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Morphological Assessment of *Stevia rebaudiana* Bertoni Accessions In IIUM's Germplasm as Initial Material for Stevia Breeding

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ABSTRACT

Background: Stevia rebaudiana Bertoni, belonging to the family Compositae, has a long and successful history of use as a commercial sweetening agent. It originated from Paraguay and was first introduced into Malaysia in 1970s. The sweetening agents are the steviol glycosides which are 150 to 350 times sweeter than sugar. Currently in IIUM's germplasm collection, there are a total of 10 stevia accessions available which are collected from all across Malaysia. **Objective:** The key step before any effective breeding program can be initiated is by studying phenotypic diversity in a germplasm. Being an introduced species, the number of stevia accessions available is still small, and therefore is inadequate for effective breeding purposes. In order to broaden its genetic base, introductions of exotic accessions have been made which include accessions from Paraguay and additional numbers of accessions collected across Malaysia. Morphological evaluation on these seventeen stevia accessions is substantial as the jumping base before further breeding process can be initiated. **Results:** All accessions are planted in an experimental plot at IIUM Kuantan, Pahang. Morphological evaluations include plant height, number of branches, number of leaves, leaf size and widest branch. From observation, results showed that accessions MS007 and MS012 have the most promising characteristics in term of plant height, number of leaves and leaf size which could be exploited for further breeding purposes. **Conclusion:** Morphological diversity observed in all seventeen stevia accessions in the germplasm showed a wide genetic variation which would be useful for future hybridization studies.

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INTRODUCTION

Stevia (*Stevia rebaudiana*Bertoni) is a perennial, herbaceous plant originating from Paraguay which the leaves produce diterpene glycosides, primarily stevioside and rebaudioside (Brandle, 2004). Malaysians, known for their 'sweet tooth', consuming a lot of sugar in most foods and this unhealthy habit is responsible for the alarming rise in diabetic cases (from 0.65% of the population in 1960 to 16-18% in 1998) (Mustaffa, 1998). Interest in this plant has picked up in recent years because of its potential as a non-calorific natural sweetener (particularly targeted for individuals with an obesity problem as well as diabetics) (Soejarto *et al*., 1982).

China currently produces more than 80% of stevia in Asia, and 90% of it goes to Japan for production of natural sweeteners (40% of the sweeteners in Japan come from stevia). Stevia cultivation has become an interest in Malaysia as a result of the sugar shortage not too long ago. The generally wet climate of Malaysia does not favour sugarcane cultivation, and the country has been largely dependent on sugar imports to satisfy local demand. To date, there is no variety that is practical and suitable for our local production in terms of leaf yield and steviol glycosides content. Therefore, there is a need to carry out appropriate breeding research towards new development of suitable local stevia varieties. This paper aims at morphological evaluations of all accessions for further breeding selection.

Botanical Description:

Stevia is amembr of theCompositae family. It is a small, dense shrubby perennial with roughly 50-80cm in height when growing wild and can reach up to 1m in height when cultivated. The leaves are intense green with lanceolate shape growing diametrically opposite on the stem. The existence of

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trichome structures on the leaf surface are of two distinct sizes, one large (4-5 µm), one small (2.5µm) (Shaffert and Chetobar, 1994). Its flowers are small (7-15mm), white in colour and arranged in an irregular cyme. The tiny white florets are perfect (hermaphrodite) having both male and female organs, borne in small corymbs of two to six florets (Goettemoller and Ching, 1999). Seeds are contained in slender achenes about 3mm in length. Each achene has about 20 persistent pappus bristles cyme (Dwivedi, 1999). The roots are fibrous and dense. Naturally, stevia is a short-day plant with a critical day length of about 13h (Zaidan *et al*., 1980). Plant can initiate flowering after a minimum of four true leaves have been produced (Carneiro, 1990).

Chemical Constituents:

Eight sweetening compounds have been identified to exist in the leaf tissue of stevia which is known as diterpeneglycisodes. The four major sweeteners are stevioside, rebaudioside-A, rebaudioside-C and dulcosides-A. The sweetness of the compounds relative to sucrose is 210, 242, 30 and 30 times respectively (Kinghorn, 1987). Two main glycosides are stevioside (5-10% of the dry weight of the leaf) and rebaudioside-A (2-4%of the dry weight of the leaf); these are the sweetest compounds (Kinghorn, 1987; Shaffert and Chetobar, 1994;Dacome *et al*., 2005; Sekaran *et al*., 2007).

Pollination Behaviour:

Stevia is self-incompatible and probably insect-pollinated (Oddone, 1997). Results of an experiment from a complete diallel cross with eight parents showed that amount of selfing ranged from 0 to 0.5%, while out-crossing ranged from 0.7 to 68.7% indicating that the self-incompatibility system is operating (Maiti and Purohit, 2008). The reproductive anatomy of the male and female gametophytes is a typical for angiosperms (Shaffert and Chetobar, 1994).

Cultivation:

Stevia is a semi-humid subtropical plant. The plant prefers a lightly textured, well-drained soil to which organic matter has been added. It requires ample water to ensure consistent moisture as the plant has poor tolerance of water stress. Semi-shade is needed in hot, sunny climate. It has little salt tolerance, so it should not be grown in saline soils or with low quality water. Short days promote flowering. Long days favour leaf yield and leaf stevioside contents (Shock, 1982).

MATERIALS AND METHOD

Fourteen stevia accessions were collected from all across Malaysia while three new introductions were from Paraguay. List of accessions are shown in Table 1.

Table 1: List of all stevia accessions used in the study

Stevia Accessions	
1) Bangi	10) Taman Pertanian
2) Nilai	11) Paraguay 1
3) Mergong	12) Paraguay 2
4) Bertam	13) Paraguay 3
5) Langat	14) WakafBharu
6) SouqBukhori	15) Kuantan 1
7) MS007	16) Kuantan 2
8) MS012	17) Kuantan 3
9) Rawang	

All stevia accessions are prepared through stem micro-cutting propagation. All the plants were subjected to the same conditions of growth and made to develop under the same climatic condition in the Kulliyyah of Science of the International Islamic University, Kuantan, Malaysia. The parameters used for evaluation include plant height, number of branches, number of leaves, leaf size and widest branch. The measurements for plant height and leaf size (LXW) were taken with the aid of a ruler calibrated in 'cm', the widest branch was measured using inelastic thread wound round the base of the stem at a region above the soil level and the length of the thread was read on the ruler, while the number of branches and number of leaves were counted visually. The data was subjected to one sample't' test statistics analysis, and level of significance at $p<0.05$ was read and recorded.

RESULTS AND DISCUSSION

Most of the evaluated accessions are different from each other morphologically, a good indicator of genetic divergence among the accessions. MS012 was the tallest among all whileNilai, SouqBukhoriand MS007were also taller than the rest (Figure 1). Besides plant height, number of branches and number of leaves are also important because a plant can be tall without enough branches and leaves. Leaf yield is another valuable characteristic in stevia, since most glycoside content are found in the leaf. Bertam despite its height and highest number of branches did not produce high number of leaves compared to the rest of the accessions (Figure 2).

Although MS007, MS012, Nilai and Souq Bukhori (Figure 2) were not significantly higher in number of branches but they produced higher number of leaves and even had bigger leaf size

compared to other accessions (Figure 3 and Figure 4). Leaf size is another good characteristic to indicate larger leaf weight which implies to higher quantity of sweetener content in the leaves. Again, in term of leaf production, accession MS007, MS012, Nilai and Souq Bukhori were higher compared to other accessions (Figure 4). While, accession MS012 had the widest branch if compared to all other accessions (Figure 5).

Overall, MS012performed well compared to other accessions especially on plant height, number of leaves, leaf size and widest branch. While MS007, Nilai and Souq Bukhori showed good qualities on plant height, number of leaves and leaf size. Bertam performed well on plant height and gave the highest number of branches among all. Morphological variation in term of plant physiology, leaf shape and leaf size variation is illustrated in Figure 6.

This study reveals that there are plentiful of useful genetic information gained from the assessment conducted. Genetic variation observed was not due to any environmental influence such as type of soil, amount of rainfall or any other climatic conditions. This is because all the plants were prepared through stem micro-cutting propagation technique and most importantly were allowed to grow under the same environmental and climatic condition all throughout the duration of the experiment.

From this,it is possible to conclude that there is suffient genetic variability among the studied accessions which could be useful for future breeding program towards development of better cultivar lines of specific traits (Sakaguchi and Kan, 1992).

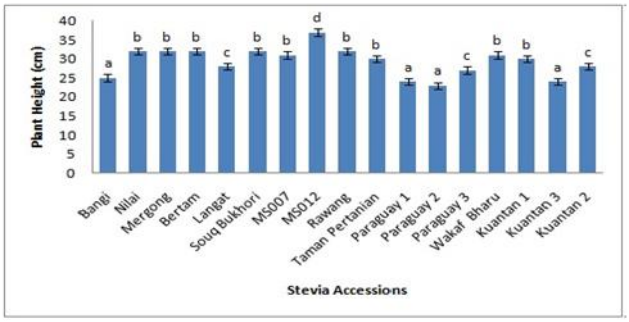


Fig. 1: Plant height in stevia accessions.

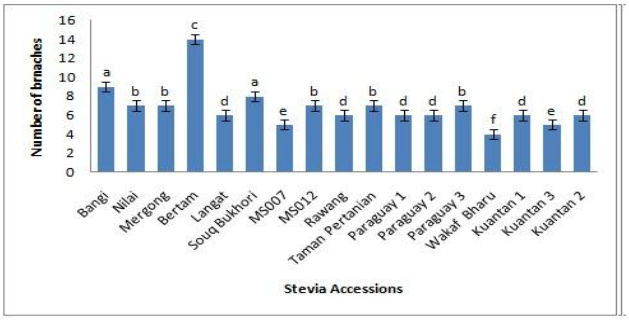


Fig. 2: Number of branches in stevia accessions.

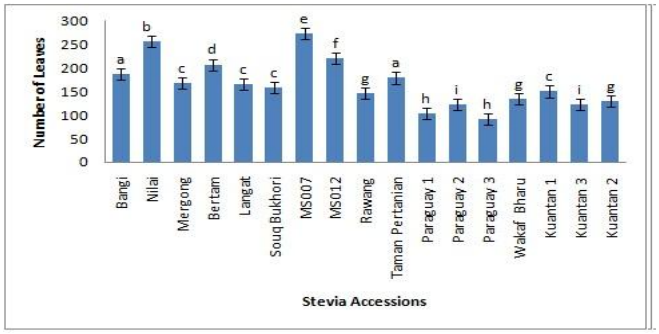


Fig. 3: Number of leaves in stevia accessions

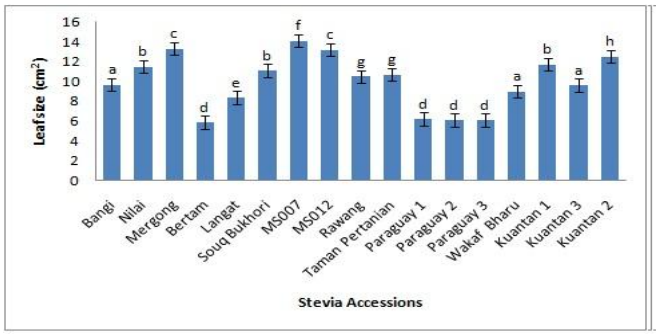


Fig. 4: Leaf size evaluation on stevia accessions

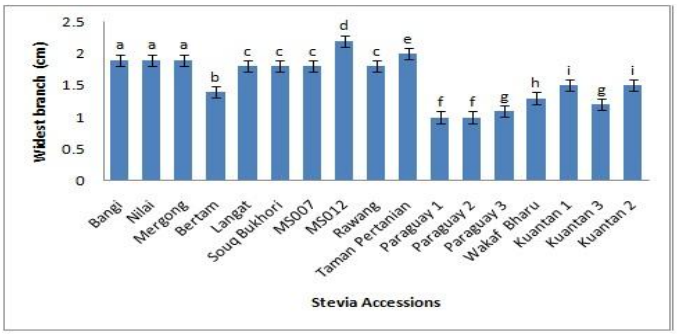


Fig. 6: Variation of stevia crop stand and leaf morphology according to respective accession

Bangi Accession

Special remark: Individual shoot less branched, Compact habit, Emergence of multiple shoots more uniform, Leaf tip acute, Leaf shape ovate

Nilai Accession

Special remark: Individual shoot less branched, Compact habit, Emergence of multiple shoots more uniform, Leaf tip acute, Leaf shape ovate

Mergong Accession

Special remark: Individual shoot less branched, Compact habit, Emergence of multiple shoots more uniform, Leaf tip obtuse, Leaf shape obovate to ovate

Bertam Accession

Special remark: Individual shoot highly branched, Loose habit, Emergence of multiple shoots less uniform, Leaf tip acute, Leaf shape ovate

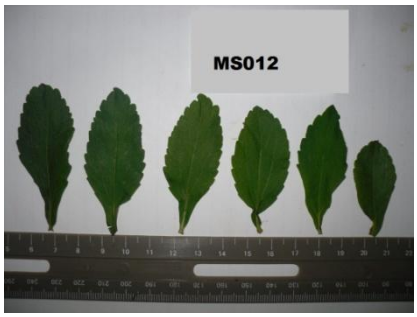
Langat Accession

Special remark: Individual shoot less branched, Compact habit, Emergence of multiple shoots more uniform, Leaf tip acute, Leaf shape ovate

MS007 Accession

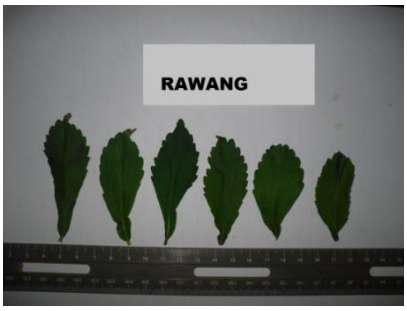
Special remark: Individual shoot less branched, Compact habit, Emergence of multiple shoots more uniform, Leaf tip obtuse, Leaf shape ovate

MS012 Accession

Special remark: Individual shoot less branched, Compact habit, Emergence of multiple shoots more uniform, Leaf tip acute, Leaf shape ovate

Rawang Accession

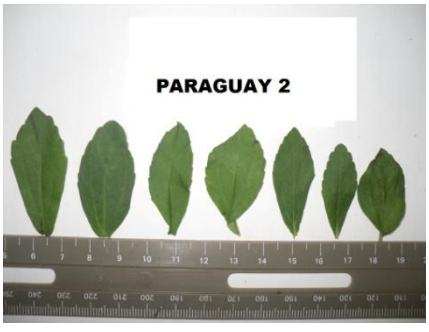



Special remark: Individual shoot less branched, Compact habit, Emergence of multiple shoots more uniform, Leaf tip obtuse, Leaf shape ovate

Tmn Pertanian Accession

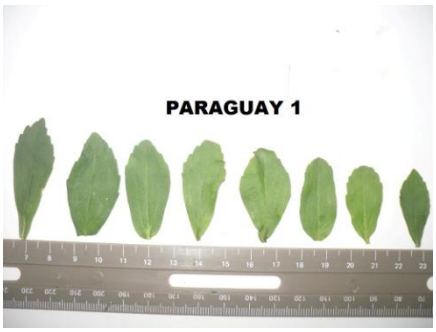

Special remark: Individual shoot less branched, Compact habit, Emergence of multiple shoots more uniform, Leaf tip obtuse, Leaf shape ovate

Paraguay 1 Accession

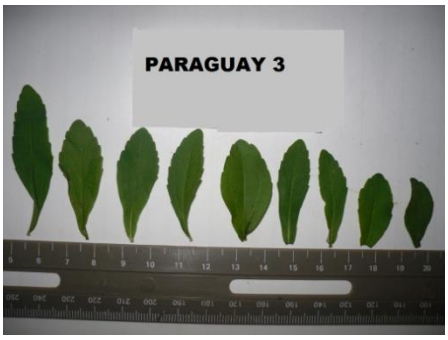
Special remark: Individual shoot less branched, Loose habit, Emergence of multiple shoots less uniform, Leaf tip obtuse, Leaf shape obovate

Paraguay 2 Accession



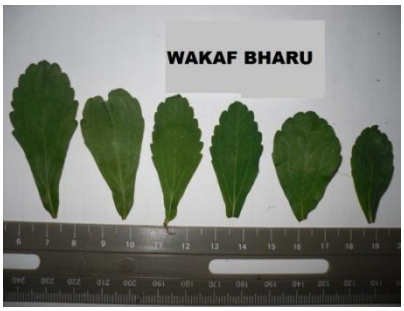
Special remark: Individual shoot less branched, Compact habit, Emergence of multiple shoots more uniform, Leaf tip acute, Leaf shape ovate

Paraguay 3 Accession



Special remark: Individual shoot highly branched, Loose habit, Emergence of multiple shoots less uniform, Leaf tip acute, Leaf shape ovate

Wakaf Bharu Accession

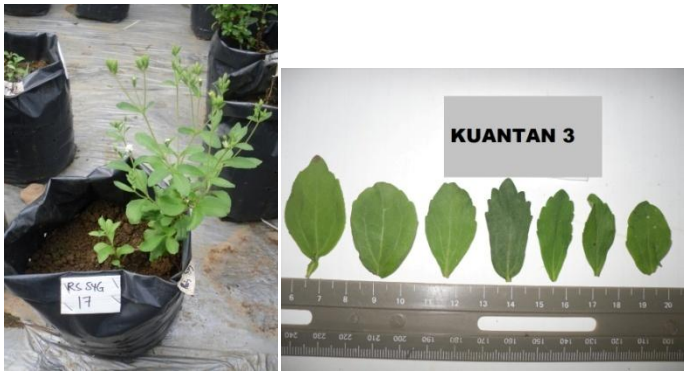


Special remark: Individual shoot less branched, Compact habit, Emergence of multiple shoots more uniform, Leaf tip acute, Leaf shape ovate

Kuantan 1 Accession

Special remark: Individual shoot less branched, Compact habit, Emergence of multiple shoots more uniform, Leaf tip acute, Leaf shape ovate

Kuantan 3 Accession



Special remark: Individual shoot less branched, Compact habit, Emergence of multiple shoots more uniform, Leaf tip acute, Leaf shape obovate to ovate

Kuantan 2 Accession

Special remark: Individual shoot less branched, Compact habit, Emergence of multiple shoots more uniform, Leaf tip acute, Leaf shape obovate

Conclusion:

The data presented in the preceding paragraphs had shown the presence of substantial variability in the stevia germplasm in IIUM. Hence, the possibility for further improvement using these variations is wide. Therefore, economical traits of stevia such as higher leaf yield which is important towards development of stevia-based industry which relies heavily on leaf production can possibly be improved for their quality and quantity through selection. Conclusively, accession MS012, MS007, Nilai and Souq Bukhori have superior and promising traits to be used for future breeding program.

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