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Effect of Cutting Position and Rooting Hormone on Propagation Ability of Stevia (*Stevia rebaudiana* Bertoni)

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ABSTRACT

To fill in existing information and knowledge gaps on asexual propagation of stevia (*Stevia rebaudiana* Bertoni), this experiment was conducted at the Wondo Genet Agricultural Research Center nursery site in a plastic chamber. The experiment consisted of two levels of cutting positions (top and middle) and three levels of rooting hormones (zero hormone, 0.2% indole-3-butyric acid (IBA) and 0.4% 1-naphthalene acetic acid (NAA)). The experiment was laid out in a 3×2 factorial arrangement in a randomized complete block design with three replications. Data on leaf number/plant, survival count and rate were recorded and analyzed. Mean squares from analysis of variance revealed the existence of a very highly significant influence ($P < 0.001$) of cutting position on leaf number and survival rate. The application of rooting hormone exerted a significant influence ($P < 0.05$) on survival rate only. Interaction effect of cutting position and hormone application did not exert an influence ($P > 0.05$) on the parameters considered. Higher but statistically similar survival rate were recorded for the control and the 0.2% IBA treatment whereas the lowest values were recorded for the 0.4% NAA treatment. Top cuttings demonstrated significantly more leaves (eight) and survival rates (80.18%) and showed a 46.78 and 28.49% increase in leaf number and survival rate compared to middle cuttings. Therefore, it is recommended that the top part be used for the propagation of stevia. The application of rooting hormone might not necessarily be an economically sound option for vegetative propagation.

Keywords: auxins, cutting part, Ethiopia, leaf number, survival rate, sweet leaf

INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is a perennial herb that belongs to the family Asteraceae (*Ahmed et al*. 2007; Ojha *et al*. 2010). It is native to South and Central America (Robinson and King 1977; Debnath 2008; Sumon *et al*. 2008; Jackson *et al*. 2009). The genus *Stevia* contains more than 154 species and the most widely utilized ones are *Stevia eupatoria*, *S. ovata*, *S. plummerae*, *S. salicifollia*, *S. serrata* and *S. rebaundiana* (Soejarto *et al*. 1983; Jackson *et al*. 2009). From these, *S. rebaundiana* is known for its significant sweetening properties (Ingle and Venugopal 2009) and this species is the focus of this particular study. The first commercial cultivation of stevia was started in Paraguay around 1964 (Sumida 1968; Katayama *et al*. 1976). Currently it is cultivated in Japan, Taiwan, Philippines, Hawaii, Malaysia and overall South America for food and pharmaceutical products (Ahmed *et al*. 2007; Debnath 2008; Sumon *et al*. 2008).

The property of the species that called attention to the plant was the intense sweet tests of the leaves and aqueous extracts (Ahmed *et al*. 2007; Jackson *et al*. 2009). Dry leaves of stevia are 30 times sweeter than table sugar with zero calories (Ojha *et al*. 2010). The leaves of stevia contain sweetening compounds namely stevioside, rebaudioside A, rebaudioside B and rebaudioside C and six other compounds which have insulin balancing properties (Moraes and Machado 2001; Meireles *et al*. 2006). These sweeteners impart 250 times sweetness than table sugar and 300 times more than sucrose (Ingle and Venugopal 2009). These glycosides are extracted from the stevia leaf as all-natural zero caloric sweeteners (Jackson *et al*. 2009); hence, stevia is a calorie-free bio-sweetener of high quality with non-fermentable and non-discoloring qualities that maintains heat stability at 100°C and has a long shelf life (Brandle 1999).

The product has been added to tea and coffee, cooked or baked goods, processed foods, beverages (Ahmed *et al*. 2007), used safely in herbal medicines, tonics, for diabeties and in the daily usage products like mouthwashes and toothpastes (Sumida 1980). It can be used in chocolates and candies not only to meet the market demand by the diabetes, but also to harvest the added advantages of this herb that it does not encourage tooth decay due to its anti-microbial property, unlike the sugar (Sumida 1980). In the Pacific Rim countries like China, Korea and Japan, stevia is regularly used in preparation of food and pharmaceutical products and currently stevia production is centered in China with major market in Japan (Lewis 1992). No negative clinical reports have papered in any of the countries where stevia is readily available (Brandle and Rosa 1992). The present scenario is that people are more inclined towards products advertised with a brand name "all natural and low carbohydrate" (Nishyama *et al*. 1991). Hence, stevia will also have wider potential utilizations. Apart from this, stevia is nutrient-rich, containing substantial amount of protein, calcium and phosphorous (Brandle 1999).

Productions of horticultural crops including stevia are affected by a number of factors. From these, propagation is the one. Stevia can be multiplied sexually by use of seeds or asexually by use of techniques such as cutting, grafting and tissue culture (Carvalho and Zaidan 1995). As stevia does not produce fertile seed in Ethiopia, asexual propagation through stem cuttings has given attention for this particular study. There are different reports about the influence of stem cuttings and rooting hormones on seedling establishment of stevia (Miyazaki and Wantanabe 1978; Carvalho and Zaidan 1995). Gvasaliye *et al*. (1990) had also reported the rooting of cutting was best in cuttings from side shoots and from tops of the main shoot in honey grass (*Melinis maitlandii* Stapf). Inaddition, cuttings from the top part of the main stem of stevia generally gave the best result (Tirtoboma 1988). Likewise, cuttings of stevia taken from new

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stems and shoots can be propagated successfully and rooting of cuttings can be stimulated, but not always, by the use of growth regulators (Bondarev 1998).

Rooting hormones are very important in the rooting process of cuttings (Ingle and Venugopal 2008). Araya (2005) also reported that the most commonly and widely used auxins for propagation are 0.2% indole-3-butyric acid (IBA) and 0.4% 1-naphthalene acetic acid (NAA) Their beneficiary effect was also confirmed by Arya *et al*. (1994), Al-Saqri and Alderson (1996), Aminah *et al*. (1997) and Hartmann *et al*. (1997).

Despite stevia being very useful in food, pharmaceutical, soft drink and stimulant-processing industries; existence of interest from processors for commercial cultivation of the crop; asexual propagation is the only options for its perpetuation; and cutting positions and rooting hormones have an impact of propagation ability of stevia, there are no documented information and knowledge regarding the propagation technologies of stevia using stem cuttings and application of rooting hormones. Due to this indispensible fact, the experiment was conducted to determine the effect of cutting position and application of rooting hormone on propagation ability of stevia.

MATERIALS AND METHODS

A stevia plant maintained at Wondo Genet Agricultural Research Center botanical garden was used for this experimentation. The experiment was conducted at Wondo Genet Agricultural Research Center nursery site during the wet season of 2010 in a plastic closed chamber. The site is located at 7°192 N latitude and 38°382 E longitude with an altitude of 1780 m a.s.l. The area receives mean annual rainfall of 1000 mm with maximum and minimum temperature of 10 and 30°C, respectively.

The experiment consisted of two levels of part used (top and middle) and three levels of rooting hormones (zero hormone, 0.2% IBA and 0.4% NAA). The experiment was laid out in 3×2 factorial arrangements in randomized complete block design with three replications according to Gomez and Gomez (1984). Each treatment in each replication included 60 cuttings.

Top and middle cuttings were taken from a six-month-old disease-free mother plant. Both the top and middle cuttings had two nodes with two pair of leaves (Zubenko 1991). For each treatment, the potting media was peat moss prepared on propagation treys. The basal parts of the cuttings were dipped in the rooting media according to the treatments concentration before planting in the propagation media. Proper weeding and watering of the experimental pots were carried out uniformly whenever required. Throughout the experimental periods, incidence of disease, insect damage, frost and storm did not occur. Data on leaf number/seedling, survival count and survival rate were recorded and analyzed for all the treatments considered. Survival rate was measured by counting the number of propagated seedlings.

To statically analyze the differences in propagation ability of stevia caused by cutting position and rooting hormone, 20 samples were taken from each treatment. Experimental data was statistically analyzed by analysis of variance (ANOVA) using SAS PROC GLM (2002) at $P < 0.05$. Differences between means were assessed using the least significance difference (LSD) test at $P < 0.05$.

RESULTS AND DISCUSSION

Variation in propagation ability of stevia with the variation in cutting position and hormone application

There was significant variation in the propagation ability of stevia with the variation in cutting position and with the application of rooting hormones (**Table 1; Figs. 1, 2**). Mean squares from analysis of variance for propagation ability of stevia are summarized in **Table 1**. The results obtained from the analysis of variance indicated that cutting position exerted a very highly significant effect ($P < 0.001$) on leaf number and survival rate there by propagation ability of stevia.

Table 1 Mean squares for leaf number and survival rate obtained from the analysis of variance tested at Wondo Genet Agricultural Research Center.

Source of variation	Df	Leaf number	Survival count	Survival rate
Replication	2	0.0022	15.38	42.74
Hormone type (HT)	2	0.4288 ns	72.05*	200.15*
Cutting position (CP)	1	30.9422***	512.00***	1422.22***
HT×PU	2	1.0688 ns	40.50 ns	112.50 ns
Error		0.4795	9.92	27.56
R^2		87.62	88.55	88.55
CV%		10.02	7.36	7.36

*** = Significant at $P < 0.01$ * = Significant at $P < 0.05$ and ns =Non significant at $P < 0.05$

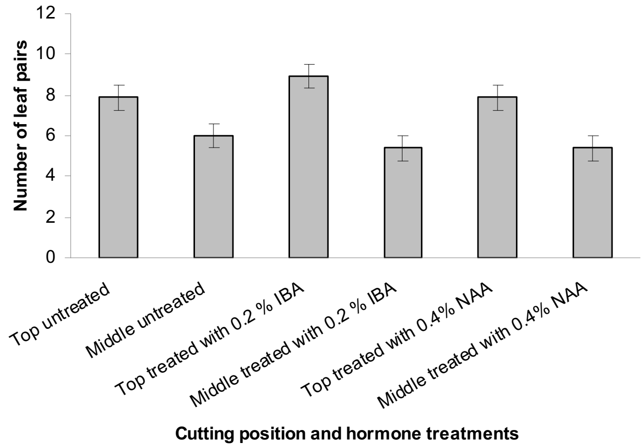

Fig. 1 Effect of cutting position and hormone application on leaf number of stevia. Values represent mean ± standard error (SE). n = 6

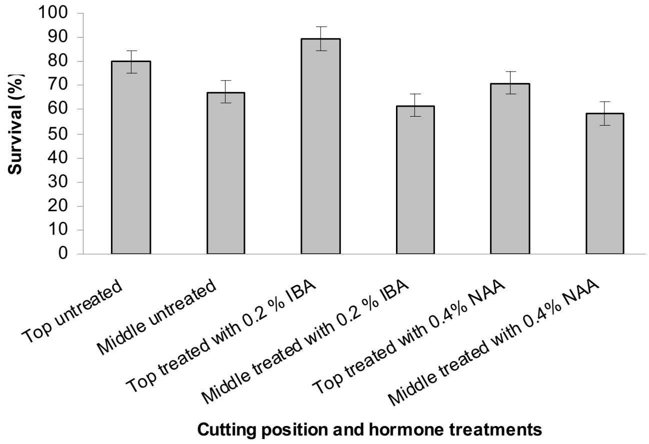

Fig. 2 Effect of cutting position and hormone application on survival rate of stevia. Values represent mean ± standard error (SE). n = 6

Application of rooting hormones also demonstrated a significant ($P < 0.05$) influence on propagation ability of stevia thorough its effect on survival rate. On the other hand interaction effect of cutting position and rooting hormone application did not have significant ($P > 0.05$) effect on leaf number and survival rate, thus the cumulative effect of cutting position and hormone application did not influence the propagation ability of stevia.

A significant variation in the propagation ability of lemon verbena (*Aloysia tryphylla* L.) using top, middle and bottom cutting position was also reported by Beemnet *et al*. (2011). Tirtoboma (1988) also reported the influence of cutting position on propagation ability and growth of stevia. Significant influence of cutting position and application of rooting hormones on the propagation ability of bush tea (*Aspalathus linearis* (NL) Burm) was also reported by Araya *et al*. (2005) and in many woody plant species by Hartman *et al*. (1997). A higher propagation ability of stevia

Table 2 Effect of cutting position and hormone application on propagation ability of stevia tested at Wondo Genet Agricultural Research Center

Treatment	Parameters		
	Leaf number	Survival count	Survival rate
Hormone type			
Control (untreated check)	6.93	44.16 a	73.61 a
IAA	7.16	45.33 a	75.55 a
NAA	6.63	38.83 b	64.72 b
$LSD_{0.05}$	0.89 ns	4.05	6.75
Cutting position			
Top part cutting	8.22 a	48.11 a	80.18 a
Middle part cutting	5.60 b	37.44 b	62.40 b
$LSD_{0.05}$	0.72	3.30	2.22
CV%	10.02	7.36	7.36

Means followed by the same letter with in the same column are statistically non significant at $P < 0.05$ according to least significant difference (LSD) test

by using 0.05% IBA was also reported by Ingle and Venugopal (2009). This could either be through their positive effect on root initiation, formation of more and uniform roots (Leakey 1990; Ofori *et al.* 1996) or through accelerating the translocation of nutrients from the upper part of the cuttings to their basal ends by increasing the activity of enzymes, which there by increases hydrolysis of carbohydrates by providing enough energy in rooting responses of the cells (Arya *et al.* 1994). This indicates that hormone treatment and cutting position are important factors to be considered during the propagation of stevia.

Effect of cutting position on propagation ability of stevia

The effect of cutting position on the propagation ability of stevia through its effect on leaf number and survival rate is presented in **Table 2** and **Figs. 1** and **2**. Variability was observed on leaf number and nursery survival rate of stevia cuttings taken from top and middle cutting position of a plant. The leaf numbers of the seedlings ranged from 5.6 to 8.22 pairs and the highest being recorded for top cuttings and the lowest for middle cutting position (**Table 2**). Survival rates varied from 62.4% for middle cuttings to 80.11% for top cuttings. For many years propagation ability has been known to vary between cuttings from different parts of the same plant, especially in woody species (Leaky and Mohammed 1985) and this was correlated with structure of the stem (Hartman *et al.* 1997) or difference in chemical composition of the plant along the stem (Hansen 1986; Hartman *et al.* 1997).

In agreement with the present study, Tirtoboma (1988) reported the advantage of top cutting position on propagation ability and growth of stevia. Likwise, Gvasaliya *et al.* (1990) reported successful propagation of stevia using top cuttings of new stems and shoots. Deen and Mohamoud (1996) also reported apical cuttings exerted more number of roots for rosemary (*Rosemarinus officinalis* L.). Similar results were also reported by Palanisamy and Kumar (1997) in rooting of neem (*Azadirachta indica* A. Juss), where cutting from the upper part of the branches rooted better than the lower ones (Wassner and Ravetta 2000). Wassner and Ravetta (2000) also reported good propagation ability for top cuttings than none of the basal cuttings rooted in *Grindelia chiloensis*. With the propagation of *Triplochiton scleroxylon* a gradual reduction in rooting percentage was recorded with distance from the apex (Leakey 1983).

This difference in propagation ability of apical and middle cuttings of stevia could be due to high concentration of endogenous root promoting substances in the apical cuttings which arise from the terminal buds and also "more cells" which are capable of becoming meristematic (Hartman and Kester 1983). According to Araya (2005), the difference in rooting due to cutting position can be related to the difference in the chemical composition of the shoots. Hartman *et al.* (1990) also indicated middle cuttings could be more mature and low meristematic activity to develop roots than the apical cuttings. In deciduous species where no carbohydrate or root promoting substances are present, cuttings from the soft shoot of the plant root best (Hartman *et al.* 1997).

In contrast to the present study, many authors (Leakey 1983; Hansen 1986, 1988; Hartman *et al.* 1990; Jawanda *et al.* 1991; Al-Saqri and Alderson 1996) reported that the best rooting of cuttings is usually found from the basal portions of shoots. This variation comes due to the variation in accumulation of photosynthetic products, mostly carbohydrates or it could be due to juvenility factors (Jawanda *et al.* 1991), species, environmental conditions, season of propagation, degree of maturity, rate of growth (Hansen 1986; Leakey and Coutts 1989).

Effect of hormone application on propagation ability of stevia

In the current study it was observed that application of hormones does not have a significant effect on leaf number (**Table 2**). Higher value and similar survival percentage was recorded for untreated and IBA treated cuttings (**Table 2, Fig. 2**). The survival percentage of the untreated cuttings demonstrated a percent increased value of 13.74 over the NAA-treated cuttings. This indicates that, application of rooting hormones on stevia cuttings does not increase the production of leave and increase the propagation ability of stevia. Hence, application of rooting hormone treatment for the propagation of stevia is not an important factor.

In difficult to root plants, rooting hormones such as auxins have an important role in the development of adventitious roots, increasing rooting percentage, improving quality of roots and uniformity in rooting of cuttings (Araya 2005). But plants that root easily do not respond well to the application of rooting hormone (Hartmann and Kester 1983). Propagation ability of bush tea was not found to respond well to the application of 0.3% IBA (Araya 2005). Similar results were also reported by Shiembo *et al.* (1996) in vegetative propagation of *Genetum africanum* Welw when IBA from 0 to 0.025% was applied. Ofori *et al.* (1996) also reported similar results in *Milicia excelsa* with the application of IBA. In this study, the non- and negative response of stevia cuttings in their propagation ability with the application of rooting hormones could be due to the high supplement of endogenous auxins in the shoots of the plant and these auxins might interact negatively with the application of exogenous rooting hormones. Similar suggestions were also drown by Ofori *et al.* (1996) in propagation ability of *Milicia excelsa*.

Generally it was observed that cutting position had an effect on propagation ability of stevia, and propagation of the plant using top cutting position can be advised to be used. Likewise, as the plat was found not responsive to the application of rooting hormones, application of rooting hormones during propagation of stevia is not economical and not advisable. However, as this was the first to its kind on the crop, effect of cutting size, season of propagation, physiology of rooting, media of propagation, etc need to be explored further.

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