 147 DAT, respectively. The highest leaf area plant $^{-1}$ was observed in N_3 (140 kg ha^{-1}) and the lowest leaf area $plant^{-1}$ was observed in N_1 (control) treatment at 21 DAT 147 DAT, respectively. The concentration of 100 ppm NAA produced the highest leaf area $plant^{-1}$ was found in H_3 (NAA 100 ppm) at 21DAT to 147 DAT, respectively. Significantly the lowest leaf area $plant^{-1}$ was found in H_1 (control) treatment at 21DAT to 147 DAT, respectively. A significant variation was found leaf area $plant^{-1}$ at 21DAT to 147 DAT by the intraction effect of nitrogen and different concentration of NAA. The highest leaf area plant was found N_3H_3 treatment (nitrogen 140 kg. ha^{-1}) × NAA 100 ppm). The lowest leaf area plant $^{-1}$ was found in N_1H_1 treatment at all growth stages. Number of primary branch of stevia was significant different in different level nitrogen and NAA application at all growth stages. The highest number of primary branch (5.10, 6.78, 8.56, 9.34, 10.65, 12.37 and 13.2) was found N_3H_3 treatment (nitrogen 140 kg ha^{-1} × NAA 100 ppm). The lowest number of primary branch (3.97, 5.22, 6.88, 8.16, 9.11, 10.22 and 12.40) was found in N_1H_1 treatment at 21DAT to 147 DAT, respectively. The intraction effect of nitrogen and NAA in most of the combination should significantly the highest number of secondary branch at all growth stages. The highest number of secondary branch was found in N_3H_3 (nitrogen 140 kg ha^{-1} × NAA 100 ppm). The lowest number of secondary branch was observed in N_1H_1 treatment at all growth stages.

Nitrogen content of stevia leaves was significantly affected by different levels of nitrogen and NAA. The highest N content (3.47%) was observed when the plot was treated with nitrogen140 kg ha^{-1} × 100 ppm NAA (N_3H_3). The lowest N content (2.56%) was recorded in the control plot which was significantly inferior to all treatments. The different parameters of stevia as K, Mg, Zn were significantly improved by the application of nitrogen and NAA compared to that without any nitrogen and NAA application. Effects of different level of nitrogen application were significant in fresh weight $plant^{-1}$ of stevia. The highest fresh weight $plant^{-1}$ was observed in N_3 (147.24 g) treatment and lowest was observed in N_1 (134.12 g) treatment. Significantly highest fresh weight $plant^{-1}$ was observed in H_3 (152.91g) treatment and lowest was observed in H_1 (122.04 g) treatment. The intraction effect of nitrogen and NAA in most of the combination should significantly the highest fresh weight $plant^{-1}$ was observed in N_3H_3 (147.43 g) treatment and lowest was observed in N_1H_1 (119.62g) treatment. The intraction effect of Nitrogen and NAA in most of the combination should significantly the highest fresh weight ha^{-1} was observed in N_3H_3 (10464.54 kg) and lowest was observed in N_1H_1 (7476.45 kg) treatment. The highest dry

weight ha^{-1} (2983.23 kg) was observed in N_3H_3 (nitrogen 140 kg ha^{-1} × NAA 100 ppm) treatment and lowest dry weight ha^{-1} (2454.65 kg) was observed in N_1H_1 (with no nitrogen application × with no NAA application) treatment.

Significantly highest fresh leaf yield ha^{-1} was observed in N_3 (5501.63 kg) treatment and lowest was observed in N_1 (5335.12 kg) treatment. Significantly highest fresh leaf yield ha^{-1} was observed in H_3 (5763.11kg) treatment and lowest was observed in H_1 (4929.32 kg) treatment. Significantly highest fresh leaf yield ha^{-1} was observed in N_3H_3 (5849.76 kg) treatment and lowest was observed in N_1H_1 (4826.43 kg) treatment. Significantly highest dry leaf yield ha^{-1} was observed in N_3 (1482.20 kg) treatment and lowest was observed in N_1 (1413.50 kg) treatment. Significantly highest dry leaf yield ha^{-1} was observed in H_3 (1588.70 kg) treatment and lowest was observed in H_1 (1277.30 kg) treatment. The intraction effect of nitrogen and NAA in most of the combination should significantly the highest dry leaf yield ha^{-1} was observed in N_3H_3 (1603.32 kg) treatment and lowest was observed in N_1H_1 (1236.91 kg) treatment.

The application of nitrogen and napthelic acitic acid had positive impact on leaf yield components resulted in higher yield of study. From the result it can be recommended that stevia nitrogen 140 kg ha^{-1} and NAA 100 ppm is might be suitable for Northern part of Bangladesh.

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Appendix I: Analysis of variance (mean square) on plant height (cm) of stevia under nitrogen with NAA

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values						
		Plant height (cm) at different days after transplanting (DAT)						
		21	42	63	84	105	126	147
Replication	2	0.447	113.581	30.356	32.717	74.036	37.895	1728.13
Factor A	4	3.122*	4.567*	5.438*	13.009*	25.794*	30.333*	8.79*
Factor B	3	113.539*	62.947*	215.874*	203.659*	220.346*	489.005*	84.05*
AB	12	0.279*	1.658*	0.358*	0.978*	0.452*	1.689*	0.32*
Error	38	7.520	9.758	13.300	20.122	21.028	20.186	17.49

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance.

Appendix II: Analysis of variance (mean square) on number of leaves plant^{-1} of stevia under nitrogen with NAA

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values						
		Number of leaves plant^{-1} at different DAS						
		21	42	63	84	105	126	147

Replication	2	38.8090	1085.45	3546.32	1890.15	621.5	580.1	1196.3
Factor A	4	3.9282*	20.65*	317.00*	269.78*	384.1*	7194.9*	452.2*
Factor B	3	24.5992*	119.35*	3854.28*	7160.75*	47679.8*	43199.1*	21107.3*
AB	12	0.3035*	1.42*	46.70*	7.83*	41.8*	811.7*	43.3*
Error	38	0.6461	3.40	86.96	17.60	48.4	189.7	61.4

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance.

Appendix III: Analysis of variance (mean square) on leaf area plant^{-1} of stevia under nitrogen with NAA

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values						
		Leaf area plant^{-1} (cm^{-2}) at different (DAT)						
		21	42	63	84	105	126	147
Replication	2	90.5677	226.3	5031.7	467	1739	1164	606

Factor A	4	51.0007*	2378.5*	7830.8*	51168*	79822*	42331*	80357*
Factor B	3	80.0373*	49777.1*	94194.8*	319455*	305325*	168746*	204314*
AB	12	1.9385*	297.0*	1149.5*	4097*	8361*	3030*	9876*
Error	38	1.4539	119.9	110.2	189	91	40	703

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance.

Appendix IV: Analysis of variance (mean square) on number of primary branches plant^{-1} of stevia under nitrogen with NAA

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values						
		Number of primary branches plant^{-1} of stevia at different DAS						
		21	42	63	84	105	126	147
Replication	2	0.93745	18.2596	60.9703	20.6654	45.2102	1.44184	37.7575
Factor A	4	0.04915*	0.4164*	0.2719*	0.3847*	0.2892*	0.91344*	0.1163*
Factor B	3	2.48514*	3.9675*	6.1604*	1.9050*	3.7213*	8.43805*	0.9115*
AB	12	0.00243*	0.0287*	0.0418*	0.0248*	0.0349*	0.08528*	0.0073*
Error	38	0.02919	0.0591	0.3122	0.2571	0.6271	1.92272	1.1767

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance.

Appendix V: Analysis of variance (mean square) on number of secondary branches plant^{-1} of stevia under nitrogen with NAA

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values						
		Number of secondary branches plant^{-1} of stevia at different DAS						
		21	42	63	84	105	126	147
Replication	2	21.0535	36.1267	45.0300	23.8711	77.8940	57.2234	64.8720
Factor A	4	4.1607*	0.7848*	0.2013*	1.1076*	2.9872*	0.6457*	1.7440*
Factor B	3	14.8303*	8.4512*	2.7383*	5.0831*	6.2861*	10.4379*	28.7724*
AB	12	0.2416*	0.0420*	0.0068*	0.0506*	0.6517*	0.0273*	0.2140*
Error	38	0.2134	0.4137	0.2602	0.4194	0.5738	0.8570	0.8510

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance

Appendix VI: Analysis of variance (mean square) on N (%), P (%), K (%), S (%), Ca (%), Mg (%) and Zn (µg g^{-1}) contents of stevia leaf under nitrogen with NAA

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values						
		Stevia leaf						
		N (%)	P (%)	K (%)	S (%)	Ca (%)	Mg (%)	Zn (µg g^{-1})
Replication	2	33.4628	0.07116	3.485E-03	0.21966	1.41512	5.346E-03	127.56
Factor A	4	0.1744*	0.00034 NS	4.190E-04 NS	1.948E-04	0.03178 NS	2.860E-04	447.82*
Factor B	3	0.7419*	0.00063 NS	2.190E-03 NS	2.138E-03	0.27958 NS	1.839E-03	2205.90*
AB	12	0.0085*	0.00002 NS	1.442E-05 NS	2.150E-06	0.00192 NS	1.175E-06	7.05*
Error	38	0.1325	0.00208	2.144E-04	1.510E-03	0.83459	7.561E-05	8.93

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance.

Appendix VII: Analysis of variance (mean square) on fresh wt. plant^{-1}(g), fresh wt. ha^{-1} (kg), dry wt. plant^{-1}(g) and dry wt. ha^{-1} (kg), leaf yield plant^{-1} (g), fresh leaf yield ha^{-1} (kg), dry leaf yield plant $^{-1}$ (g) and dry leaf yield ha^{-1} (kg) of stevia of under nitrogen with NAA

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values							
		Stevia plant weight				Stevia leaf yield			
		Fresh wt. plant^{-1} (g)	Fresh wt. ha^{-1} (kg)	Dry wt. plant^{-1}(g)	Dry wt. ha^{-1} (kg)	Fresh leaf yield plant^{-1} (g)	Fresh leaf yield ha^{-1} (kg)	Dry leaf yield plant $^{-1}$ (g)	Dry leaf yield ha^{-1} (kg)
Replication	2	2488.23	1754517	71.4420	1875169	20.869	166228	118.828	3158
Factor A	4	338.65*	1323037*	8.5607*	33381*	15.272*	59126*	2.467*	9616*
Factor B	3	2743.29*	1.072E+07	88.4932*	345589*	555.699*	2171282*	73.125*	285651*
AB	12	55.46*	216721*	0.7416*	2899*	0.795*	3101*	0.171*	664*
Error	38	18.18	104591	23.4186	5303	24.667	201151	6.269	1968

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance.

Appendix VIII: Analysis of variance (mean square) on nutrient status of post harvest soil (pH, OM, N (%), P (%), K (%), S (%), Zn (µg g^{-1}), B% and Mg (%) of stevia field under nitrogen with NAA

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values								
		Post harvest soil of stevia field								
		pH	OM	N (%)	P (%)	K (%)	S (%)	Zn (µg g^{-1})	B%	Mg (%)
Replication	2	1.22018	0.45000	0.01742	355.16	70.46	262.305	2.6572	0.05618	0.09248
Factor A	4	0.91703*	3.72816*	0.14851*	1962.99*	1023.47*	242.965*	24.6201*	1.57011*	6.87835*

Factor B	3	0.00994*	0.00620*	0.00108*	35.67*	9.96*	2.408*	0.2028*	0.03810*	0.16796*
AB	12	0.01476*	0.05575*	0.00321*	20.67*	15.82*	4.080*	0.1237*	0.01762*	0.03740*
Error	38	0.01420	0.04345	0.00259	5.79	7.77	4.813	0.0462	0.05670	0.02592

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance.