UNIVERSITI TEKNOLOGI MARA

MACRONUTRIENTS SCREENING ON THE GROWTH AND DEVELOPMENT OF *Stevia rebaudiana* (Bertoni) *in vitro* SHOOT CULTURE AND THE YIELDING OF STEVIOSIDE AND REBAUDIOSIDE A.

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Thesis submitted in fulfilment
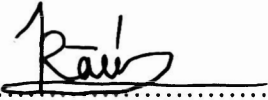of the requirements for the degree of
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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification

I, hereby acknowledge that I have been supplied with the academic rules and regulation for Post Graduates, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Stevia rebaudiana (Bertoni) is a herbaceous plant which has an increasing demand in agricultural industry worldwide. This is due to the presence of the sweet glycosides in the leaves that are hundreds times sweeter than sugar and have zero calories. Previously, studies were focused on the increasing its yield such as growth regulator manipulation, light, water and temperature controlling in greenhouse and genetic modification of the herb. However, no study had been done on the macronutrients manipulation in *Stevia rebaudiana* (Bertoni). Therefore, the novelty of this study is, it is the first study in discovering the effects of macronutrients manipulation in *Stevia rebaudiana* (Bertoni) *in vitro* plantlets. This study aimed at screening the growth and development of *Stevia rebaudiana* (Bertoni), and the stevioside and rebaudioside A yield in different concentration strength of macronutrients. It was done by using one-factor-at-a-time (OFAT) technique. Different parts of the *in vivo* plants were separated and extracted in different solvents and analysed by using HPLC. Meanwhile, the sterile shoot tip explants were cultured onto MS basal media. After 1 month, several of these sterile plantlets were extracted in different solvents and analysed by using HPLC. The remained sterile plantlets were continued regenerated and multiplied to increase their quantities. Media with manipulated macronutrients (N, K, Ca, Mg, P and S) were prepared in different concentrations strength (0, 0.5x, 1x, 2x, 4x and 8x) for each macronutrient element. The sterile plantlets were then cultured onto these modified media. After 1 month, the whole plantlets were oven dried and ground into powder for extraction in different solvents and analysed by using HPLC. Findings showed that *in vitro* shoots contain significantly high concentration of stevioside (22.63±0.25mg/ml), while *in vivo* leaves contain significantly high concentration of rebaudioside A (2230.40±26.72 mg/ml) as compared to other parts of *Stevia rebaudiana* (Bertoni). Results from this study also showed that the aqueous solvent was the best solvent to extract stevioside while methanol was the best solvent to extract rebaudioside A. Moreover, 4x K concentration produced highest number of leaves (22.00±2.21), highest number of nodes (9.40±0.85), highest fresh biomass (0.07±0.01g) and highest dry biomass (0.006±0.001g), while 8x S produced the highest shoot length (3.83±0.39cm). There is no direct relationship between macronutrients concentration increment towards physical characteristics and biomasses of *Stevia rebaudiana* (Bertoni) shoot culture. The proposed macronutrient concentrations in optimizing the growth and development of *Stevia rebaudiana* (Bertoni) shoot culture are 1x N, 4x K, 8x Ca, 4x Mg, 8x P and 1x S. Furthermore, media without Mg (0x concentration strength) yielded the highest stevioside concentration (4.57±0.02mg/ml), while media without Ca (0x concentration strength) yielded the highest rebaudioside A concentration (1709.10±6.44mg/ml). There is no direct relationship between the macronutrients concentration increment towards the stevioside and rebaudioside A yield. The proposed macronutrients concentration strength in yielding the highest stevioside concentration are 8x N, 0 K, 0.5x Ca, 0 Mg, 8x P and 2x S while the proposed macronutrient concentration strength in yielding the highest rebaudioside A concentration are 4x N, 2x K, 0 Ca, 2x Mg, 0 P and 0.5x S. Findings from this study also showed that there is no direct relationship between the physical characteristics and biomass towards the stevioside and rebaudioside A yield.

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CHAPTER ONE
INTRODUCTION

1.1   BACKGROUND OF STUDY

Human life depends on plants for building materials, fabric materials, furniture, papers and source of nutrients. In addition to basic nutrients like proteins, fats or carbohydrates, plants are a source of pharmaceuticals, cosmetics, food ingredients, wood, cellulose, agrochemicals, flavors, insecticides and pigments which these compounds were known to originate from plant secondary metabolites. These secondary metabolites are not crucial for plant growth, but play a vital role for the plants survival in environment. Within this perspective, the plant kingdom has the potential to be the best, non-polluting chemical factory for secondary metabolites (Vasconsuelo and Boland, 2007).

Stevia rebaudiana (Bertoni) that was selected for this research study is a perennial herb belongs to the Asteraceae family. It is a small green plant bearing leaves which have a pleasant and revitalizing taste that can be 300 times sweeter than sugar. The leaf of this plant is a natural alternative source to traditional sugar and has received a greater attention because of its sweet secondary metabolites. These secondary metabolites that have high demand for their sweet property are stevioside and rebaudiose A. *Stevia rebaudiana* (Bertoni) leaves and whole leaf concentrate are nutritious, natural dietary supplements offering numerous health benefits (Kinghorn, 2002). Besides acting as a sweetening agent, these secondary metabolites have many health benefits such as antioxidant, anti-inflammatory, immunomodulatory, bactericidal, enhancing renal function, vasodilator, hypoglycaemic agent, anti-viral, anticancer activity (Madan et al., 2010) and also has been widely used in food industry (Mizutani et al., 1989). Various studies have found that the leaf contain proteins, fibers, carbohydrates, iron, phosphorus, calcium, potassium, sodium, magnesium, zinc, rutin (a flavionoid), true vitamin A, vitamin C and an oil which contains 53 other constituents, besides the intensely sweet glycosides (steviosides, rebaudiosides and a dulcoside) (Kinghorn, 2002). As a result of these beneficial substances inside the *Stevia rebaudiana* (Bertoni) leaf, it becomes one of the ingredients in the herbal drinks