

Vegetative Propagation of Stevia (*Stevia rebaudiana* Bertoni Hemsl) Using Stem Tip Cuttings in Different Growing Media

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The study was intended to teach students the essence of recycling farm wastes to support the cultivation of valuable crops like stevia (*Stevia rebaudiana* Bertoni Hemsl). The study was undertaken to evaluate different combinations of growing media in the asexual propagation of stevia, specifically on the rooting and survival of stem tip cuttings, and to evaluate the best growing medium that can enhance survival and production. The different growing media combinations were recycled from the farm, namely: spent mushroom compost (M), chicken manure compost (Ck), fine sand (FS), and garden soil (GS). The earliest emergence of root initiation of cuttings (8.00 ± 1.73 to 8.67 ± 1.15 days) was recorded in T5 (1Ck:1FS v/v), T6 (3Ck + FS v/v) and T7 (1GS:3Ck v/v). The poor survival of T5 stevia was deemed attributable to the poor root initiation (22.23 ± 9.93) of cuttings. Longer roots were observed in cuttings grown in T5 and T6 (6.40 ± 0.26 to 6.40 ± 1.00). The highest number of shoots produced per cutting ($7.27^a \pm 0.50$) was recorded in T9 (3GS + 1Ck v/v), while T8 (1GS:1Ck v/v) produced the lowest number of shoots. Stevia cuttings grown in T3 (3GS:CMS (3:1 v/v)) registered the highest mean survival rate (82.22 ± 38.95), and comparable to T1 (66.67 ± 5.80), T6 (65.53 ± 6.93), T8 (55.57 ± 16.43), and T9 (71.10 ± 6.96), all markedly higher relative to the control group (21.13 ± 5.10). Present findings point to survival of stevia being enriched in growing medium comprising GS, M and CK. In light of these findings, experimentation on the combination of animal manure compost, spent mushroom compost and garden soil, and studies on the quality of leaves produced including chemical analysis are highly recommended.

Key words: Agrivet Sciences Institute, rooting media, Salikneta Farm, stevia cuttings

INTRODUCTION

Stevia is a herb that grows naturally in tropical climates like Paraguay and Brazil, where it grows wild as a small shrub and has long been used as a sweetener (Muth 2011; Madan et al. 2010). There are more than 100 species of stevia plant belonging to family Asteraceae, but *Stevia*

rebaudiana Bertoni Hemsl stands out for its excellent properties as a sweetener (Barreda et al. 2015). Stevia's sweetening property is attributed to its component glycosides. These include rebaudioside A, the sweetest-flavored leafy component that is chemically similar to sugar in onset, intensity, and duration of sweetness without aftertaste. Another component is the white crystalline compound stevioside, a natural herbal sweetener with

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no calories and 100-300 times sweeter than table sugar (Goyal & Samsher Goyal 2010; Bayraktar et al. 2016).

Benefits of *S. rebaudiana* are manifested in the regulation of glucose (Singh et al. 1996), and in renal protection in rats (Assaei et al. 2016; Shivanna et al. 2012). In studies on human subjects, stevia has been documented to improve glucose tolerance and induces the release of insulin from pancreas and thus, is regarded as a potential mediator for type 2 diabetes treatment (Goyal & Samsher Goyal 2010). However, there are toxicological findings based on test-tube and animal studies with stevioside component causing malignancies, such as chromosomal damage, genetic mutations, and DNA breakage, that are contributory to cancer (Kobylewski & Eckhart 2008). Interestingly, numerous clinical studies in Japan had generated data in support of the safety of stevia for human use (Muth 2011). Nonetheless, the dearth of long-term studies on the use and effects of stevia needs to be worked out further to substantiate earlier discordant findings.

At present, despite conflicting findings, stevia has become a major source of high-potency inducements for the burgeoning natural food market as tea, sugar, source of organic extract that comes in flavours and in salads, and as a food supplement in food products and beverage sweetener (Goyal & Samsher Goyal 2010). Stevia is still considered a very popular sugar substitute and thus, its sustained propagation is imperative.

Stevia plants are small and delicate and produce seeds that are difficult to handle. Expansion of the production of herbs in nurseries and farms requires growing media with the essential ingredients for plant growth and development like organic manure and bio-fertilizers (Murthy et al. 2010; Vessey 2003). These pre-requisites could enhance the yield and quality of stevia (Umesha et al. 2011; Koppad & Gouda 2010). Increasing higher stevia productivity had been recorded using seed germination, stem cuttings, and micro-propagation. In micro-propagated leaf extracts, Smitha & Umesha (2012) documented 84.6% callus induction compared to 60% in stem cutting under natural and greenhouse condition with the application of indole butyric acid solutions dipped in different plant growth regulators solution, growth of stevia stem cuttings vary. While germination rates between while fresh and Gy gamma-irradiated seeds do not manifest marked difference in their germination rate, the viability of fresh seeds at post-harvest is higher (Khalil et al. 2014).

In light of the increasing demands for stevia production, and considering the difficulty of its propagation using seeds, the present study sought to evaluate different combinations of growing media in the asexual propagation, specifically on the rooting and survival of stem tip cuttings, and to evaluate the best growing medium

that can enhance survival and production. It also aimed to teach the students the essence of recycling farm wastes to support the cultivation of valuable crops like stevia.

MATERIALS AND METHODS

Study Site, Procurement of Stevia Seedlings, and Management of Seedlings as Mother Plants

The study was conducted at Salikneta Farm (San Jose Del Monte, Bulacan), which services a school that nurtures the skills of Filipino scholars from Luzon, Visayas, and Mindanao in agriculture. Nine hundred (900) stevia seedlings, about one inch in height per seedling, were individually sowed in small plastic trays. The seedlings (priced at Php 9.00 each) were procured from mother plants of stevia grower at Santa Maria Subdivision, (San Francisco, Mabalacat, Pampanga, Philippines), and were transported to the farm on the same day the seedlings were delivered to the Sciences and Technology Research Center of De La Salle University-Manila. The seedlings were transplanted in the greenhouse and at two months post-transplanting were used as mother plants (Fig. 1). The mother plants provided the source of stem tip cuttings used in the experimentation carried out.

Preparation of Growing Media and Transplantation of Stem Tip/Bare Root Cuttings

A total of 900 cuttings of stevia were prepared by cutting the stem tip with three nodes from mother plants (Fig. 1). Slanted/diagonal cuts at 1 cm length below the terminal node were made where lateral roots of cutting emerged. Cuttings were treated with the rooting hormone-alpha-naphthalene acetic acid (ANAA) by dipping the end base for about 60 s prior to planting. Cuttings were planted individually into the holes previously prepared on the growing media. These served as community container until the cuttings were already established about one month after planting i.e., the cuttings had already rooted and produced shoots. One month later, rooted cuttings were transplanted to black plastic bags containing different combinations of growing media (Fig. 2). The growth media comprised a combination of garden soil, fine sand, spent compost of *Pleurotus* sp. grown in Salikneta, and/or chicken manure compost procured from a nearby poultry farm.

Per growing medium, a total of 30 rooted stevia were used at 10 plants per trial and in triplicates (Fig. 3). A total of 30 black plastic bags (9cm x 15cm) were used, divided into three (3) groups of 10 bags representing each replicate for each of the three combinations of growing media used (Fig. 4). Figure 5 shows samples of transplanted rooted stevia stem tip cuttings used in the final observation on survival rate, length of root, and stem and number of shoots per cutting.

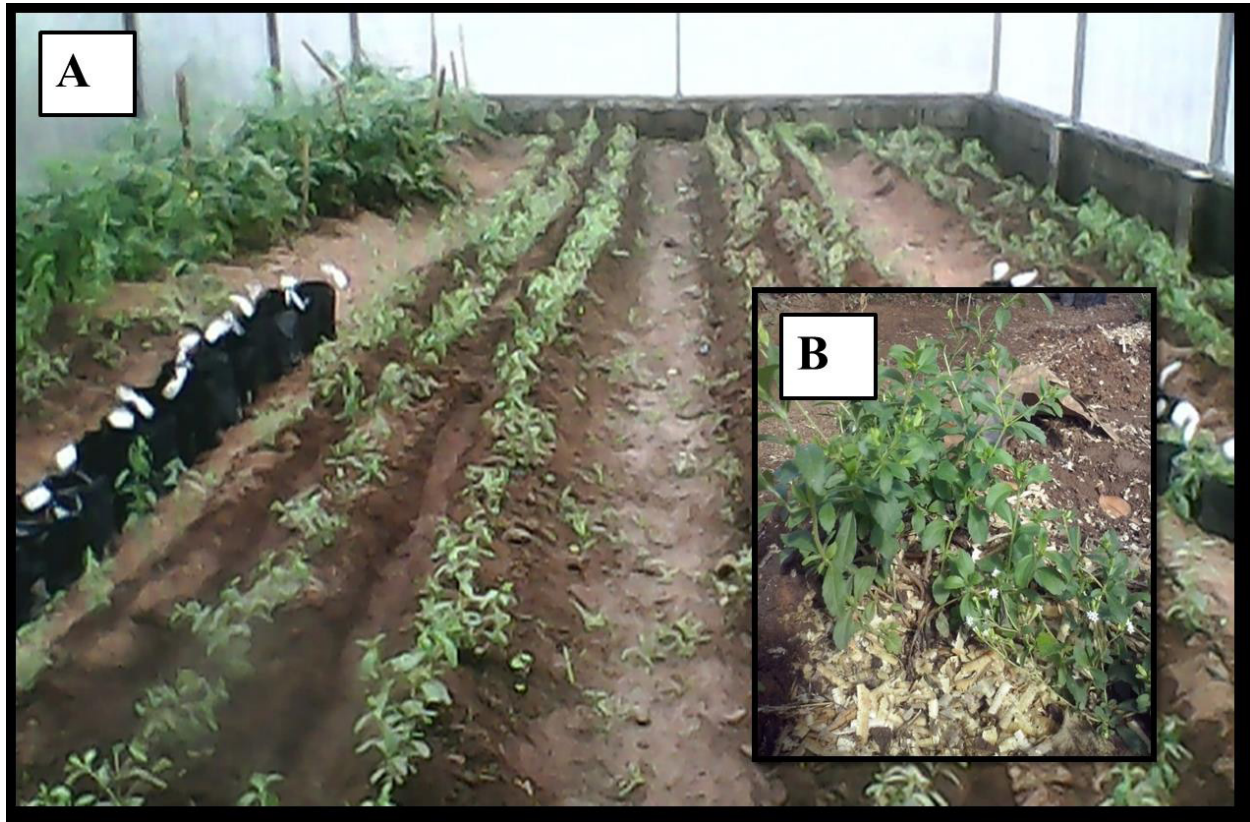


Figure 1. Transplanted purchased stevia seedlings in the greenhouse (A, B) cultivated as mother plants for cutting materials used in the experiment (two-months post-transplanting).



Figure 2. Black plastic bag containing the different growing media used for rooting of stevia stem tip cuttings. (A) chicken manure compost + fine sand (1:1 v/v); (B) garden soil + chicken manure compost (1:3 v/v); (C) chicken manure compost + spent mushroom compost (1:1 v/v); (D) garden soil + chicken manure compost + fine sand (1:1:1 v/v).

Data Analysis

One month post-transplanting in different growing media, the days of root initiation from transplanting, length of root and stem, number of shoots per cutting and survival were recorded, and analyzed using ANOVA

and Duncan's multiple range test (DMRT) at 5% level of significance.

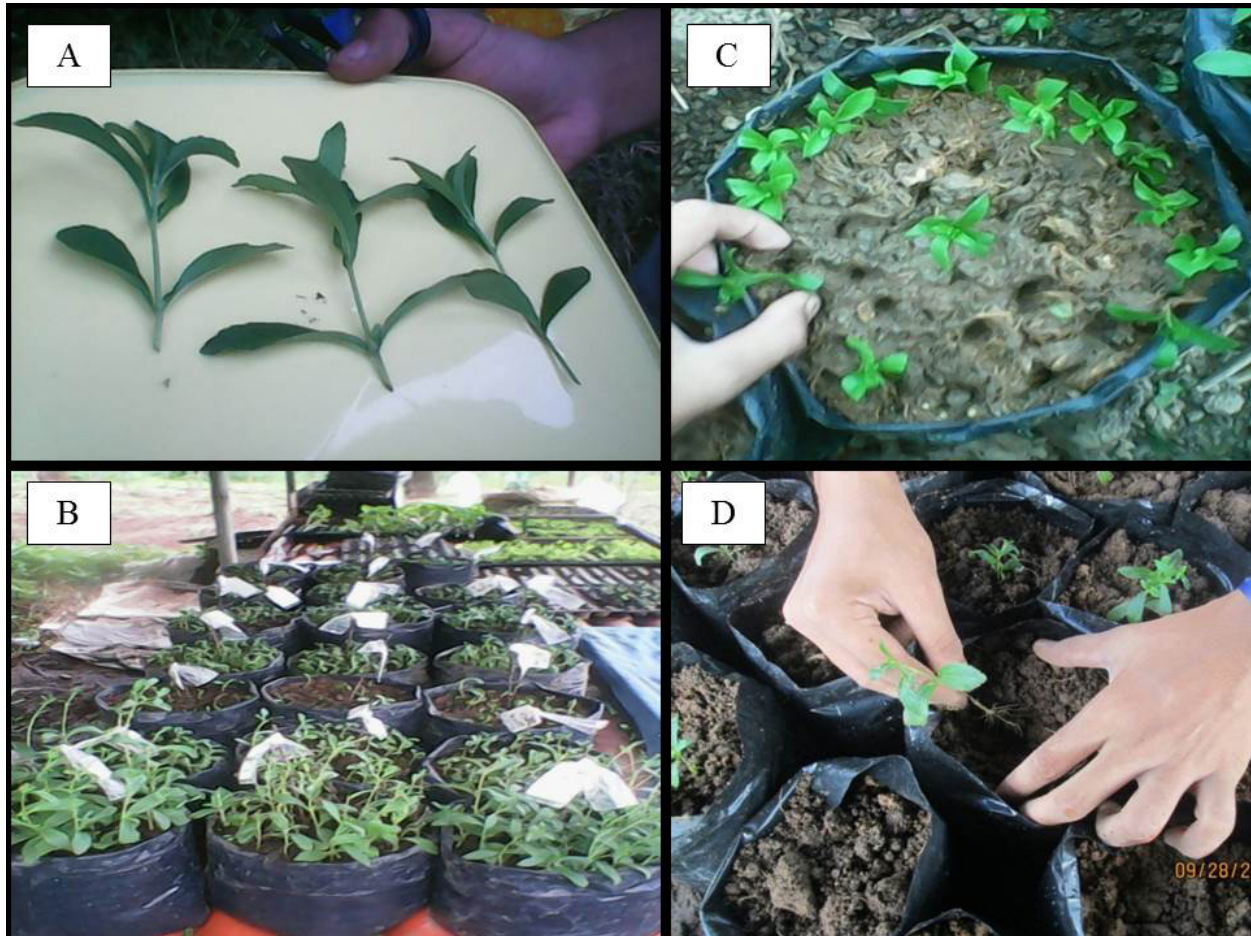


Figure 3. Stem tip cuttings from mother plants (A) established in different growing media (B) and gently uprooted and transplanted to individual black bags (C & D).



Figure 4. Set-up of individually transplanted stevia stem tip cuttings grown in different growth media.

RESULTS AND DISCUSSION

The earliest emergence of root initiation of cuttings was recorded in T5, T6, and T7 at days ranging from 8.00 ± 1.73 to 8.67 ± 1.15 , four days earlier compared to T1 (Table 1; Fig. 7). The shorter duration indicates the porosity and better aeration in fine sand and rich organic materials present in the decomposed chicken

manure which may have speeded up the development of root structure. Present results are in agreement with the reports that stevia cuttings are best grown in media with adequate nutrients and good draining capacity (Cedo et al. 2014; Aquino 2011). Longer roots were observed from stevia cuttings grown in T5 and T6 (6.40 ± 0.26 to 6.40 ± 1.00 cm), 2.62cm longer than cuttings grown in T3, suggesting that the development of roots in stem tip



Figure 5. Transplanted rooted stevia stem tip cuttings for final observation on survival rate, length of root and stem, and number of shoots per cutting.

cuttings is not dependent on the type/combinations of growing media. The highest number of shoots produced per cuttings was recorded in T9 (7.27 ± 0.50), while T8 registered the lowest number of shoots. The longest stems were recorded in T4 (Table 1; Fig. 5). While stevia cuttings grown in T3 had the highest mean survival rate of 82.22 ± 38.95 , 61.1% higher than the control group, T3 percent survival is statistically insignificant compared to T1, T6, T8, and T9. However, survival of stevia stem tip cuttings in the presence of composted manure animal and crop

manure in the present study is higher compared to those earlier reports on stem cuttings (Shahid et al. 2014).

Stevia grown in garden soil, fine sand, and composted chicken manure (representing the control group), had the lowest survival rate (21.13 ± 5.10 days) compared to the experimental group where only one type of soil was used (either garden soil or fine sand) with composted manure; this suggests that stevia needs organic materials in their nourishment. This account is corroborated by Fianza et al. (2010), who had asserted that mushroom compost applied or incorporated into the soil improves soil structure, and water status, as it reduces soil compaction, increases plant nutrient availability, and soil microbes.

The survival of stevia stem tip cuttings in the presence of composted animal and crop manure in the present study is higher compared to similar earlier reports (Shahid et al. 2014). The overall healthy growth performance of stevia in the present study where rooting hormone was used proved beneficial to growth induction which is consistent with earlier studies exhibiting 76.8% sprouting of stevia dipped in different plant growth hormone prior to planting (Smitha & Umesha 2012; Shahid et al. 2014).

CONCLUSION AND RECOMMENDATIONS

Growth medium containing garden soil and spent composted mushroom supported higher survival relative to other mixtures. Experimentation on the use of garden soil, composted mushroom spent, and combination of animal manure composts (chicken, cow, water buffalo and/or carabao manure), including studies on the

Table 1. Comparison of the parameters of the growth of stevia cuttings in different growing media (mean \pm SD).

Treatment ¹	Days to Root Initiation	Root Length (cm)	Stem Length (cm)	Number of Shoots per Cutting ²	Percent (%)
					Survival ²
GS + Ck+ FS (1:1:)	9.00 ^a \pm 1.73	4.90 \pm 0.92	7.87 \pm 1.70	4.53 ^b \pm 0.31	21.13 ^c \pm 5.10
GS + M (1:3)	12.00 ^b \pm 0.00	4.87 \pm 0.84	6.23 \pm 1.31	5.53 ^{ab} \pm 1.33	66.67 ^{ab} \pm 5.80
GS + M (1:1)	10.00 ^{ab} \pm 0.00	4.95 \pm 1.18	8.23 \pm 0.73	5.93 ^{ab} \pm 1.80	47.40 ^{abc} \pm 38.95
GS+ M (3:1)	10.00 ^{ab} \pm 0.00	3.76 \pm 1.50	8.83 \pm 1.90	6.80 ^a \pm 0.20	82.22 ^a \pm 38.95
Ck + FS (1:3)	8.67 ^a \pm 1.15	5.10 \pm 2.10	12.93 \pm 9.13	5.00 ^b \pm 1.21	40.00 ^b \pm 28.87
Ck + FS (1:1)	8.00 ^a \pm 1.73	6.40 \pm 1.00	7.93 \pm 1.10	4.87 ^b \pm 0.90	22.23 ^c \pm 6.93
Ck + FS (3:1)	8.00 ^a \pm 1.73	6.40 \pm 0.26	6.17 \pm 1.20	5.00 ^{ab} \pm 0.70	65.53 ^{ab} \pm 6.93
GS + Ck (1:3)	8.00 ^a \pm 1.73	5.80 \pm 0.61	6.17 \pm 2.00	4.33 ^b \pm 0.50	47.77 ^{abc} \pm 22.67
GS + Ck (1:1)	9.00 ^a \pm 1.73	5.87 \pm 0.50	5.87 \pm 0.50	4.13 ^b \pm 0.70	55.57 ^{abc} \pm 16.43
GS + Ck (3:1)	10.00 ^{ab} \pm 0.00	5.16 \pm 0.81	9.23 \pm 1.2	7.27 ^a \pm 0.50	71.10 ^{ab} \pm 6.96

¹Garden soil (GS); Fine sand (FS); Spent mushroom compost (M); Chicken manure compost (Ck)

²Means followed by the same letter are not significantly different at 5%.

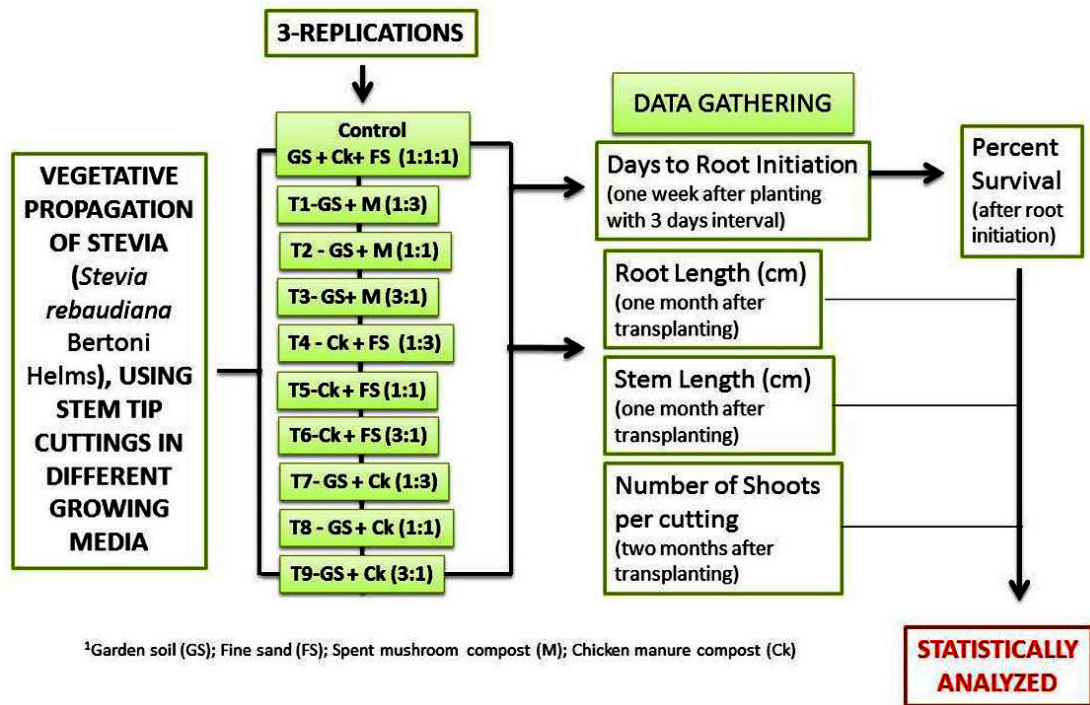


Figure 6. Summary of materials and sequence of execution of the experimentation.

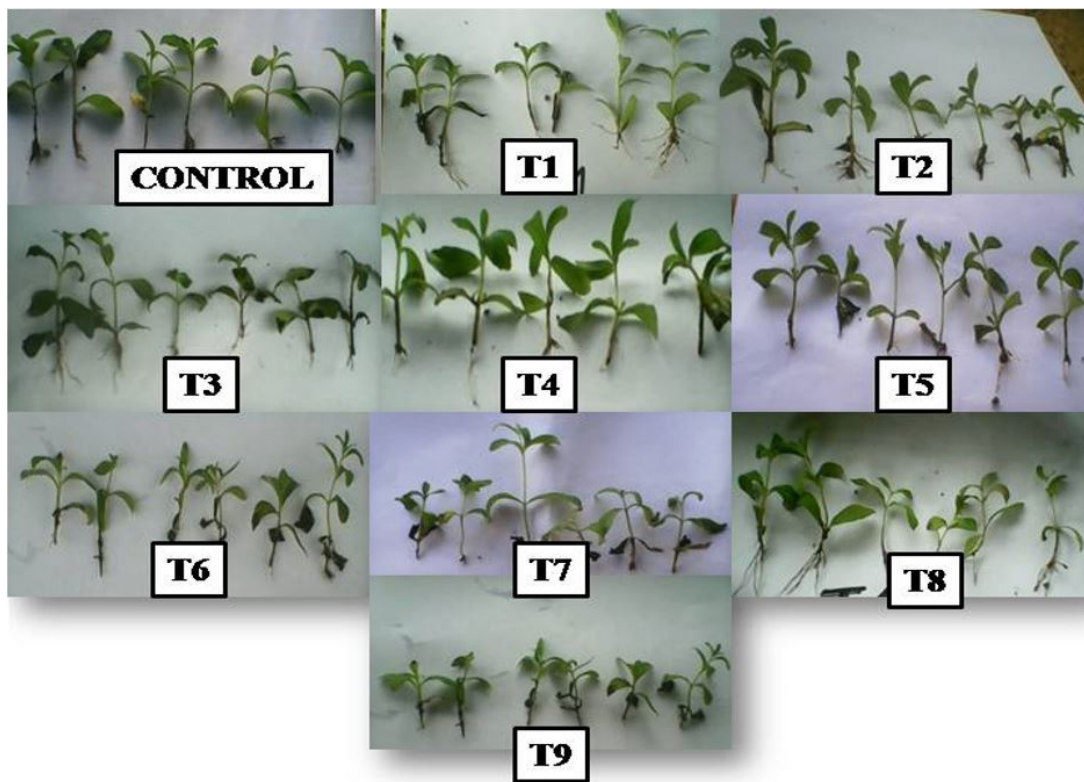


Figure 7. Representative samples of rooted stevia cuttings grown in different growing media. Control (GS + Ck+ FS (1:1), and Experimental: GS + M (1:3), GS + M (1:1), GS+ M (3:1), Ck + FS (1:3), Ck + FS (1:1), Ck + FS (3:1), GS + Ck (1:3), GS + Ck (1:1), GS + Ck (3:1).

quality of leaves produced plus chemical analysis are highly recommended. Future investigation designed to determine bulk density of all growth media used is likewise suggested.

ACKNOWLEDGMENTS

The researchers extend their gratitude to the Agrivet Sciences Institute, Salikneta, De La Salle Araneta University for the scholarship awarded to the main author. The primary author wishes to extend his deepest appreciation and thanks to Br. Jun Erguiza Jr., FSC, and to many other individuals who had helped to complete this small but useful project in a farm setting, which has led to the expansion and evaluation of its uses for income generation in the farm.

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