Growth Performance of Stevia (Stevia Rebaudiana Bert.) as Influenced by Clonal Propagation Methods and Growing Media

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Abstract- The study was conducted to evaluate the growth performance of Stevia as influenced by clonal propagation methods and growing media at the Nursery House of the City Agriculture Office, Casisang, Malaybalay City, Bukidnon, Philippines from September 2015 to November 2015. The study was carried-out in a 3 x 5 factorial arrangement in Completely Randomized Design (CRD) with three replicates and twenty plants per replication with cutting methods (A1 – shoot tip cuttings, A2 – nodal cuttings and A3 – wedge stem cuttings) as Factor A and growing media (B1 – pure garden soil, B2 – ½ garden soil + ½ vermicast, B3 – pure vermicast, B4 – ½ garden soil + ½ sand, and B5 – 1/3 garden soil + 1/3 sand + 1/3 vermicast) as Factor B. Results revealed that shoot tip cuttings in combination with 1/3 garden soil + 1/3 sand + 1/3 vermicast in plastic cups two months after planting significantly produced the tallest plants (13.72 cm), highest survival rates (96% for Factor A and 97.33% for Factor B), least incidence of leaf curling and leaf dark spot (both 1.22 ratings for Factor B), and most number of leaves (26.67) and branches (3.25). However, the combinations of nodal cuttings and pure garden soil showed very poor growth performance with the shortest height, least survival rate, highest incidence of leaf curling and leaf dark spot, and least number of leaves and branches. Thus, propagating Stevias using shoot tip cuttings with 1/3 garden soil + 1/3 sand + 1/3 vermicast media mixture using plastic cups for two months showed the best growth performance. Hence, recommended for mass propagation and herbage production.

Index Terms- Stevia, vermicast, survival rates, wedge stems and nodal cuttings

I. Introduction

Stevia (*Stevia rebaudiana* Bertoni) commonly known as sweet leaf or sugar leaf is a natural and healthy alternative to sugar and artificial sweeteners. It is a famous perennial shrub of Asteraceae family (Geuns, 2007). It is extensively grown in the subtropical regions, Stevia has long been widely used as sweetener in beverages and mask the bitter taste of certain herbal medicinal plants in several countries like Brazil, Japan, Paraguay, etc. (Parsons and Cuthberston, 2001), however, it is not well known in the Philippines.

The leaves of Stevia are the sources of stevioside, a natural sweetener and is 250-300 times sweeter than sucrose, heat stable, pH stable, non-fermentable and has no effect on blood sugar (Geuns, 2003; Goyal and Samsher, 2010); hence considered and allowed to be called as sweetener by the Food and Drug Administration (FDA) of the US in 2008. Stevia alleviates hypoglycaemia and type 2 diabetes (Soejorto et al., 2002; Ramesh et al, 2006), nourishes pancreas and thereby helps restore its normal function. It also contains high percentage of phenols that reduce the cardiac and cancer diseases (Dragovi-Uzelac et al., 2010) and flavonoids which have high antioxidant activity (Tadhani et al., 2007; Shukla et al., 2009).

Apart from sweetness, Stevia is imbued with stevioside related compounds of rebaudioside A and steviol that offer therapeutic benefits like antihypertensive, antidiabetic, anti-inflammatory, anti-tumor, antioxidant, antidiarrheal, diuretic and immunomodulatory actions. Steviol interacts with the drug transporters, hence proposed as drug modulator (Goyal and Samsher, 2010).

The economic importance, health benefits and high demand of Stevia in both local and export market provide great production, income and livelihood opportunities for farmers, hobbyists and households. Moreover, the crop is adapted over a wide range of climatic conditions in the country and apparently been successfully grown abroad (Qui et al., 2000).

At present, little is known about Stevia as well as its propagation, postharvest handling and processing. There are no protocols on rapid clonal propagation and growing media. particularly in Mindanao or Bukidnon are available.

Thus, the objective of this study was to evaluate the growth performance of Stevia (S*tevia rebaudiana* Bert.) as influenced by methods of clonal propagation and growing media.

II. Materials and Methods

The study was conducted at the Nursery House of the City Agriculture Office, Casisang, Malaybalay City, Bukidnon for two (2) months starting September 2015 to November 2015.

The study utilized Stevia mother plants (*Stevia rebaudiana* Bert.), B-net screened nursery structures (9.5 m long x 5.5 m wide x 3 m high) with 50-60% shade, disposable cups (7 oz), cutting propagation tools, hose, garden soil, sand, vermicast, water sources, sprinklers, garden tools, wooden sticks, scissors/cutting tools, plastic twines, rulers/meter stick, weighing scales, cent-o gram balance, polyethylene pots/bags, stevia leaves and stems, blender, empty sacks/mesh nets, packaging/processing tools and equipment.

This study was laid-out in a 3 x 5 Factorial Arrangement in Completely Randomized Design (CRD) replicated 3 times with 20 samples per replication. The three types of cuttings of Stevia served as Factor A and the five combinations of growing media as Factor B, as indicated:

Factor A (Types of Cutting)	Factor B (Growing Media)
A_1 - Shoot tip	B_1 - Pure garden soil (control)
A_2 - Nodal	B_2 - 1/2 Garden soil + 1/2Vermicast
A_3 - Wedge stem	B_3 - Pure Vermicast
	B_4 - 1/2 Vermicast +1/2 Sand
	B_5 - 1/3Vermicast+1/3Garden soil+1/3 Sand

The different treatments and treatment combinations are shown in Table 1.

Table 1. Treatments and treatment combinations

TYPES OF CUTTING (FACTOR A)	GROWING MEDIA (FACTOR B)	TREATMENT COMBINATIONS/ CODES	TREAT-MENTS
Shoot tip (A_1)	▪ Pure garden soil (Control –B_1)	A_1B_1	T_1
	▪ ½ Garden soil + ½ Vermicast (B_2)	A_1B_2	T_2
	▪ Pure vermicast (B_3)	A_1B_3	T_3
	▪ ½ Vermicast + ½ Sand (B_4)	A_1B_4	T_4
	▪ 1/3 Vermicast + 1/3 Garden soil + 1/3 Sand (B_5)	A_1B_5	T_5
Nodal (A_2)	▪ Pure garden soil (Control –B_1)	A_2B_1	T_6
	▪ ½ garden soil + ½ Vermicast (B_2)	A_2B_2	T_7
	▪ Pure vermicast (B_3)	A_2B_3	T_8
	▪ ½ Vermicast + ½ Sand (B_4)	A_2B_4	T_9
	▪ 1/3 Vermicast + 1/3 Garden soil + 1/3 Sand (B_5)	A_2B_5	T_{10}
Wedge stem (A_3)	▪ Pure garden soil (Control –B_1)	A_3B_1	T_{11}
	▪ ½ Garden soil + ½ Vermicast (B_2)	A_3B_2	T_{12}
	▪ Pure vermicast (B_3)	A_3B_3	T_{13}
	▪ ½ Vermicast + ½ Sand (B_4)	A_3B_4	T_{14}
	▪ 1/3 Vermicast + 1/3 Garden soil + 1/3 Sand (B_5)	A_3B_5	T_{15}

A two-layer 2-ply B-net nursery was established with a height of 3 meters and a considerable 50-60% shade in the inside condition. It has a strong support of metal and wooden frames including the door. The ground terrain inside the nursery was elevated and was compartmentalized with cemented boxes for the set-ups (Appendix Figure 1). The terrain was rolling; hence, canals were defined along the cemented boxes.

The study utilized 10 sq.m. area (10 m long and 1 m wide) and was enclosed by a screened nursery. The area was divided into 45 small plots of equal sizes (0.31 m x 0.38 m or an area of 0.12 sq.m. each plot) corresponding fifteen (15) treatments with an alleyway between plots of 0.076 m. as well as 0.5 m from plots to the nursery wall. The lay-out is shown in Appendix Fig. 2.

A composite sample of 1 kg for each of the growing media was taken and air-dried for chemical analysis. These air-dried samples were then submitted to the City Soil Testing Laboratory, City Agriculture Office, Casisang, Malaybalay City for routine analysis such as organic matter (OM), phosphorus (P), potassium (K) and pH.

All the growing media were sterilized using hot water treatment prior to polypotting.

Stevia planting materials were purchased from a nursery in Baungon, Bukidnon and the plastic cups (7 oz) from an agricultural supply at Malaybalay City. The garden soil and sand were procured from the garden area of the City Agriculture Office, Casisang, Malaybalay City, while the vermicast from an agricultural supply in Malaybalay City.

Each growing medium was weighed equally prior to polypotting with the vermicast as the basis for all the growing media combinations for all the pots. For the growing media combinations, a 100% pure garden soil, 1/2 garden soil + 1/2 vermicast, 100%

vermicast, 1/2 vermicast + 1/2 sand and 1/3 vermicast + 1/3 garden soil + 1/3 sand were allocated to various treatment samples. Twenty plastic cups filled with media mixtures were placed according to their respective lay-out per treatment per replication.
For the types of cuttings: three nodes shoot tip cuttings, one node nodal cutting and one inch wedge stem cutting for each sample was prepared. Nodal and wedge stem cuttings were taken from medium to matured stems of Stevia mother plants. All the cuttings were dipped in tap water prior to planting. One cutting was planted per pot.

A total of nine hundred (900) cuttings were planted for the entire study with one cutting per pot. The potted plastic cups were watered using the sprinkler before planting to prevent the cuttings from washing-out if sprinkling is done after planting. Labeling was done according to respective treatments.

A 10 m long x 1.25 m wide transparent polyethylene cellophane was established 0.5 m above the experimental set-up after planting the cuttings to the pots to prevent the young cuttings from direct heat of the sun and heavy rains.

Watering. Water was applied to the cuttings 2 to 3 times a day or as needed. The soil was kept moist all the time, but not water soaked, to prevent the cuttings from wilting.

Cultivation. The growing media were cultivated from time to time where the cuttings were grown to facilitate proper aeration for the cuttings to produce roots.

Diseases Control. Diseases attacking the plants were not controlled, but were being observed and recorded.

Among the data gathered were the average plant height, survival rate, incidence of leaf curling and leaf dark spot, and number of branches and leaves per plant.

The analysis of variance (ANOVA) using factorial in Completely Randomized Design (CRD) was used to solve for the level of significance. The Tukey Test was used to compare significant differences among treatment means.

III. Results and Discussion

Average Plant Height (cm)

The average plant height of Stevia cuttings at 60 days after planting (DAP) in response to growing media is presented in Table 2. Statistical analysis revealed that heights of stevia cuttings upon termination of the study were highly influenced by the propagation methods (Factor A). Similarly in Factor B, the respective heights of the plants were affected by the growing media used. However, no significant interaction effect was observed between the two factors.

The types of cuttings in propagating Stevias produced different heights at the end of the study. Shoot tip cuttings had the tallest plants with an average height of 13.72 cm which were significantly different from those propagated using wedge stem and nodal cuttings with 4.84 cm and 4.51 cm, respectively (Fig. 1). Since the shoot tip cuttings used at the start of the study had an average height of 5 cm containing 3 nodes from the tip while the nodal and wedge stem cuttings just bulging the bud eyes from the stems, it is therefore expected that those grown using shoot tips had unprecedented leap of the heights compared to the latter two months after the study started. Shoot tip cuttings bind to the idea that growth is fast due to the controlling power of auxin for apical dominance (Hartman and Kester, 2013).

Table 2. Average plant height (cm) of Stevia (*Stevia rebaudiana* Bert.) cuttings at 60 day after planting (DAP) in response to different growing media

GROWING MEDIA (B)	PROPAGATION METHODS (A)			Mean (B)*
	Shoot Tip Cutting	Nodal Cutting	Wedge Stem Cutting	
Pure Garden Soil	11.43	3.73	3.90	6.35^{b}
½ Garden Soil + ½ Vermicast	16.23	4.97	5.23	8.81^{a}
Pure Vermicast	13.81	4.33	4.43	7.52^{ab}
½ Sand + ½ Vermicast	12.48	4.57	4.80	7.28^{ab}
1/3 Garden Soil + 1/3 Vermicast + 1/3 Sand	14.67	4.93	5.83	8.48^{ab}
MEAN (A)**	13.72^{a}	4.51^{b}	4.84^{b}	
F-test: A**, B*, A x B ^{ns}				
CV: 20.53%				

Means within the same column and row followed by common letters are not significantly different at 5% level of significance based on Tukey's Test.
*** - highly significant,* - significant, ns - non significant*

According to Lab (2012) that auxin induces shoot apical dominance; the axillary buds are inhibited by auxin, as a high concentration of auxin directly stimulates ethylene synthesis in axillary buds, causing inhibition of their growth and potentiation of apical dominance. When the apex of the plant is removed, the inhibitory effect is removed and the growth of lateral buds is enhanced. Auxin is sent to the part of the plant facing away from the light, where it promotes cell elongation. If there is no cutting happened in the apical part or the shoot tip, the growth would be faster since auxin is polar, hence, more of its concentration focuses on the apex part.

The growth and proliferation of the cuttings has something to do with the various growing media used based on the independent interaction effects of each factor.

For the various growing media, it showed also that cuttings grown in ½ garden soil + ½ vermicast had the tallest plant with 8.81 cm upon the termination of the study (Fig. 2). It was, however, not significantly different from those grown in (1/3 garden soil + 1/3 vermicast + 1/3 sand) and 100% pure vermicast with 8.48 cm and 7.53 cm, respectively; but were statistically far from those grown in pure garden soil with 6.35 cm only. Based from the soil analysis of the growing media and the kinds of the media themselves, nutrients are contributory to the growth performance of the cuttings especially plant height. According to Hartman and Kester (2013) and Bautista (1994), the 3 macronutrients such as nitrogen, phosphorus and potassium have special roles on the growth and development of the plants which were contained in four organic growing media except with few amounts in pure garden soil alone.

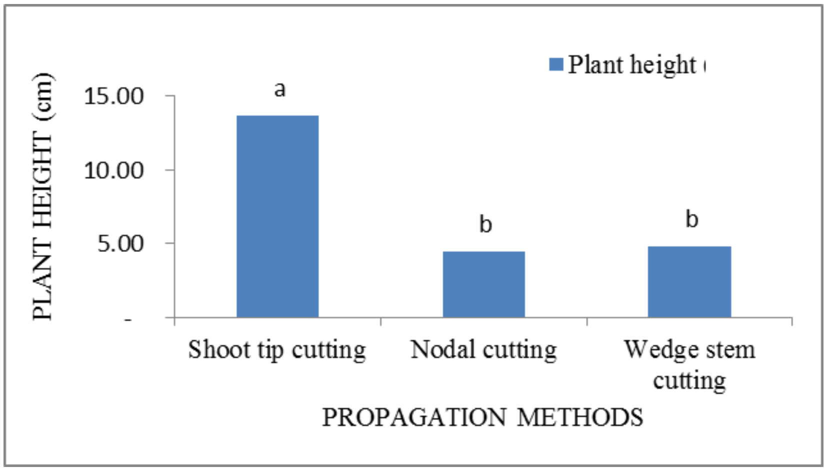

Fig. 1. Average plant height (cm) of Stevia at 60 DAP as affected by propagation methods

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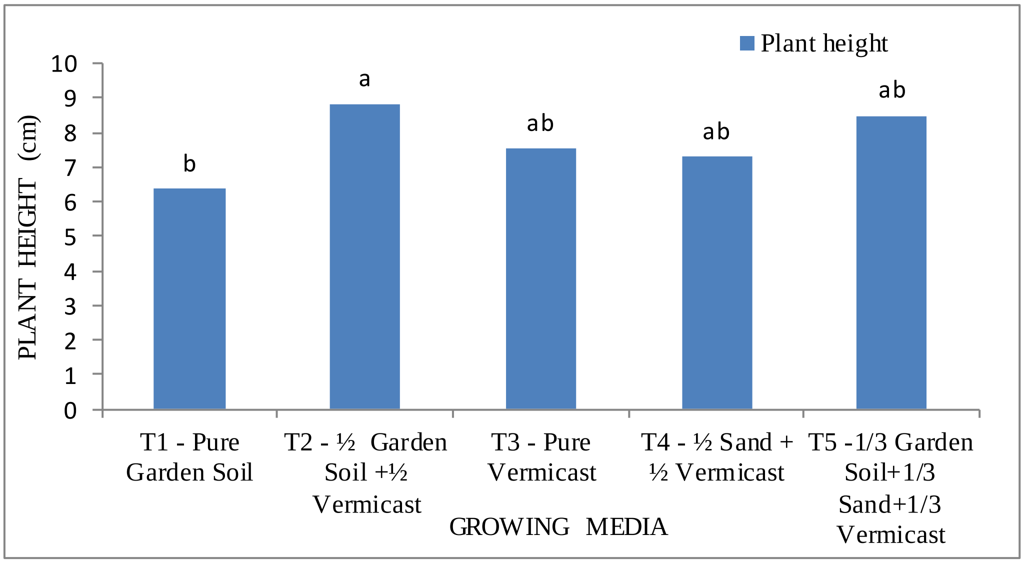

Fig. 2. Average plant height (cm) of Stevia at 60 DAP as affected by growing media

Survival Rate

The percent survival of Stevia in response to types of cuttings and growing media is presented in Table 3. Statistical analysis showed no significant difference observed regardless of the types of cuttings (Factor A) and the growing media (Factor B) used. No interaction effect was likewise observed between the two factors.

However, the combination of shoot tip cuttings with 1/3 garden soil + 1/3 vermicast + 1/3 sand as growing media obtained the highest survival rate with 96% for the former and 97.33% for the latter, respectively, though their differences were not that significantly different from the rest of the treatment combinations.

Stevia plants using nodal cuttings in Factor A obtained the lowest survival rate with 92.07%, while those grown in pure garden soil in Factor B had 92.22% survival rate.

According to Kassahun et al. (2013), top cuttings or shoot tip cuttings with three nodes among Stevia plants demonstrated significantly higher values of survival rate (82.5%), but are not that far from cuttings taken at the bottom. Consistently in this study, shoot tip cuttings also obtained the highest survival rate at 96% compared to nodal and wedge stem which had only 92.07% and 93.67%, respectively. Considering that the whole set-ups were placed under screened house or B-net nursery, survival rate had been high for all the treatments.

Table 3. Survival rate (%) of Stevia (*Stevia rebaudiana* Bert.) cuttings in response to growing media

TREATMENTS	SURVIVAL RATE (%)
Propagation Methods (A)	
Shoot Tip Cutting	96.00
Nodal Cutting	92.07
Wedge Stem Cutting	93.67
Growing Media (B)	
Garden Soil	92.22
½ Garden Soil + ½ Vermicast	96.11
Vermicast	92.78
½ Sand + ½ Vermicast	91.11
1/3 Garden Soil + 1/3 Vermicast + 1/3 Sand	97.33
F-test: A^{ns}, B^{ns}, A x B^{ns}	
C.V. (%)	6.20

ns - non significant

Leaf Curling and Leaf Dark Spot Incidence

The incidence of leaf curling and leaf dark spots among Stevia cuttings propagated using different growing media are shown in Table 4. No significant differences were observed regardless of the types of cuttings and growing media as well as their respective interaction effects.

Leaf curling occurred in plants especially Stevia in the younger stage due to deficiency in calcium in the growing media, thus making the leaves to have downward position (Cuenen, 2010). Thus, as shown in Table 5, the highest incidence of leaf curling was observed in propagated cuttings (Factor B) grown in garden soil with 2.0, while the lowest was on cuttings grown in media with 1/3 garden soil + 1/3 vermicast + 1/3 sand with 1.22 incidence per plot. However, their differences were not that significant regardless of the growing media used.

For the propagation methods (Factor A), it was revealed that Stevia cuttings using nodes obtained the highest incidence of leaf curling per plot with 1.80, while the lowest was with those propagated using wedge stems with 1.40 occurrences. Their differences, however, were found not significantly different from each other.

For leaf dark spots, it showed that the lower incidence was observed on cuttings propagated using nodes and wedge stems (both 1.53) in Factor A, with a bit higher incidence on those propagated using shoot tips with 1.73. However, their differences were said to be not significantly far from each other. Likewise, it was revealed in Factor B that stevia cuttings grown in pure garden soil had the most number of leaf dark spots with 1.89, while the least with those grown in 1/3 garden soil + 1/3 vermicast + 1/3 sand with 1.22 plants infected per plot. According to Cuenen (2010), leaf dark spots among stevia plants are due to lack of calcium resulting to chlorosis and necrosis of leaves.

The incidence of both leaf curling and leaf dark spots in Stevia plants only ranged from 1 to 2 plants having these deficiency symptoms per plot with a total of 20 plants in a plot (or only 5 to 10% of the plants in the plot). The degree of damage was just slight and insignificant since only few leaves in selected plants were infected with the symptoms.

Table 4. Leaf curling and leaf dark spot incidence in Stevia (*Stevia rebaudiana* Bert.) cuttings in response to growing media

TREATMENTS	LEAF CURLING INCIDENCE	LEAF DARK SPOT INCIDENCE
Propagation Methods (A)		
Shoot Tip Cutting	1.47	1.73
Nodal Cutting	1.80	1.53
Wedge Stem Cutting	1.40	1.53
Growing Media (B)		
Garden Soil	2.00	1.89
½ Garden Soil + ½ Vermicast	1.56	1.67
Vermicast	1.44	1.67
½ Sand + ½ Vermicast	1.56	1.56
1/3 Garden Soil + 1/3 Vermicast + 1/3 Sand	1.22	1.22
F-test:		
A	ns	ns
B	ns	ns
A x B	ns	ns
C.V. (%)	14.55	19.46

ns – non significant

Average Number of Branches per Rooted Cutting

Table 5 reflects the average number of branches per rooted cutting or per plant of Stevia grown in different growing media. Statistical analysis revealed that propagation methods (Factor A) and growing media (Factor B) highly influenced the number of branches per rooted cutting. No interaction effect was found between these factors.

In Factor A, Stevia grown using shoot tip cuttings obtained the most number of branches per plant with 2.69 and was significantly different from those using wedge stem and nodal cuttings with 1.17 and 0.99, respectively (Fig. 3). Propagating Stevia using wedge stem cuttings was not statistically different from those using nodal cuttings. As Gvasaliye et al. (1990) also reported that the rooting of cuttings was best in cuttings taken from side shoots and from tops of the main shoot in honey grass. Cuttings from the top part of the main stem of Stevia generally gave the best result (Tirtoboma, 1988). Top cuttings with three nodes demonstrated significantly higher values of number of branches per seedling (Kassahun et al., 2013).

According to Hartman and Kester (2013), apical dominance is apparent among shoot tips with high concentration of auxin, thus, promoting growth faster. Shoot tips were already defined having 3 nodes from the tip which were used in the study as compared to nodal and wedge stem cuttings where the buds were still bulging and whose stem nodes have yet to be defined.

Similarly in Factor B, stevia cuttings grown in 1/3 garden soil + 1/3 vermicast + 1/3 sand had the most average number of branches per plant with 2.04. It was, however, not significantly different from those grown in ½ garden soil + ½ vermicast, ½ sand + ½ vermicast and pure vermicast with 1.73, 1.69 and 1.62 branches, respectively (Fig. 4). Those grown in pure garden soil only obtained the least with 0.99 branch per plant. Hartman and Kester (2013) emphasized the roles of nutrients such as nitrogen, phosphorus and potassium in the cell division, differentiation and elongation as well as nutrient uptake and transport to different growth regions in plants; thus, giving rise to the development of many nodal points and branches.

Top cuttings with three nodes demonstrated significantly higher values of number of branches/seedling (7), number of leaves/branch (15) and number of leaves/seedling (56) and lowest values of these parameters were recorded for bottom cuttings (Kassahun et al., 2013).

Table 5. Average number of branches per rooted cutting of Stevia (*Stevia rebaudiana* Bert.) cuttings in response to growing media

GROWING MEDIA (B)	PROPAGATION METHODS (A)			Mean (B)*
	Shoot Tip Cutting	Nodal Cutting	Wedge Stem Cutting	
Pure Garden Soil	2.43	0.23	0.50	0.99[b]
½ Garden Soil + ½ Vermicast	2.63	1.17	1.40	1.73[a]
Pure Vermicast	2.71	1.00	1.17	1.62[a]
½ Sand + ½ Vermicast	2.61	1.27	1.20	1.69[a]
1/3 Garden Soil + 1/3 Vermicast + 1/3 Sand	3.25	1.30	1.57	2.04[a]

MEAN (A)**		2.69^{a}	0.99^{b}	1.17^{b}
F-test:	A**, B*, A x B^{ns}			
CV:	26.86%			

Means within the same column and row followed by a common letter are not significantly different at 5% level of significance based on Tukey's Test.
*** - highly significant, ns - non significant*

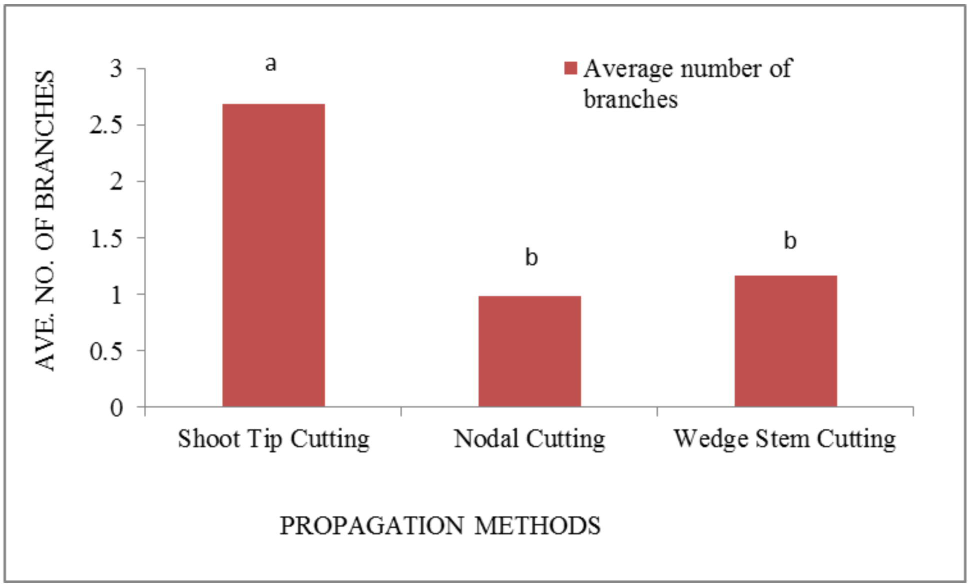

Fig. 3. Average number of branches per rooted cutting of Stevia as affected by propagation methods

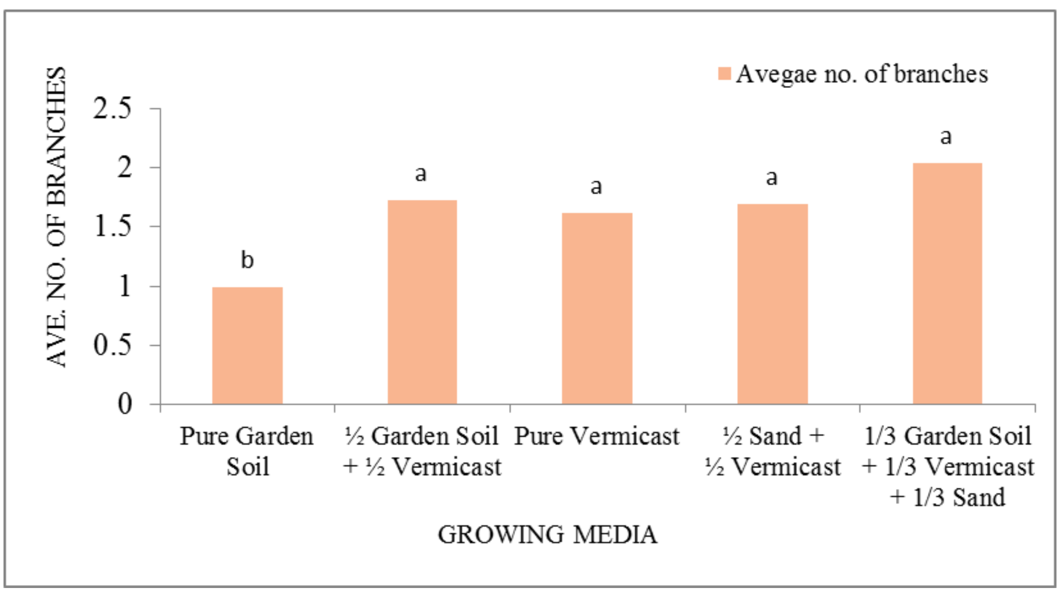

Fig. 4. Average number of branches per rooted cutting of Stevia as affected by growing media

Average Number of Leaves per Plant

Propagation methods (Factor A) as well as growing media (Factor B) greatly influenced the production of leaves of Stevia two months after planting with no interaction effect found between the two factors (Table 6) .

The production of leaves conforms to the production of branches (Table 5) on propagation methods and growing media as well as their respective interaction effects. The number of branches likewise determines the numbers of leaves since nodes are borne to every branch.

On propagation methods, it showed that Stevia propagated through shoot tip cuttings obtained the most number of leaves with 22 and was significantly different from those grown using nodal and wedge stem cuttings with 15.87 and 14.73, respectively (Fig. 5). It is also evident that as more branches were produced using shoot tip cuttings, more leaves were likewise being borne because many nodes were also produced regardless of the sizes of the leaves. Faster growth was also observed among shoot tip cuttings as compared to nodal and wedge stems. Cuttings from the top part of the main stem of stevia generally gave the best result (Tirtoboma, 1988).

Results on growing media revealed that the combination of 1/3 garden soil + 1/3 vermicast + 1/3 sand produced the most number of leaves per plant with 20.56 regardless of the independent interaction effects of the two factors (Fig. 6). It was, however, not significantly different from those grown using ½ garden soil + ½ vermicast with 19.22, but is statistically different from the rest of the treatment combinations.

Those grown using pure garden soil only obtained 13.56 leaves per plot. Stevia grows well in sandy loam soils with an enough supply of water during the month of October as exhibited in the balanced media combinations of 1/3 garden soil + 1/3 sand + 1/3 vermicast. Stevia prefers acidic to neutral soil with a pH range of 6.5-7.5 for its best growth. Saline soils should be avoided as stevia plant is susceptible to water logged conditions. (http://stevia.blogspot.com). Stevia will not grow in saline soils (Todd, 2010).

Table 6. Average number of leaves per plant of Stevia (*Stevia rebaudiana* Bert.) cuttings in response to growing media

GROWING MEDIA (B)	PROPAGATION METHODS (A)			Mean (B)*
	Shoot Tip Cutting	Nodal Cutting	Wedge Stem Cutting	
Pure Garden Soil	18.00	10.67	12.00	13.56[d]
½ Garden Soil + ½ Vermicast	22.67	17.33	17.67	19.22[ab]
Pure Vermicast	21.67	14.33	14.67	16.89[c]
½ Sand + ½ Vermicast	21.00	15.33	16.00	17.44[bc]
1/3 Garden Soil + 1/3 Vermicast + 1/3 Sand	26.67	16.00	19.00	20.56[a]
MEAN (A)**	22.00[a]	14.73[b]	15.87[b]	
F-test: A**, B*, A x B[ns]				
CV: 8.84%				

Means within the same columns and rows followed by common letters are not significantly different at 5% level of significance based on Tukey's Test.

*** - highly significant, ns - non significant*

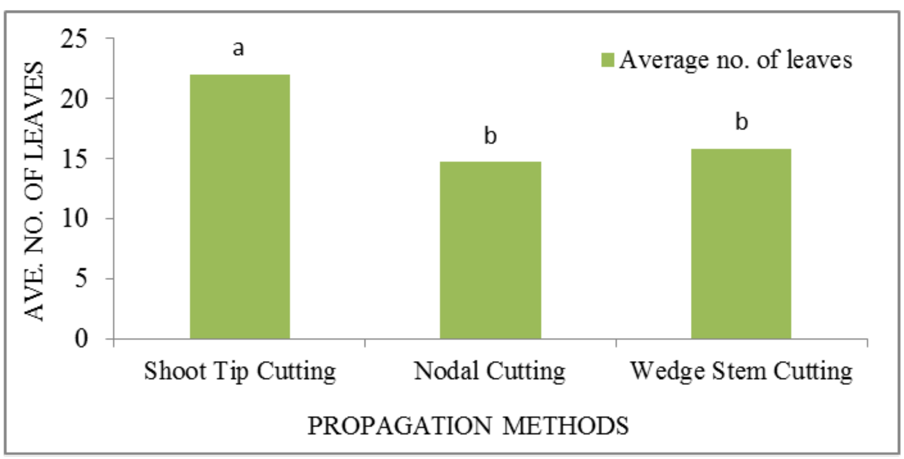

Fig. 5. Average number of leaves per plant of Stevia as affected by propagation methods

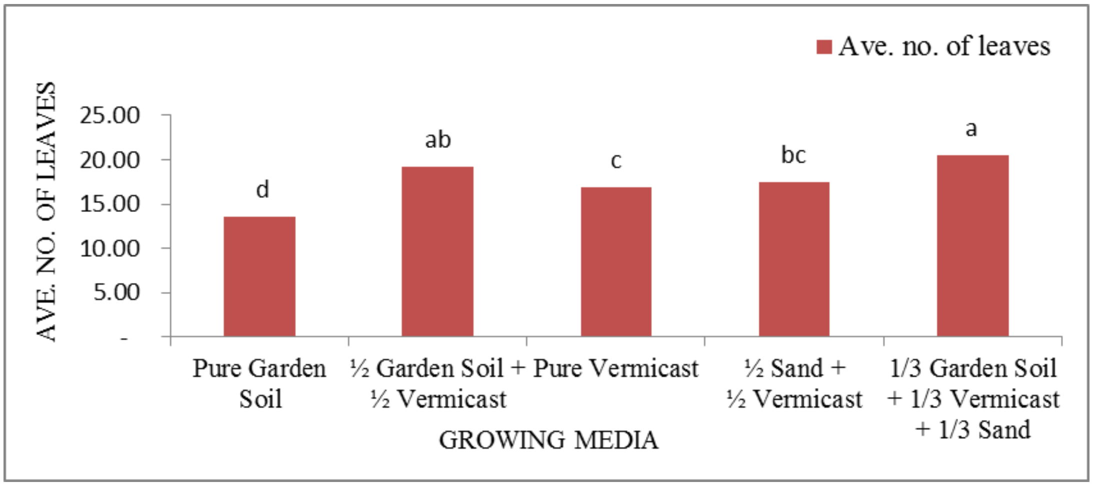

Fig. 6. Average number of leaves per plant of Stevia as affected by growing media

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