UNIVERSITI PUTRA MALAYSIA

IMPACT OF NITROGEN FERTILIZATION ON GROWTH, YIELD AND GLYCOSIDE CONTENT OF STEVIA (Stevia rebaudiana Bertoni)

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By

ROSNANI ABD GHANI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

October 2014

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I am dedicating this thesis to my beloved :

Husband, Mohammad Fauzi. . . Bonda, Maimun Hassan. . . Children, Syabil Faiq and Aniq Isyad . .

For their endless love, support and sacrifices. .

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

IMPACT OF NITROGEN FERTILIZATION ON GROWTH, YIELD AND GLYCOSIDE CONTENT OF STEVIA (*Stevia rebaudiana* Bertoni)

By

ROSNANI ABD GHANI

October 2014

Chairman : Associate Professor Mohd. Ridzwan Abd Halim, PhD

Faculty : Agriculture

Stevia, a well-known crop with sweet taste and zero calorie is an ideal substitute for cane sugar. There is currently an increasing demand for non-sugar sweeteners such as stevia due to the heightened health consciousness among consumers to reduce sugar intake to combat diabetes. Nitrogen is an important nutrient in stevia cultivation as it promotes leaf growth which is the economic component of stevia. Two glasshouse experiments were carried out in this study. The first experiment was conducted to determine optimum rate of nitrogen (N) and to compare two frequencies of N application for stevia. Urea was used as a source of N fertilizer in this study. The plant was planted as a ratoon crop and a total of four harvests were taken. Factorial combinations of seven N rates (0, 50, 100, 150, 200, 250 and 300 kg ha^{-1}) and two application frequencies (every 30 days and every 60 days) were evaluated for their effects on stevia yield and sweetness quality.

Results showed that N rates and fertilizer application frequencies had significant influence on biomass yield, glycosides content, glycosides yield and sweetness quality of stevia. Based on biomass yield the best N rate for stevia was 260 kg ha^{-1} and the application interval of 30 days was better than 60 days. The total fresh yield and dry yield from four harvests were 37 t ha^{-1} and 6.2 t ha^{-1}, respectively. On the other hand, the glycoside content was superior at low N rates. The highest stevioside (ST) and rebaudioside-A (Reb-A) concentration was obtained at 0 N (control) (85 mg g^{-1} for ST, 21 mg g^{-1} for Reb-A). When biomass yield was multiplied with glycoside content to give glycoside yield, the best N rate was 160 – 210 kg N ha^{-1} with 60 days interval of application that gave ST yield of 410 kg ha^{-1} and Reb-A yield of 85 kg ha^{-1}. Nevertheless, the

most important factor in stevia production is their sweetness quality. With higher concentration of Reb-A, the sweetness and quality of stevia is much greater. Based on the sweetness quality, 50 kg N ha^{-1} was the best rate for stevia with fertilizer applied at 30 days interval. At this rate and frequency, the ratio Reb-A to the ST was 0.4.

The second experiment was carried out to evaluate response of stevia to the different sources of nitrogen fertilizer. The treatments comprised of a control (0 N), one inorganic source (urea) and three organic sources of N which were chicken dung, goat dung and worm casting. Biomass yield was higher in plants treated with urea compared to those treated with organic fertilizers. The glycoside content ST was significantly higher at 0 N (113.60 mg g^{-1}) followed by chicken dung (110.76 mg g^{-1}). However, the highest Reb-A content was in plants treated with chicken dung (10.41 mg g^{-1}) followed by 0 N treatment. The application of chicken dung also produced significantly higher amount of ST yield (333.23 kg ha^{-1}) and Reb-A yield (31.30 kg ha^{-1}). The sweetness quality was better when chicken dung and goat dung were used with ratio of Reb-A to the ST respectively at 0.094 and 0.091.

In conclusion, optimum N rate in stevia production was 260 kg ha^{-1} based on biomass yield and 50 kg ha^{-1} based on sweetness quality. The fertilizer application interval of 30 days was more suitable for stevia compared to application of every 60 days. The use of chicken dung or goat dung gave high sweetness quality of stevia and it was recommended as a better nitrogen fertilizer source compared to the inorganic source, urea.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN PEMBAJAAN NITROGEN TERHADAP PERTUMBUHAN, HASIL DAN KANDUNGAN GLIKOSIDA PADA STEVIA (*Stevia rebaudiana* Bertoni)

Oleh

ROSNANI ABD GHANI

Oktober 2014

Pengerusi : Professor Madya Mohd. Ridzwan Abd Halim, PhD

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Stevia, tanaman yang terkenal dengan rasa manis dan tiada kalori serta sesuai menggantikan gula tebu. Permintaan yang semakin meningkat terhadap pemanis bukan gula seperti stevia dewasa ini adalah disebabkan kesedaran terhadap kesihatan telah meningkat di kalangan pengguna untuk mengurangkan pengambilan gula bagi bertarung dengan penyakit kencing manis. Nitrogen adalah nutrien penting dalam penanaman stevia kerana ia membantu meningkatkan pertumbuhan daun yang mana daun merupakan komponen ekonomik bagi stevia. Dua eksperimen di dalam rumah kaca telah dijalankan dalam kajian ini. Eksperimen pertama telah dijalankan bagi menentukan kadar optimum baja nitrogen (N) dan membandingkan dua kekerapan pembajaan (F) N bagi tanaman stevia. Urea telah digunakan sebagai sumber baja N dalam kajian ini. Pokok telah ditanam secara ratun dan jumlah empat tuaian telah diambil. Kombinasi faktorial dengan tujuh kadar N (0, 50, 100 , 150 , 200 , 250 dan 300 kg ha^{-1}) dan dua kekerapan aplikasi (setiap 30 hari dan setiap 60 hari) telah dinilai terhadap hasil dan kualiti kemanisan stevia.

Keputusan menunjukkan bahawa kadar N dan kekerapan aplikasi pembajaan mempengaruhi secara signifikan ke atas hasil biomas, kandungan glikosida, hasil glikosida dan kualiti kemanisan stevia. Berdasarkan hasil biomas, kadar N terbaik untuk stevia adalah 260 kg ha^{-1} dan selangan aplikasi pembajaan 30 hari adalah lebih baik daripada 60 hari. Jumlah hasil basah dan hasil kering dari empat tuaian adalah masing-masing sebanyak 37 t ha^{-1} dan 6.2 t ha^{-1}. Sebaliknya, kandungan glikosida adalah lebih baik pada kadar N yang

rendah. Kandungan glikosida stevioside (ST) dan rebaudioside-A (Reb-A) yang tertinggi didapati pada 0 N (kawalan) (85 mg g^{-1} for ST, 21 mg g^{-1} for Reb-A). Apabila hasil biomas didarab dengan kandungan glikosida untuk mendapatkan hasil glikosida, kadar N terbaik adalah 160 – 210 kg N ha^{-1} dengan selangan aplikasi pembajaan selama 60 hari memberi hasil ST sebanyak 410 kg ha^{-1} dan hasil Reb-A sebanyak 85 kg ha^{-1}. Walau bagaimanapun, faktor yang paling penting dalam pengeluaran stevia adalah kualiti kemanisannya. Dengan tingginya kandungan Reb-A, kemanisan dan kualiti stevia adalah lebih baik. Berdasarkan kualiti kemanisan, 50 kg N ha^{-1} adalah kadar yang terbaik bagi stevia dengan baja diberi pada selangan 30 hari. Pada kadar dan kekerapan pembajaan ini, nisbah Reb-A kepada ST adalah 0.4.

Kajian kedua telah dijalankan untuk menilai tindakbalas stevia terhadap sumber baja N yang berbeza. Rawatan kajian adalah terdiri dari kawalan (0 N), satu dari sumber bukan organik (urea) dan tiga dari sumber organik N iaitu tinja ayam, tinja kambing, dan tinja cacing. Hasil biomas lebih tinggi pada pokok dirawat dengan urea berbanding pokok yang dirawat dengan baja organik. Kandungan glikosida ST adalah tertinggi dan signifikan pada 0 N (113.60 mg g^{-1}) diikuti tinja ayam (110.76 mg g^{-1}). Walau bagaimanapun, kandungan Reb-A yang tertinggi adalah pada pokok yang dirawat dengan tinja ayam (10.41 mg g^{-1}) diikuti oleh rawatan 0 N. Penggunaan tinja ayam juga menghasilkan jumlah yang tinggi dan signifikan bagi ST yield (333.23 kg ha^{-1}) dan Reb-A (31.30 kg ha^{-1}). Kualiti kemanisan adalah lebih baik apabila tinja ayam dan tinja kambing digunakan dengan nisbah Reb-A kepada ST masing-masing adalah 0.094 and 0.091.

Kesimpulannya, kadar N yang optimum untuk pengeluaran stevia adalah sebanyak 260 kg ha^{-1} berdasarkan hasil biomas dan 50 kg ha^{-1} berdasarkan kualiti kemanisan. Selangan aplikasi baja selama 30 hari adalah lebih sesuai untuk stevia berbanding aplikasi setiap 60 hari. Penggunaan tinja ayam atau tinja kambing memberi kualiti kemanisan yang tinggi bagi stevia dan disyorkan sebagai sumber baja nitrogen yang lebih baik berbanding sumber bukan organik, urea.

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I certify that an Examination Committee has met on 15 October 2014 to conduct the final examination of Rosnani Abd Ghani on her thesis entitled "Impact Of Nitrogen Fertilization On Growth, Yield And Glycoside Content Of Stevia (*Stevia Rebaudiana* Bertoni)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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ANOVA	:	Analysis of Variance
B	:	Boron
Ca	:	Kalsium
CEC	:	Cation exchange capacity
Cu	:	Cuprum
DAP	:	Diammonium phosphate
DAS	:	Day after sowing
DMRT	:	Duncan's Multiple Range Test
ETOH	:	Ethanol
H	:	Harvest
HCl	:	Hydrochloric acid
H_2O_2	:	Hydrogen peroxide
HPLC	:	High Performance Liquid Chromatography
Ltd	:	Limited
K	:	Potassium
MOP	:	Muriate of Potash
$KSbOC_4H_4O_6$	:	Antimonyl tartrate
MARDI	:	Malaysian Agricultural Research and Development Institute
Mg	:	Magnesium
Mn	:	Mangan
Mo	:	Molybdenum
N	:	Nitrogen
NaOH	:	Sodium hydroxide
NH_3	:	Ammonia

NH_4S	:	Ammonium sulfide
$(NH_4)_6Mo_7O_{24})$	:	Ammonium molybdate
NH_4OAc	:	Ammonium acetate
P	:	Phosphorus
PCNB	:	Pentachloronitrobenzene
PROC REG	:	Procedure regression
ppm	:	parts per million
P_2O_5	:	Triple Super Phosphate
R^2	:	Regression coefficients
RCBD	:	Randomized complete block design
rpm	:	revolutions per minute
S	:	Sulphur
UPM	:	Universiti Putra Malaysia
USA	:	United State of America
Zn	:	Zink

CHAPTER 1

GENERAL INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is commonly known as sweet herb, honey leaf and sugar leaf. It is native to Paraguay and have been used as a source of natural sweetener by Paraguayan Guarani Indians for hundreds of years. The native Guarani Indians have their local name for stevia namely Ka-a He-e, used as herbal tea and described it as a sweet herb of Paraguay (Lewis, 1992).

Stevia is from the Asteraceae family which is the same family with well-known plants such as dandelion, sunflower and chicory. About 240 stevia species are well known, among them *Stevia rebaudiana* was proven to give the sweetest essence (Soejarto *et al.*, 1983).

Among plant parts of stevia, the leaf is the commercial part which has high phytochemical content. Stevia has an alternate leaf arrangement and herbaceous growth habit with flowers arranged in indeterminate head. Leaves are small, oblong, lanceolate and serrate (Dwivedi, 1999). Meanwhile, the stem of stevia has very low phytochemical content compared to the leaf. The stem is annual, subligneous, more or less graft (Sakaguchi and Kan, 1982). The root system of stevia is perennial, fibrous and filiform (Schmeling, 1967). The root forming abundant stock that is hardly ramified and does not deepen and distribute itself to the land surface. The fine roots congregate around the soil-surface and thicker roots in the deepest zones. Roots of stevia is the only part of the plant that does not contain any phytochemical (Ramesh *et al.*, 2006).

The sweetness of stevia is derived from compounds known as diterpene glycosides that have been used as a sweetener for foods and baverages for many years in Paraguay (Geuns, 2003a). There are about 10 sweet glycosides in stevia, but only two are important, namely, stevioside and rebaudioside-A (Singh and Rao, 2005). Stevioside, is the sweet glycoside that is found in the highest concentration in stevia contributing about 60 – 70% of total glycosides (Yadav *et al.*, 2011). Stevioside has been estimated to be about 250 – 300 times sweeter than cane sugar (Crammer and Ikan, 1986) but it has an after taste (licorice taste) (Midmore and Rank, 2002). However, rebaudioside-A the other important glycoside contributing about 30 – 40% of total glycosides in stevia is assessed as 350 – 450 times sweeter than cane sugar (Crammer and Ikan,1986) and has no after taste (Midmore and Rank, 2002).

There is an increasing trend towards consuming non-chemical products and living a natural life all over the world. Human lifestyles have changed so much that sweeteners have become integral part in daily diet. Sweeteners like high calorie natural, processed sugar or high potency and low calorie sweeteners such as aspartame are not good for our health. These sweeteners will lead to obesity and diabetic problems (Anon, 2004).

Stevia is a new crop that is very popular among all type of sweetener users, which is ideal substitute for sugar. This is because sweeteners from stevia such as stevioside and rebaudioside have no calorie although they are much sweeter than sugar (Crammer and Ikan, 1986).

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 (Mohamad Ghawas *et al.*, 2009). Malaysia can provide the plant with all these conditions except for the day length, which leads to early flowering. The day length of Malaysia can reach a maximum of only 12 hours. This problem can reduce the yield and glycosides content of stevia, which lowers its sweetness. Thus, other factors should be considered to increase the glycoside content 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 a 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 recommendations can be made. Thus, specific recommendation for nutrient especially nitrogen requirement should be developed under local condition.

The general objective of the research was to determine the nitrogen requirement of stevia for optimum yield and quality. The specific objectives were:

i. to determine optimum rate of nitrogen fertilizer for stevia
ii. to determine suitable frequency of fertilizer application
iii. to evaluate response of stevia to the different sources of nitrogen fertilizer
iv. to determine the impact of nitrogen fertilization on sweetness quality of stevia

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