Influence of Genetic Variation on Morphological Diversity in Accessions of Stevia Rebaudiana Bertoni

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Abstract

Stevia rebaudiana, a non caloric natural sweetener is currently the available substitute to sugar. It is a genus of about 150 species of herbs and shrubs, a Compositae and a native to Paraguay. The aim of this study was to evaluate collected accessions of stevia and characterize their genetic divergence. In 2010, 10 stevia accessions were collected across different locations in Malaysia. At maturity they were evaluated using morphological parameters and collected data were subjected to't' test analysis at p<.05 as follows-(i) Plant heights: MS012 & SBK were significant at p<.001 and p<.002 respectively. (ii) Number of branches: BGI & SBK were significant at p<.001 and p<.008 respectively.(iii) Number of corymbs: AZI was significant at p<.000, while BGI was significant at p<.033. (iv) Number of leaves: MS012 & MS007 were respectively significant at p<.000 and p<.008. (v) Plant leaf size: AZI was significant at p<.003, MS007 at p<.005, and MS012 at p<.019 and (vi) stem girth: MS012 & MRG were also significantly different at p<.000 and p<.001 respectively. MSO12, MS007 & SBK had loads of promising genetic traits in studied accessions.

Keywords: Stevia rebaudiana, Non caloric, Natural, Sweetener, Morphology, Parameters, Genetic divergence

1. Introduction

The Worldwide demand for a substitute and a high potency sweetener, to the artificially synthesized sugar, increases year in year out, more so as a high percentage of the world population becomes increasingly diabetic (Marc, 2008). The alternative natural sweetener at hand has been Stevia (*Stevia rebaudiana* Bertoni.) plant. Stevia, also known as sweet leaf, or sugar leaf is a genus of about 150 species of herbs and shrubs (Robert, 2010), a member of the family Compositae and a native to Paraguay (Mark, 2009).

Stevia rebaudiana Bert. is one of 154 members of the genus Stevia and one of only two that produce sweet steviol glycosides (Soejarto et al. 1982, 1983). It is native to subtropical and tropical South America and Central America (Robert, 2010). Stevia was first brought to the attention of Europeans in 1887 when M.S. Bertoni learned of its unique properties from the Paraguayan Indians and Mestizos (Lewis, 1992). Various reports cited by Lewis (1992) indicate that it was long known to the Guarani Indians of the Paraguayan highlands who called it caá-êhê, meaning sweet herb. The leaves were used either to sweeten maté or as a general sweetening agent.

It is considered as a good substitute of sugar. Diabetic patients can take herbal powder of stevia available in market as it lowers down the sugar level, giving the sweet taste of sugar. It is a non-caloric sweetener, the sweet compounds pass through the digestive process of the body without chemically breaking down, thus making Stevia a safe substance for consumption for people who need to reduce the sugar content of their Blood (Strauss, 1995).

From most of the previous work, Stevia has been reported to have no adverse effect on humans (Brandle and Rosa, 1992). The leaves could be eaten fresh or when dried and it could be boiled in tea to release the sweetener.

A large effort aimed at establishing stevia as a crop in Japan was begun by Sumida (1968). Since then, stevia has been introduced as a crop in a number of countries including Brazil, Korea, Mexico, United States, Indonesia, Tanzania, and, since 1990 Canada (Donalisio et al. 1982; Goenadi, 1983; Shock 1982; Saxena and Ming 1988; Brandle and Rosa 1992; Fors 1995). Since the 1970s, stevia extracts have been widely used in many countries as a sugar substitute. In Japan, for instance, stevia extracts account for about 5.6% of the sweetener market (Strauss 1995).

1.1 Plant Description

Stevia is a member of the Compositae family and it exists as both herbs and shrubs (Robert, 2010). The plant, under cultivation can reach up to 1 m or more in height (Shock, 1982).It possesses an extensive root system and brittle stems producing small, elliptic leaves. The leaves are sessile, oppositely arranged lanceolate to oblancoelate in shape, and 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 tiny white florets are perfect, borne in small corymbs of 2–6 florets. Corymbs are arranged in loose panicles. Oddone (1997) considers stevia to be self-incompatible and insect pollinated. Additionally, he considers “clear” seeds to be infertile. Seeds are contained in slender achenes, about 3 mm in length. Each achene has about 20 persistent pappus bristles.

1.2 Glycosides &Chemical Constituents

A substance called rebiana which is a trade name for zero-calorie sweetener containing mainly steviol glycoside is extracted from stevia. (Mark, 2009).Stevia is also rich in flavonoids and terpenes. Other constituents are stevioside (C38 H60 O18) which is considered sweetest in stevia, steviolbioside, rebaudiosides (C44 H70 O23) A-E, dulcoside ; chemicals like caffeic acid, campesterol, chlorogenic acid, chlorophyll, diterpene glycosides, dulcosides A-B, and formic acid. An extract of one or more of the glycosides may be up to 300 times sweeter than sugar (Morita et al, 2009; Duke, 1993).

1.3 Photoperiodism

Light has primarily two functions during plant growth and development. First, light influences plant growth (stem thickness, rooting, branching, etc.) through the process of photosynthesis. Secondly, light influences several developmental processes, such as seed germination and flowering. During crop production, growers can manipulate the length of the day to influence flowering of crops sensitive to photoperiod, and add supplemental lighting to a crop to increase the amount of photosynthesis and thus plant growth.

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. Stevia requires short days for flowering (Brandle et al, 2000). Some short-day plants perceive light at 1 foot-candle or even lower. Researchers have been able to establish that plants that flower earlier when grown under short days will have delayed flowering if exposed to long days (Runkle, 2007). Plants can initiate flowering after a minimum of four true leaves have been produced.

1.4 Cytogenetic and Crossings

Stevia is diploid and has 11 chromosome pairs, which is characteristic for most of the South American members of the genus (Brandle et al, 2000)**.** 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 out crossing 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.

1.5 Cultivation

Stevia is now cultivated in most parts of the world. Being a sugar substitute and seeing the percentage of diabetes patients in the world, this herb has gained popularity widely. It is commercially cultivated in many parts of Brazil, Paraguay and Uruguay, Central America, Thailand, India and china. Stevia prefers moist, sandy and loamy soil with vconsiderate summer sunshine. (Rhonda, 2004)

Stevia is grown as a perennial in subtropical regions including parts of the United States, but must be grown as an annual in mid to high latitude regions, where longer days favor leaf yield and stevioside contents (Goettemoeller and Ching, 1999). Propagation of stevia is usually by stem cuttings which root easily, but require high labour inputs. Poor seed germination (36.3%) is one of the factors limiting large-scale cultivation (Goettemoeller and Ching, 1999).

Shock (1982), Duke (1993), and Carneiro (1997), all mention poor production of viable seeds. Propagation is a special concern for northern growers who must grow stevia as an annual. Leaves of stevia are dehydrated in sunshine or heaters and if eaten fresh give bitter taste. Dry leaves are further powdered to be used as a sweetener. Leaves are preferably collected in autumn. Dead leaves and black leaf spots diseases occur specifically to Stevia and are caused by Septoria fungus and Alternaria, these fungi live in soil. (Morita et al 2009).

Stevia has the potential to become a general substitute for sugar for the Malaysian population, its products for certain niche markets (e.g. for medicinal value) are even more promising, e.g. there are > 3 million diabetic patients in the country who are likely to benefit from it. However, Malaysia still lacks suitable varieties and production technologies, while some suitable stevia varieties have been successfully developed in few countries (e.g. Japan and India). Therefore, there is the need to carry out appropriate research and development (R&D) program in order to develop suitable local varieties.

The aim of this study is to evaluate collected accessions and characterize their genetic divergence in order to make selection for future hybridization programs in stevia.

2. Materials and Methods

Ten stevia accessions were collected across different locations in Malaysia. The accessions were named either after the areas where they were collected or after the collector. These are shown on table1. New plants were raised from these accessions; there were ten plants per an accession and 100 in all.

Stem cuttings were made from each accession and were treated with Indole butyric acetic acid (IBA) hormone in order to stimulate the cuttings to sprout roots in a mist chamber. Upon rooting the young plants were transferred to a nursery section in small polythene bags under a shade (fig.5) and were made to remain there for two weeks. This stage was necessary to harden the young growing plants.

The soil type used in this experiment was a mixture of sand and loam; 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.

After two weeks they were transferred to a larger polythene bags and finally taken to field. When the plants became three months old they were evaluated on the field.

The parameters used for evaluation include (i) plant height, (ii) number of branches, (iii) number of corymbs, (iv) number of leaves, (v) leaf size and (vi) stem girth.

The measurements for plant height and leaf size (LXB) were taken with the aid of a ruler calibrated in 'cm', the stem girth 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, number of corymbs and number of leaves were counted.

The data was subjected to one sample 't' test statistics analysis, where individual mean for a particular trait was compared to population mean of such trait and level of significance at $p<.05$ was read and recorded.

Histograms and superimposed line graphs were also plotted in order to graphically represent the data collected. These were placed in figures.

Pictures of the accessions were taken and also represented in figures.

3. Results

Data collected are presented in table 2.

The one sample't' test statistics analysis revealed that considering the plant heights, accessions MS012 and SBK were significantly taller than other collected accessions at $p<.001$ and $p<.002$ respectively. Others were not significantly different. Analysis on number of branches showed that accessions BG and SB were also significantly different at $p<.001$ and $p<.008$ respectively. As for the number of corymbs AZI was significant at $p<.000$, while BGI was significant at $p<.033$. With the number of leaves, accession MS012 and MS007 were respectively significant at $p<.000$ and $p<.008$, while others were not. AZI was significant at $p<.003$; MS007 at $p<.005$, and MS012 at $p<.019$ with the trait, plant leaf size, while with stem girth, two accessions namely MS012 and MRG were also significantly different at $p<.000$ and $p<.001$ respectively.

Figures 1 to 4 are graphical expressions of data collected on plant heights, number of branches, and number of leaves and size of leaves respectively in stevia accessions. Figure1 showed that 20% of the plant accessions had between 20 to <30cm, 40% had between 30 to < 35cm, another 20% had between 35 to <40cm, while still another 20% had between 40 to45cm in height. The plants with the highest height were MS012 (43cm) and BGI (42cm).

In figure 2, 20% of the plants (TPT and AZI) had least number of branches ranging from four to five. Most (70%) of the plants (MS007, MS012, RWG, SBK, MPG, MRG and LGT) had between six to ten, while the remaining 10 % (BGI) had 11 number of branches.

Figure 3 showed that 10% of the plant accessions had between 50 to <100 leaves (TPT), 40% had between100 to 150 leaves; these include SBK, RWG, AZI, and MPG; 10% had >150 to <200 (LGT), 30% had 200 to <250(MS007, BGI and MRG), while 10% had between 250 to <300 number of leaves (MS012). The highest number of leaves was recorded in MS012.

For size of leaves in figure 4, 10% of the plant accessions had between 6 to <8cm (MRG); 60% had 8 to <12cm (LGT, TPT, MPG, BGI, SBK and RWG); 10% had 12 to <14cm (MS012); while 20% had between 14 to <16cm (MS007 and AZI). The highest values for leaf size were recorded for MS007 and AZI, followed closely by MS012.

Figure-5 showed picture of stevia accessions being raised in the nursery through stem cuttings.

4. Discussions

From the above results it would be observed that most of the evaluated accessions differ from one another morphologically, indicating genetic divergence across the collections. The observed variation could not have been due to any other factor such as soil types, age differences among plants, amount of rainfall, environmental or climatic conditions, this is because new plants were raised from collected accessions through stem micro-cutting propagation (Sakaguchi and Kan, 1982) and were allowed to grow under the same environmental and climatic conditions throughout the period of the experiment.

MS012 and SBK were taller than other accessions. Number of branches and leaves are also very important because at times a plant may be tall without enough branches and leaves.

High leave yield especially is a valued trait in this plant, since the sweetener compositions are found in the leaves. In this case though MS012 was not significantly higher in number of branches than accessions BGI and SBK but it produced the highest number of leaves over other accessions (table 2). While SBK despite its height, and also BGI and SBK with their higher number of branches did not produce significant number of leaves when compared to MS012 and MS007.

On the corymbs production, AZI and BGI had higher numbers than other accessions. Corymbs constitute the floral part of stevia plant, corymbs may harbor from two to four or more of tiny ‘flowers’ called florets which are the reproductive structure of the plant. When a plant begins to flower the rate of growth of the vegetative body reduces, such that the development of branches and leaves are affected. A higher production of corymbs therefore in accessions like AZI and BGI are not of advantage to farmers and consumers, as the leaf yield which is a major desired quality in stevia may be affected.

Plant leaf size is also a good trait because large leaf size implies larger weight than small leaf size and may connote possibility of higher quantity of leaf sweetener content since the sweetener are found on the leaves. AZI, MS007 and MS012 were highest in leaf size production than other accessions. MS012 and SBK also performed better in size of stem girth than in other accessions.

It would be observed that MS012 performed well over other accessions in desired traits, especially on plant heights and number of leaves, while MS007 also showed good qualities on number leaves and leaf size. SBK performed well on plant height and higher number of branches. This evaluation revealed that there are useful genetic traits in studied accessions.

The information revealed from this research work gives an insight that the three accessions mentioned above could be used for future hybridization program so as to develop better cultivar lines of specific genotypes, which could then be propagated by stem micro-cutting method in order to maintain the line or lines (Sakaguchi and Kan, 1982).

5. Conclusion

MSO12, MS007, and SBK had useful genetic traits in studied accessions and could be selected for future hybridization programs in stevia.

References

Brandle, J.E.; Starratt, A.N. and Gijzen, M. (2000). Stevia rebaudiana Its biological, chemical and agricultural properties. *Agriculture and AgriFood Canada,* Southern Crop Protection and Food Research Centre, 1391 Sandford St., London, Ontario N5V 4T3.

Brandle, J.E. and N. Rosa. (1992). Heritability for yield, leaf-stem ratio and stevioside content estimated from a landrace cultivar of Stevia rebaudiana. *Can. J. Plant Sci*., 72:1263–1266.

Carneiro, J.W.P., A.S. Muniz, and T.A. Guedes. (1997). Greenhouse bedding plant production of Stevia rebaudiana (Bert) bertoni. *Can. J. Plant Sci.*, 77:473–474.

Donalisio, M.G.R., Duarte, F.R., Pinto, A.J.D.A., and Souza, C.J. (1982). Stevia rebaudiana. *Agronomico,* 34: 65-68.

Duke, J. (1993). *Stevia rebaudiana*. In: J. Duke, CRC handbook of alternative cash crops. CRC Press, Boca Raton, FL. p. 422–424.

Fors, A. (1995). A new character in the sweetener scenario. *Sugar J.*, 58: 30.

Goenadi, D.H. (1983). Water tension and fertilization of Stevia rebaudiana Bertoni on Oxic Tropudalf (English abstr.). *Menara Perkebunan,* 51: 85-90.

Goettemoeller, J. and A. Ching. (1999). Seed germination in *Stevia rebaudiana*. p. 510–511. In: J. Janick (ed.), Perspectives on new crops and new uses. ASHS Press, Alexandria, VA.

Lewis, W.H. (1992). Early uses of Stevia rebaudiana (Asteraceae) leaves as a sweetener in Paraguay. *Econ. Bot.*, 46: 336-337. [Online] Available: http://dx.doi.org/10.1007/BF02866633

Marc Santora. (2008). *Concern Grows over Increase in diabetes Around World.* The New York Times Washington. (2010). World population increasing over Diabetics.

Mark Stibich. (2009). About Stevia sweetener-Is it better than sugar? [Online] Available: http://longevity.about.com/od/lifelongnutrition/a/stevia-extract.htm

Morita, T., Morita, K., and Koichiro, K. (2009). Variety of *Stevia rebaudiana* Bertoni with a high content of Rebaudioside – A plant. Publication No:US 2009/0214753 A1. United State Patent Application Publication.

Oddone, B. (1997). *How to grow stevia. Technical manual.* Guarani Botanicals, Pawtucket, CT.

Rhonda Janke. (2004). *Farming a few Acres of Herbs*: Stevia. Kansas State University. [Online] Available: http://www.oznet.ksu.edu

Robert C. Artkins. (2010). *New Diet revolution*. Healthier-Harvest 2009. [Online] Available: http://healthier-harvest.com/news-articles/nutritional_information/stevia.htm

Runkle, E. (2007). Technically speaking: Providing long days. *Greenhouse product news*, 17 (1): 66. [Online] Available: www.gpnmag.com

Sakaguchi, M. and T. Kan. (1992). Japanese researches on stevia rebaudiana (Bertoni and stevioside. *Ci, Cult.*, 34: 235-248.

Saxena, N.C., and Ming, L.S. (1988). Preliminary harvesting characteristics of stevia. *Phys. Prop. Agric. Mat. Prod*. 3: 299-303.

Shock, C.C. (1982). Experimental cultivation of Rebaudi's stevia in California. Univ. California, *Davis Agron. Progr. Rep.* 122.

Soejarto, D.D., Compadre, C.M., Medon, P.J., Kamath, S.K., and Kinghorn, A.D. (1983). Potential sweetening agents of plant origin. II. Field search for sweet-tasting Stevia species. *Econ. Bot,* 37: 71-79. [Online] Available: http://dx.doi.org/10.1007/BF02859308

Soejarto, D.D., Kinghorn, A.D., and Farnsworth, N.R. (1982). Potential sweetening agents of plant origin. III. Organoleptic evaluation of stevia leaf herbarium samples for sweetness. *J. Nat. Prod*, 45: 590-599. [Online] Available: http://dx.doi.org/10.1021/np50023a013

Strauss, S. (1995). The perfect sweetener? *Technol. Rev,* 98:18–20. Whitaker, J. 1995. Sweet justice: FDA relents on stevia. Human Events 51:11.

Table 1. Showed name of accessions, accession codes, area of collection/name of collectors and Global Positioning System (GPS) coordinates & position

S/N	NAMES OF ACCESSIONS	ACCESSION CODES	AREA OF COLLECTION/ COLLECTOR	GPS COORDINATES & POSITION
1.	MARDI STEVIA 007	MS007	Malaysian Agricultural and Research Development Institute.	N2 58.909 E101 41.891
2.	MARDI STEVIA 012	MS012	Malaysian Agricultural and Research Development Institute.	N2 58.909 E101 41.891
3.	AZIZ	AZI	AZIZ	N 2° 59'0.1.01" E 101° 42'23.58"
4.	RAWANG	RWG	RAWANG	N 03° 18.892' E 101° 34.631'.
5.	SOUQ BUKHARI	SBK	BUKHARI MARKET	N 6007'53.58" E 100^{0}23'27.68"
6.	BANGI	BGI	BANGI	N 2^{O}55'18.08" E 101^{0}47'03.38"
7.	MATANG PAGAR	MPG	MATANG PAGAR	27/80 [Lat:3°0'32" Lng:101°34'1"]
8.	TAMAN PERTANIAN	TPT	TAMAN PERTANIAN	N 03° 05.687' E 101° 30.683'
9.	MERGONG	MRG	MERGONG	Lat- (lat): 6°8'0"N Lng- (lon): 100°20'0"E Elevation (approx.): 3m
10	LANGAT	LGT	LANGAT	N309.774 E101 49.599

Table 2. Showed collected accessions and evaluation of morphological characteristics in stevia

S/N	CHARACTERISTICS	COLLECTED STEVIA ACCESSIONS									
		MS007	MS012	AZI	RWG	SBK	BGI	MPG	TPT	MRG	LGT
1.	Plant height.	33	43	37	33	37	42	32	23	30	29
2.	Number of branches.	06	08	05	06	10	11	07	04	10	08
3.	Number of corymbs.	15	57	147	05	43	88	103	07	05	04
4.	Number of leaves.	245	290	135	123	120	200	130	50	210	185
5.	Leaf size.	14.05	13.53	14.46	11.04	11.47	9.45	8.37	11.72	6.10	8.79
6.	Stem girth.	2.55	3.50	2.70	2.65	2.45	2.60	2.40	1.80	3.40	2.00

KEY- MS: Mardi stevia; AZI: Aziz; RWG: Rawang; SBK: Souq Bukhari; BGI: Bangi; MPG: Matan Pagar; TPT: Taman Pertanian; MRG: Mergong; LGT: Langat.

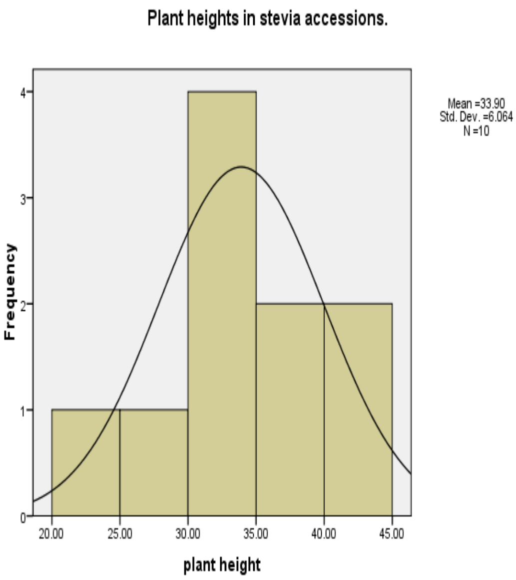

Figure 1. Plant heights in stevia accessions

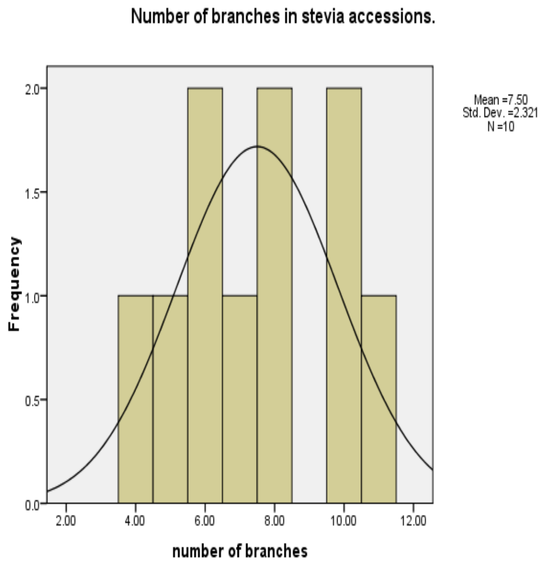

Figure 2. Number of branches in stevia accessions

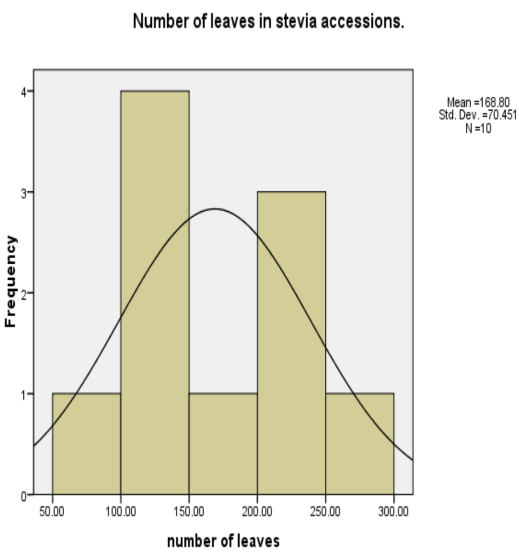

Figure 3. Number of leaves in stevia accessions

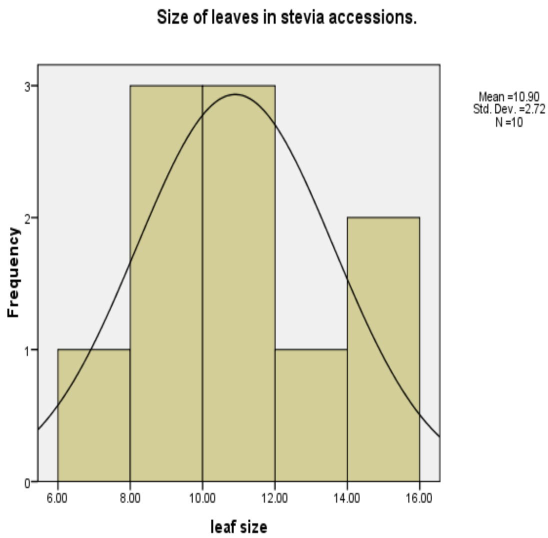

Figure 4. Size of leaves in stevia accessions

5a 

5b 

Figure5a. Accessions of *Stevia rebaudiana*. & 5b. New set of stevia accessions being raised in the nursery (through stem cutting)

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