

## IBA APPLICATION TO STEVIA STEM CUTTING: ITS PRODUCTIVITY AND QUALITY OF PLANT AND RATOON CROPS.

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### Abstract

Four experiments were carried out in the Agricultural Research Center Experimental Station – Giza – Egypt. Two glasshouse experiments were conducted during 2001 and 2002 seasons to investigate the effect of stem cutting position, IBA application on stem cutting and their interaction on stevia propagation. Also, two field experiments were designed to study the effect of propagation method, nitrogen fertilizer and their interaction on stevia plant yield and quality for plant crop during 2002/2003 season and ratoon crop of 2003/2004. Five cuts were obtained for each growing season. Basal cuttings were superior in all traits giving 87.56 % for survived plants, 1.23 g for leaves fresh weight, 1.07 g for leaves dry weight, 2.31 g for stem fresh weight, 0.70 g for stem dry weight, 0.81 g for root fresh weight and 0.49g for root dry weight. The effect of IBA application on stem cuttings was significant only on cutting survival percentages which were increased with increasing IBA concentration from 0 to 500 and 1000 ppm. giving 74.44, 82.44, and 86.89 %, respectively. Maximum value of cutting survival percentage (95.33 %) was obtained from basal cuttings treated with IBA concentration of 1000 ppm. while the lowest value was obtained from tip cuttings with 0 ppm. IBA (70.00 %). These results showed that in both plant crop and ratoon crop, root cutting propagated plants exceeded both seed and tissue culture propagated plants for all studied characters. Meanwhile, application of 40 kg N/fed/cut gave maximum values over both 0 (control) and 20 kg N/fed/cut. Root cutting propagated plants of the fifth cut that received 40 kg N/fed/cut gave the maximum values of leaves dry weight (1.13 t/fed for plant crop and 1.15 t/fed for ratoon crop), total leaves dry weight (4.39 t/fed/year for plant crop and 4.58 t/fed/year for ratoon crop), stevioside percentage (24.60 % for plant crop and 28.50 % for ratoon crop) and stevioside yield (279.60 kg/fed for plant crop and 327.75 kg/fed for ratoon crop). Total stevioside yield was 962.43 kg/fed/year for plant crop and 1035.58 kg/fed/year for ratoon crop.

### INTRODUCTION

The sweet herb of Paraguay, *Stevia rebaudiana* Bertoni produces, natural sweeteners in its leaves, just such an alternative with the added advantage that stevia

sweeteners are natural plant products. The established uses for stevia products cover all those of artificial low-calorie (non-sucrose) sweeteners, as well as most other purposes for which sugar can be used. The primary use is as a sweetener to enhance the palatability for foods and drinks. Stevia sweeteners are heat stable to 200 °C, acid stable and don't ferment, making them suitable for use in a wide range of products including baked and/or cooked foods. Stevia is a small perennial herb shrub with green leaves that belongs to the aster family (Asteraceae). The sweet component of the plant is 200 to 300 times sweeter than sucrose and has similar tasteful properties. The necessary steps to expand stevia cultivation in Egypt are the development of seeds, care of seedlings and appropriate agri-practices, including information on optimized crop inputs. Understanding of the biology and agricultural practices of stevia is prerequisite for the adoption and successful cultivation of stevia to be a new economic cultivated crop in Egypt (Allam *et al.*, 2001).

Nitrogen requirement of stevia varies from one region to another around the world: In Ontario, Canada, total biomass production of 7500kg ha<sup>-1</sup> would require approximately 105 kg N (Katayama *et al.*, 1976). In Brazil, the production of one ton of dry leaves of stevia required 64.6 kg. N (Lima – Filho *et al.*, 1997). In Canadian sandy soils, the recommended N fertilizer program was 140 kg urea/ha in a split application (Columbus, 1997). In Korea, leaf yield was increased as moderate applications of nitrogen was applied (100kg N/ha) (Lee *et al.*, 1979). Meaning that, amount of nitrogen required for optimizing the yield varied in different countries and different types of soils. The suitable amount of fertilizer under Egyptian conditions at different types of soils must be investigated. Following such trend, Allam *et al.* (2001) reported that a linear and significant increase in fresh and dry leaf weights, fresh and dry yield of stem, growth of new tiller and lateral branches, fresh yield and stevioside content were observed as nitrogen rates were increased up to 30 kg/fed/cut. *Stevia rebaudiana*; Bertoni strains obtained in vitro were used to study the ability of rooting. It was found that shoot tip propagation is a useful method in plant production. Propagation of stevia plant through seed or cuttings is recommended and plants can remain productive for 4-6 years (Donalisio *et al.*, 1982). Cuttings showed least variation while seed showed the greatest variation among individual plants in chemical composition. Plants cultivated by seeds were less productive in the first year than those from cuttings (Lee *et al.*, 1979). Seeds are usually very small and as a result, seedlings are slow to develop, reaching a size suitable for transplanting to the field at 45-60 days. Environmental conditions such as short days induce flowering, whereas, optimum yield (biomass) and stevioside quality and quantity are satisfactory just prior to flowering (Columbus, 1997). Ratoon crop of stevia was found to be significantly

superior to the plant crop in respect of higher daily leaf yield ( $39.39 \text{ kg ha}^{-1} \text{ day}^{-1}$ ) (Chalapathi *et al.*, 1999). Treating stevia cuttings with IBA (50 mg/l) for 6 hours gave a significant increase in rooting (Morcavillaca, 1978). In a study to evaluate cuttings taken from different parts of the shoot, rooting was maximum (96.7%) in cuttings from the tip of the shoot with 4 internodes. The location on the plant from which cuttings are taken can also affect growth and rooting, cuttings from the top half and with 4 internodes showed distinguished performance (Tirtoboma, 1988).

The present work was carried out to investigate the effect of different propagation methods (seed, root division and tissue culture), nitrogen fertilizer rates (0, 20 and 40 kg N/fed/cut) and their interaction on stevia plant (plant crop and first ratoon crop) productivity and quality. Also, to investigate the success of propagating stevia plant throughout stem cuttings as affected by cutting position and IBA application.

## MATERIALS AND METHODS

Two glasshouse and two field experiments were carried out in the Agricultural Research Center Experimental Station – Giza – Egypt. The first two experiments were conducted in glasshouse during 2001 and 2002 seasons to study the effect of stem cutting position, IBA application on stem cutting and their interaction on stevia propagation. The second two experiments were carried out to study the effect of propagation method, nitrogen fertilizer and their interaction on stevia plant yield and quality for plant crop of 2002/2003 season and ratoon crop of 2003/2004 season during the five cuts of each season.

### **I- Effect of stem cutting position, IBA application and their interaction on cutting success of *Stevia rebaudiana* Bertoni.**

The first two experiments were carried out to investigate the success of stevia plant propagation through stem cuttings under glasshouse conditions during the two successive seasons 2001 and 2002. Cuttings were taken from the main branches only and were defoliated to four leaves each cut. The following treatments were applied:

#### **A- Stem cutting position:**

- 1- Tip cuttings (15 cm. length).
- 2- Mid cuttings (15 cm. length).
- 3- Basal cuttings (15 cm. length).

**B- Indole buteric acid (IBA) concentration:**

Stem cuttings were dipped in indole buteric acid for 30 seconds with the following concentrations:

- 1- 0ppm. (control).
- 2- 500ppm.
- 3- 1000ppm.

All cuttings were approximately 15cm long, planted in March 15 in both seasons (2002 and 2003) in peatmoss in wooden boxes, covered with transparent plastic sheets and immediately irrigated after planting. Cuttings were irrigated daily and the following data were recorded after 30 days from planting:

- 1- Survived cuttings percentage.
- 2- Leaves fresh weight (g).
- 3- Leaves dry weight (g, air dried).
- 4- Stem fresh weight (g).
- 5- Stem dry weight (g, air dried).
- 6- Root fresh weight (g).
- 7- Root dry weight (g, air dried).

**Statistical analysis**

A complete randomized design was used with three replications each of 50 cuttings. Since there was homogeneity between both seasons, a combined analysis was performed according to Snedecor and Cochran (1969). For means comparison, LSD values were calculated at 5% level of significance following the method of Gomez and Gomez (1984).

**II- Effect of propagation method and nitrogen fertilization on stevia 2002/2003 plant crop and its first ratoon crop (2003/2004):**

The second two experiments were carried out during the two successive seasons 2002/2003 (plant crop/ plantation of first year) and 2003/2004 (first ratoon crop) to evaluate the effect of different stevia plant propagation methods, nitrogen fertilizer rates and their interaction on stevia plant yield and quality. Other cultural practices such as hoeing, irrigation....etc. were maintained at

levels to assure optimum production. Physical and chemical properties of experimental soil are shown in Table (1).

Table 1. Physical and chemical properties of the experimental soil

Sand %	12.16
Silt %	48.85
Clay %	38.99
Soil texture	Silt clay loam
pH	8.10
E.C. (mmohs/cm)	2.65
CaCO <sub>3</sub> %	1.81
Available N (ppm)	11.00
Available P (ppm)	9.12
Available K (ppm)	35.86

#### The experimental treatments:

##### A- Propagation method:

Three propagation methods were used in this study (seed, tissue culture and root division) during cultivation of plant crop (2002/2003). Plants of each propagation method were left in the soil for ratoon crop (2003/2004).

##### 1- Root division (root cuttings)

Plants of one-year-old were taken out from the soil, where roots were separated, cleaned and divided each into three parts and replanted in field at the same day.

##### 2- Tissue culture.

Plants were propagated in the Agricultural Genetic Engineering Research Institut (AGERI-ARC) through shoot apex culture technique (Tamura *et al*,1984).

##### 3- Seeds.

Seeds of stevia variety Spanti imported from Spain and locally regenerated vegetatively (1g. of seeds contained 1640 seeds of 5% germination) were obtained from Sugar Crops Research Institute (SCI) and preserved in the refrigerator at 5 C<sup>0</sup>, then it was planted in the glasshouse in January 15<sup>th</sup> in fine clay soil covered with thin layer of soil. Plantlets were transplanted into open field 75 days later.

All propagated plants of the three different methods discribed previously were transplanted on 1<sup>st</sup> of April in the first season (2002/2003) on rows and were maintained in the soil during the second season 2003/2004 (first ratoon crop).Plot area was 10.5m<sup>2</sup> (6 ridges, 3.5m long and 50cm apart) and spacing

between hills was 30cm to reach a population of 28000 plants per feddan (4200m<sup>2</sup>).

**B- Nitrogen fertilizer rates:**

0 kg / fed / cut.

20 kg / fed / cut.

40 kg / fed / cut.

These rates were added after each cut at two equal doses for both plant crop and ratoon crop. The first dose was added after the first cut and before the first irrigation and the second dose was added before the next irrigation. Meanwhile, same rates were added in two equal doses in the beginning of the growth season before the first and second irrigation. Nitrogen was added as urea fertilizer (46.5 % nitrogen).

Five harvests were taken per year starting 90 days after planting, where, cutting date, plant age, season and day length during each cut are presented in (Table 2). Cutting was carried out 10 cm above soil surface on mentioned dates.

Table 2. Cutting date, plant age, season and day length for each of the five cuts for each year (2001/2002 and 2002/2003)

Cut No.	Cutting date	Plant age	Season	Day length (hr.)
*1 <sup>st</sup>	July 1 <sup>st</sup>	90 days	Summer	13.53
*2 <sup>nd</sup>	October 1 <sup>st</sup>	180 days	Autumn	11.44
**3 <sup>rd</sup>	December 1 <sup>st</sup>	240 days	Autumn	10.19
**4 <sup>th</sup>	February 1 <sup>st</sup>	300 days	Winter	10.58
*5 <sup>th</sup>	April 1 <sup>st</sup>	360 days	Spring	12.11

\*Cuts of vegetative parts (stem + leaves) whereas, growth/regrowth occurred during warm season and day length > or = 11 hours.

\*\*Cuts of vegetative parts (stem + leaves) whereas, growth occurred during low temperature season and day length <11 hours.

**Parameters determined:**

The whole plot was harvested each cut and the following parameters were determined:

- 1- Plant height (cm).
- 2- Number of branches/plant.
- 3- Percentage of flowering plants.

A plant was considered as a flowering plant if it had one flowering branch.

- 4- Leaves fresh weight (t/fed).

5- Leaves dry weight (air dried) (t/fed/cut).

Collected leaves were air dried in shade under natural conditions.

6- Accumulated leaves dry weight (air dried) (t/fed/year).

Leaves air dried weight (t/fed/cut) of the five cuts/season were summed to determine total yield of dry leaves.

7- Stevioside%

Stevioside standard determination was carried out according to Nishiyama *et al.* (1992) using HPLC. Pure stevioside powder was obtained from N. U. Natural Inc., USA as a standard. Stevioside extraction from leaves was carried out by soaking 1 gram of dry leaves in 1.0 liter water at 85 °C for 30 minutes. The resulting liquid fraction was separated by Buchner filtration and the residue was washed with an additional volume of hot water (50 ml). The aqueous solution was concentrated by lyophilization (Edwards Model Ef03, England) to 50 ml and defatted by ethyl acetate then extracted with isobutyle alcohol (150 ml). The aqueous phase was discarded and the organic solution was evaporated by rotary evaporator (Type 349, James Jobling and Co. Ltd., England) at 70 °C until drying. The dried extract was dissolved in hot methanol (100 ml) and kept over night to crystallize. The crystals were separated by filtration and re-dissolved again in boiling methanol (50 ml). This solution was clarified with active charcoal (B.D.H. Laboratory Chemicals Division, Poole, England) and left to recrystallize. The procedure was repeated three times until the formation of colorless crystals were obtained. An isocratic mobile phase with 30% H<sub>2</sub>O/methanol (50:50) and 70% acetonitrile was utilized. The flow rate was set at 1 mL/min, the quantity of injected sample was 20 mL, the drift tube temperature was 90°C and the flow of nebulising gas was 2.20 SLPM.

8- Stevioside yield (kg/fed/cut).

Stevioside yield (kg/fed/cut.) was calculated by multiplying air dried leaves yield of each cut by stevioside percentage determined for the same cut.

9- Accumulated stevioside yield (kg/year).

Stevioside yield (kg/year) was calculated by summing the stevioside yield (kg/fed/cut) of the five cuts taken every season.

Statistical analysis

A split plot design with 3 replications was used where propagation method was put in the main plots and nitrogen rates were distributed randomly in the sub-plots. Data were exposed to statistical analysis. For means comparison LSD values were calculated at 5% level of significance following the method of Gomez and Gomez (1984).

## RESULTS AND DISCUSSION

### **I- Effect of stem cutting position, IBA application and their interaction on cutting success of *Stevia rebaudiana* Bertoni.**

#### **a- Effect of cutting position:**

Cutting position had a significant effect on all studied characters (Table 3) where the basal cuttings were superior in all traits giving 87.56 % for survived plants percentage, 1.23 g for leaves fresh weight, 1.07 g for leaves air dried weight, 2.31 for stem fresh weight, 0.70g for stem dry weight, 0.81 for root fresh weight and 0.49 for root dry weight. This result may be due to the effect of the basal cuttings with thicker leaves that could act well rather than the tip thin watery cuttings. Another reason of this effect may be due to the relatively more materials stored in the basal cuttings than that in mid and tip cuttings.

#### **b- Effect of IBA**

The effect of IBA application on stem cuttings was significant only on cutting survival percentages which were increased with increasing IBA concentration from 0 to 500 and 1000 ppm. giving 74.44, 82.44, and 86.89 %, respectively. Effect of IBA demonstrates its role in inducing rooting of perennial plant cuttings. Meanwhile, IBA application did not affect any of the other studied traits (Tables 3). This can be attributed to the promoting effect of IBA on cell division and differentiation but not on biomass production after rooting.

#### **c- Effect of interaction**

The interaction between cutting position and IBA concentrations was significant for cutting survival percentage only. Maximum value of cutting survival was obtained from basal cuttings treated with IBA concentration of 1000 ppm. (95.33 %) while the lowest value was obtained from tip cuttings with 0 ppm. IBA (70%). Such finding may be due to the ability of basal cuttings to absorb more hormone solution during dipping through its larger base and its more phloem tubes. Concerning other studied traits, results did not show any significant meaning that neither cutting position nor IBA concentration could interact with the effect of each other.

Several researchers indicated the significance of cutting position on survived cuttings including Tirtoboma (1988) and Gavasaliya *et al.* (1990). Concerning hormone application it was concluded that it had a role in stimulating cutting rooting by Bondarev and K-A; (1998). Meanwhile, Shock (1982) stated that the propagation by cuttings is an alternative method that is (but not in general) a method of good economic feasibility giving the rapidity of propagation, in addition, the possibility to be carried out with little inputs.



## **II- Effect of propagation method and nitrogen fertilization on stevia 2002/2003 plant crop and its first ratoon crop (2003/2004):**

Plants were remained in the land after the fifth cut of 2002/2003 plant crop (plants of one year old) to continue its growth during the second year (ratoon crop, 2003/2004) where the following results were obtained:

### **1-plant height**

Propagation method had significant effect on stevia plant height in both plant crop (second and fifth cuts) and ratoon crop in the first and last cut (Table 4). Tissue culture and root cutting propagated plants exceeded seed propagated plants in plant height of plant crop (51.90 and 55.33 cm. in the second cut and 52.10 and 52.51 cm. in the fifth cut, respectively) while ratoon crop gave (55.43 and 56.46cm in the first cut and 57.10 and 57.76 cm in the fifth cut, respectively). This result means that during low temperature season, propagation method did not affect plant height because of unsuitable low temperature which, in turn, affected plant growth and productivity. The significant effect of propagation method in the first cut in ratoon crop (in the contrary with plant crop) may be due to the slow growth rate after transplanting in the first year of plant life (plant crop). Application of 40 kg nitrogen/fed/cut to plant crop gave maximum values of plant height in first, second and fifth cuts (53.85, 54.20 and 53.03 cm.), respectively, in contrast with plant crop where no significant effect of nitrogen fertilization on plant height of ratoon crop was detected in all cuts (Table 4). This result means that ratoon crop plants may have larger root system deep in the soil so, any additional application of nitrogen fertilizer did not show significance. This may be due to the greater root surface of ratoon crop that has more ability of absorbing nutrients from soil. In both plant and ratoon crops, warm season cuts (after 90, 180 and 360 days) gave maximum values of plant height (52.50, 52.68 and 40.01 cm. in plant crop and 53.81, 51.88 and 55.88 cm in ratoon crop, respectively) while plants of winter cuts (after 240 and 300 days) were much shorter (25.13 and 21.00 cm. in plant crop and 26.25 and 21.07 cm. in ratoon crop, respectively). This result indicates that temperature of growing season had a notable effect on plant height, plant growth rate, production and consequently stevia biomass.

### **2- Number of branches/plant**

No significant effect of propagation method on stevia number of branches/plant was shown in all ratoon cuts except the second cut (after 180 days from the last cut of plant crop) while in plant crop, propagation method had significant effects in first, second and fifth cuts (warm season cuts) (Table 5). However, in the second cut of ratoon crop, root cutting propagated plants significantly had more branches/plant (21.33 branches/plant). This result indicates that in ratoon

crop the three propagation methods tended to give almost the same number of branches/plant. This is due to the fact that all propagated plants produced by different propagation methods had been established and reached the same biomass after the first year of growth (plant crop). Nitrogen fertilization did not show any significant effect on number of branches/plant of ratoon crop plants except in the second cut where application of 40 kg N/fed/cut gave maximum number of branches/plant (14.46), (Table 5). Meanwhile, plants of first year (plant crop) did not show the same trend since propagation method had significant effects on first, third and fifth cuts (after 90, 270 and 360 days from transplanting). However, the non-significant effect of nitrogen fertilization in most of cutting dates of ratoon crop may be due to the high growth rate of plants of ratoon crop which indicate the low nitrogen requirements of stevia plant especially of ratoon crop as mentioned by Allam *et al.* (2001). Maximum number of branches/plant was obtained during the first cut of ratoon crop plants (23.74) while in plant crop, maximum number of branches/plant recorded its in the last cut (20.39) (Table 5). However, in both plant and ratoon crops, low temperature season cuts (third and fourth cuts) gave 12.97 and 13.32 branches/plant in plant crop and 13.91 and 15.65 branches/plant in ratoon crop, respectively. During warm weather, number of branches/plant was increased because of notable sprouting of basal buds in the spring from subsoil, while low temperature season cuts had less number of branches/plant because of low temperature effect during this period.

### **3-Percentage of flowering plants**

In both plant and ratoon crop seasons, propagation method did not affect the percentage of flowering plants except in the first cut after 90 days (Table 6). However, seed propagated plants gave minimum values of flowering plants percentage in the first cut in both plant and ratoon crops (40.38 and 40.77 %, respectively). These data show the effect of short day length on stevia flowering since all plants gave 100% flowering at late summer and during winter (after 180, 240 and 300 days). No significant effect of nitrogen fertilization on flowering plants percentage was observed at any of the cuts all year round neither in plant nor in ratoon crops. This result shows that nitrogen application could not overcome the effect of day length on plant transition towards flowering. Data in Table (6) show differences among cuts for flowering plant percentage revealing that stevia plants flowered most of the year when days become shorter than 12 hours. However, some plants flowered in summer and spring cuts (45.05 and 25.71 % in plant crop and 43.05 and 26.08 % in ratoon crop, respectively).

Table 3. Effect of cutting position, IBA application and their interaction on survived cutting %, leaves fresh weight (g.), leaves air dried weight (g.), stem fresh weight (g.), stem air dried weight (g.), root fresh weight (g.) and root air dried weight (g.) of stevia cuttings (combined data of 2002 and 2003 seasons).

Treatment	Survived cuttings %	Leaves fresh weight (g.)	Leaves air dried weight (g.)	Stem fresh weight (g.)	Stem air dried weight (g.)	Root fresh weight (g.)	Root air dried weight (g.)
Cutting position	Tip	74.89	0.90	0.65	1.67	0.61	0.37
	Mid	81.33	1.25	0.89	1.96	0.72	0.43
	Basal	87.56	1.23	1.07	2.31	0.81	0.49
L.S.D		2.19	0.09	0.07	0.10	0.03	0.04
IBA (ppm)	0	74.44	1.22	0.86	1.97	0.70	0.42
	500	82.44	1.21	0.87	1.98	0.60	0.44
	1000	86.79	0.90	0.88	1.97	0.59	0.43
L.S.D		2.19	N.S	N.S	N.S	N.S	N.S
Interaction							
Tip	0	70.00	0.90	0.64	1.65	0.50	0.37
	500	75.33	0.92	0.65	1.65	0.50	0.38
	1000	79.33	0.90	0.66	1.70	0.51	0.36
Mid	0	75.33	1.25	0.88	1.95	0.59	0.44
	500	82.67	1.25	0.88	1.97	0.60	0.43
	1000	86.00	1.24	0.91	1.96	0.60	0.43
Basal	0	78.00	1.53	1.07	2.33	0.71	0.46
	500	89.33	1.51	1.07	2.34	0.70	0.50
	1000	95.33	1.51	1.06	2.27	0.69	0.50
L.S.D		3.79	N.S	N.S	N.S	N.S	N.S

Table 4. Effect of propagation method, nitrogen fertilization and their interaction on plant height (cm.) of plant crop (2002-2003) and ratoon crop (2003-2004) seasons during five respective cuts each year.

Treatment		Cutting age (days)																			
		Plant crop (2002-2003)					Ratoon crop (2003 - 2004)														
		90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )					
Propagation Method (M)	Seed	51.86	50.81	23.89	20.40	50.18	49.53	50.05	25.21	20.56	52.77	52.32	51.90	26.02	21.18	52.10	55.43	26.34	20.89	57.10	
	Tissue culture	52.95	55.33	25.49	21.43	52.51	56.46	53.38	27.20	21.76	57.76	N.S	1.45	N.S	N.S	1.82	1.23	N.S	N.S	3.66	
	Root cutting	50.85	51.48	24.85	20.31	49.80	52.78	50.68	25.49	20.55	53.49	52.42	52.35	25.32	21.42	51.95	54.32	26.43	21.12	56.51	
Nitrogen (Kg/fed./cut) (N)	0	53.85	54.20	25.22	21.23	53.03	54.32	52.72	26.84	21.54	57.63	1.54	1.40	N.S	1.32	N.S	N.S	N.S	N.S	57.63	
	20	49.61	49.44	23.34	19.55	50.31	48.17	48.54	24.25	20.13	50.10	52.10	50.37	23.44	20.89	49.99	50.21	25.68	20.50	53.64	
	40	53.86	52.61	24.90	20.76	50.23	50.20	51.34	25.71	21.04	54.58	50.73	50.98	25.81	21.01	49.50	54.31	25.80	20.08	55.18	
Tissue culture	0	52.23	51.01	26.50	21.43	52.73	56.00	52.41	26.14	21.03	57.23	54.00	53.72	25.75	21.11	54.06	55.99	27.09	21.56	58.89	
	20	54.00	53.72	25.75	21.11	54.06	55.99	53.00	27.09	21.56	58.89	52.21	54.03	25.41	20.36	49.60	55.86	26.41	21.43	55.19	
	40	52.21	54.03	25.41	20.36	49.60	55.86	52.30	26.41	21.43	55.19	52.94	55.68	26.03	22.02	53.14	56.74	27.46	21.84	58.67	
Root cutting	0	54.80	56.27	25.02	21.88	54.80	57.77	53.82	27.72	22.01	59.42	N.S	N.S	N.S	N.S	2.28	N.S	N.S	N.S	59.42	
	20	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	52.50	52.68	25.13	21.00	40.01	53.81	26.25	21.07	55.88	
	40	52.50	52.68	25.13	21.00	40.01	53.81	51.88	26.25	21.07	55.88	Interaction MxN									
L.S.D at 0.05	Mean	52.50	52.68	25.13	21.00	40.01	53.81	51.88	26.25	21.07	55.88	Interaction MxN									
	0	49.61	49.44	23.34	19.55	50.31	48.17	48.54	24.25	20.13	50.10	Interaction MxN									
	20	52.10	50.37	23.44	20.89	49.99	50.21	50.26	25.68	20.50	53.64	Interaction MxN									
Seed	40	53.86	52.61	24.90	20.76	50.23	50.20	51.34	25.71	21.04	54.58	Interaction MxN									
	0	50.73	50.98	25.81	21.01	49.50	54.31	51.20	25.80	20.08	55.18	Interaction MxN									
	20	52.23	51.01	26.50	21.43	52.73	56.00	52.41	26.14	21.03	57.23	Interaction MxN									
Tissue culture	40	54.00	53.72	25.75	21.11	54.06	55.99	53.00	27.09	21.56	58.89	Interaction MxN									
	0	52.21	54.03	25.41	20.36	49.60	55.86	52.30	26.41	21.43	55.19	Interaction MxN									
	20	52.94	55.68	26.03	22.02	53.14	56.74	54.01	27.46	21.84	58.67	Interaction MxN									
Root cutting	40	54.80	56.27	25.02	21.88	54.80	57.77	53.82	27.72	22.01	59.42	Interaction MxN									
	0	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	Interaction MxN									
	20	52.50	52.68	25.13	21.00	40.01	53.81	51.88	26.25	21.07	55.88	Interaction MxN									
L.S.D at 0.05	Mean	52.50	52.68	25.13	21.00	40.01	53.81	51.88	26.25	21.07	55.88	Interaction MxN									
	0	49.61	49.44	23.34	19.55	50.31	48.17	48.54	24.25	20.13	50.10	Interaction MxN									
	20	52.10	50.37	23.44	20.89	49.99	50.21	50.26	25.68	20.50	53.64	Interaction MxN									
Seed	40	53.86	52.61	24.90	20.76	50.23	50.20	51.34	25.71	21.04	54.58	Interaction MxN									
	0	50.73	50.98	25.81	21.01	49.50	54.31	51.20	25.80	20.08	55.18	Interaction MxN									
	20	52.23	51.01	26.50	21.43	52.73	56.00	52.41	26.14	21.03	57.23	Interaction MxN									
Tissue culture	40	54.00	53.72	25.75	21.11	54.06	55.99	53.00	27.09	21.56	58.89	Interaction MxN									
	0	52.21	54.03	25.41	20.36	49.60	55.86	52.30	26.41	21.43	55.19	Interaction MxN									
	20	52.94	55.68	26.03	22.02	53.14	56.74	54.01	27.46	21.84	58.67	Interaction MxN									
Root cutting	40	54.80	56.27	25.02	21.88	54.80	57.77	53.82	27.72	22.01	59.42	Interaction MxN									
	0	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	Interaction MxN									
	20	52.50	52.68	25.13	21.00	40.01	53.81	51.88	26.25	21.07	55.88	Interaction MxN									

Table 5. Effect of propagation method, nitrogen fertilization and their interaction on number of branches/plant of plant crop (2002-2003) and ratoon crop (2003-2004) seasons during five respective cuts each year.

treatment	Cutting age (days)																			
	Plant crop (2002-2003)					Ratoon crop (2003-2004)					Interaction MxN									
	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )					
Propagation Method (M)	7.74	13.90	12.84	13.15	19.86	23.29	18.79	13.42	14.62	21.16	16.78	18.32	13.09	13.45	20.49	23.98	20.26	13.97	16.05	22.85
Root cutting	16.70	19.30	12.97	13.36	20.82	23.95	21.33	14.35	16.27	22.88	1.88	1.08	N.S	N.S	0.57	N.S	0.94	N.S	N.S	N.S
L.S.D at 0.05	12.48	16.38	11.09	11.95	19.33	23.02	19.46	12.92	14.67	21.60	13.79	17.47	13.64	13.71	20.33	23.98	20.45	14.36	15.92	22.48
Nitrogen (K <sub>2</sub> O/fed./cut) (N)	14.94	17.68	14.17	14.31	21.51	24.21	20.47	14.46	16.36	22.81	1.25	N.S	1.13	N.S	1.12	N.S	N.S	1.81	N.S	N.S
L.S.D at 0.05	7.25	13.50	10.89	11.25	18.76	22.50	18.42	12.86	13.46	20.26	7.11	14.12	13.65	13.66	19.83	23.20	19.01	13.41	14.81	21.73
Seed	8.86	14.09	14.00	14.54	20.99	24.16	18.93	14.00	15.60	21.50	17.02	18.66	13.72	14.09	20.22	24.90	20.54	14.82	16.57	22.61
Tissue culture	17.00	18.09	14.44	14.21	21.74	24.18	20.68	14.63	16.66	23.49	13.88	17.43	11.26	12.53	19.73	23.71	20.39	13.45	15.63	22.09
Root cutting	17.24	19.63	13.54	13.37	20.49	23.83	21.80	14.84	16.37	23.10	2.16	N.S	20.85	14.09	14.18	21.80	24.30	14.76	16.81	23.45
L.S.D at 0.05	13.73	15.15	12.97	13.32	20.39	23.74	20.12	13.91	15.65	22.30	2.16	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
Mean	13.73	15.15	12.97	13.32	20.39	23.74	20.12	13.91	15.65	22.30	13.73	15.15	12.97	13.32	20.39	23.74	20.12	13.91	15.65	22.30

Table 6. Effect of propagation method, nitrogen fertilization and their interaction on flowering plants percentage of plant crop (2002-2003) and ratoon crop (2003-2004) seasons during five respective cuts each year.

Treatment		Cutting age (days)														
		Plant crop (2002-2003)					Ratoon crop (2003-2004)									
		90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )
Propagation Method (M)	Seed	40.38	100.00	100.00	100.00	100.00	40.77	100.00	100.00	100.00	100.00	40.77	100.00	100.00	100.00	25.70
	Tissue culture	40.04	100.00	100.00	100.00	100.00	42.93	100.00	100.00	100.00	100.00	42.93	100.00	100.00	100.00	26.25
	Root cutting	54.71	100.00	100.00	100.00	100.00	45.45	100.00	100.00	100.00	100.00	45.45	100.00	100.00	100.00	26.29
L.S.D at 0.05		1.45	N.S	N.S	N.S	N.S	3.10	N.S	N.S	N.S	3.10	N.S	N.S	N.S	N.S	N.S
Nitrogen (Kg/bed./cut) (N)	0	42.95	100.00	100.00	100.00	100.00	27.63	100.00	100.00	100.00	42.87	100.00	100.00	100.00	100.00	25.16
	20	45.28	100.00	100.00	100.00	100.00	25.34	100.00	100.00	100.00	42.97	100.00	100.00	100.00	100.00	26.18
	40	46.90	100.00	100.00	100.00	100.00	27.09	100.00	100.00	100.00	43.31	100.00	100.00	100.00	100.00	26.90
L.S.D at 0.05		1.80	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
		Interaction MxN														
Seed	0	40.01	100.00	100.00	100.00	100.00	24.40	100.00	100.00	100.00	39.53	100.00	100.00	100.00	100.00	24.56
	20	40.13	100.00	100.00	100.00	100.00	25.81	100.00	100.00	100.00	41.24	100.00	100.00	100.00	100.00	26.18
	40	41.01	100.00	100.00	100.00	100.00	28.55	100.00	100.00	100.00	41.53	100.00	100.00	100.00	100.00	26.36
Tissue culture	0	38.42	100.00	100.00	100.00	100.00	25.14	100.00	100.00	100.00	42.47	100.00	100.00	100.00	100.00	25.49
	20	40.40	100.00	100.00	100.00	100.00	25.06	100.00	100.00	100.00	42.83	100.00	100.00	100.00	100.00	26.06
	40	41.30	100.00	100.00	100.00	100.00	26.39	100.00	100.00	100.00	43.50	100.00	100.00	100.00	100.00	27.20
Root cutting	0	50.43	100.00	100.00	100.00	100.00	24.63	100.00	100.00	100.00	46.60	100.00	100.00	100.00	100.00	25.43
	20	55.32	100.00	100.00	100.00	100.00	25.14	100.00	100.00	100.00	44.83	100.00	100.00	100.00	100.00	26.30
	40	58.40	100.00	100.00	100.00	100.00	26.32	100.00	100.00	100.00	44.91	100.00	100.00	100.00	100.00	27.13
L.S.D at 0.05		3.11	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
Mean		45.05	100.00	100.00	100.00	100.00	25.71	100.00	100.00	100.00	43.05	100.00	100.00	100.00	10.00	26.08

#### 4- Leaves fresh weight

A significant effect of propagation method on leaves fresh weight in all cutting ages in both plant and ratoon crops (except in the last cut after 360 days of ratoon crop) is shown in Table (7). Meanwhile, root cutting propagated plants gave significantly more fresh leaves yields (2.75, 2.99, 1.55, 1.58 and 3.27 t/fed) in the five respective cuts of plant crop and (3.29, 3.44, 1.71 and 1.63 t/fed) in first, second, third and fourth cuts of ratoon crop, respectively. The variability within seed propagated plants after 90 days of plant crop and ratoon crop was documented and indicated previously by Donalizio *et al.*, (1982). A significant effect of nitrogen fertilization on stevia leaves fresh weight in all cuts of plant crop and only in the second, third and fifth cuts of ratoon crop is shown in Table (7). However, plants that received 40 kg N/fed/cut gave maximum values of leaves fresh weight i.e. 2.28, 2.91, 1.56, 1.57 and 3.38 t/fed in the five respective cuts of plant crop and 3.35, 1.67 and 3.52 t/fed for the second, third and fifth cuts of ratoon crop, respectively. This result shows that, in the first ratoon, plants started to have closer values of leaves fresh weight than in the first year of plant life (plant crop) as a response to nitrogen fertilization levels. In plant crop, fifth cut had the maximum value of leaves fresh weight (3.29 t/fed). It is notable that there was very close values of leaves fresh weight in first, second and last cuts of ratoon crop (warm season cuts) giving 3.23, 3.20 and 3.49 t/fed, respectively. On the other hand, third and fourth cuts (winter cuts) gave 1.64 and 1.59 t/fed, respectively, (Table 7).

#### 5-Leaves air dried weight

Results (Table 8) show the significant effect of propagation method on leaves air dried weight in all cuts of plant crop and only the first three cuts of ratoon crop. In both plant and ratoon crops, plants propagated through root cuttings gave maximum values of leaves air dried weight (0.91, 0.99, 0.52, 0.53 and 1.09 t/fed) for the five respective cuts of plant crop and (1.06, 1.14 and 0.56 t/fed) in the first, second and third cuts of ratoon crop, respectively. This result shows the gradual narrowing among the three propagation methods by time where values gave the same values of air dried weight of leaves by the end of the second season of plant life. This may be due to the greater biomass produced by time. Nitrogen fertilization had significant effect on stevia leaves air dried weight in all cutting dates of plant crop except in the last cut, meanwhile, nitrogen fertilization did not have any significant effect on ratoon crop except in the second cut (Table 8). Application of 40 kg N/fed/cut gave maximum values of stevia leaves air dried weight (0.76, 0.96, 0.52 and 0.52 t/fed) in the respective first four cuts of plant crop and (1.11 t/fed) in the second cut of ratoon crop, respectively. This result indicates the low fertilization requirements of stevia

plants during its second year of plant life, these plants may have its requirements from available nitrogen already exists in the soil. In the first, second and fifth cuts (warm season cuts) maximum values of leaves air dried weight were (0.68, 0.90 and 1.09 t/fed) in plant crop and (0.99, 1.04 and 1.13 t/fed) in ratoon crop, respectively while the lowest values were obtained in low temperature season cuts (after 240 and 300 days) which gave 0.51 and 0.69 in plant crop and 0.54 and 0.51 t/fed in ratoon crop, respectively. These results show the effect of temperature and growing season on leaves air dried weight of stevia plant which was previously mentioned by Donalizio *et al.*, (1982).

#### **6- Accumulated leaves air dried weight**

In both plant crop and ratoon crop, maximum values of total leaves air dried weight were obtained from root propagated plants giving values of 4.04 t/fed in plant crop and 4.42 t/fed in ratoon crop (Table 9). This result indicates the increase in total leaves air dried weight by time which may be due to the great root size formed and more branches production especially in the first cut. Nitrogen fertilization had a significant effect on stevia total leaves air dried weight only in plant crop. However, in both plant and ratoon crops, maximum values were obtained from application of 40 kg N/fed/cut (3.88 t/fed in plant crop and 4.36 t/fed in ratoon crop). From result, it is obvious that nitrogen application affected plants of the first year of its life but in the second year (ratoon crop) plants became able to absorb more nitrogen from soil showing less response to nitrogen application.

#### **7- Stevioside percentage**

Differences among the three propagation methods of first ratoon crop were smaller than the differences among the three propagation methods in the first year of plant life in the five cuts (Table 9). However, root cutting propagated plants were still superior than plants of the other two propagation methods (21.12, 22.25, 16.42, 16.21 and 24.17 %) for plant crop and (19.70, 21.53, 16.40, 18.20 and 27.57%) for ratoon crop of the five respective cuts. This result may be due to the great biomass produced of plant propagated by root cuttings causing stevioside to be increased in plants that produced greater biomass of plant crop and ratoon crop. Stevioside percentage was maximum with increasing nitrogen fertilization (Table 9). However, application of 20 kg N/fed/cut gave close values of stevioside percentage with those obtained from plants received 40 kg N/fed/cut. Meanwhile, by the end of the season, stevioside content gave its maximum values in the fifth cut. Stevioside percentages varied from 16.66 and 18.01% with 0 kg N/fed/cut after 90 days to 22.44 and 24.36% with 40kg N/fed/cut after 360 days for plant crop and ratoon crop, respectively. Data in



Table (9) show the differences among the five cutting ages of stevia plant in stevioside percentage. From data, it could be noted that the differences in stevioside percentage are due to the different environmental conditions throughout the season. Maximum mean value of stevioside percentage in the leaves was obtained at the end of the plant crop and ratoon crop (21.95 and 23.41%) followed by the second cut after 180 days from the beginning of plant crop and ratoon crop (19.78 and 20.27 %), respectively. Several researchers indicated the effect of environmental conditions on stevioside content and that high temperatures lead to high stevioside percentage, among them Columbus, (1997).

#### **8- Stevioside yield**

In all cutting dates, root cutting propagated plants exceeded both tissue culture and seed propagated plants (Table 10) in stevioside yield. Maximum value of stevioside yield was obtained from root cutting propagated plants in the last cut (263.50 kg/fed) in plant crop and (314.29 kg/fed) in ratoon crop. Result shows that both tissue culture and root cutting propagated plants gave close values of stevioside yield/fed but higher than seed propagated plants, also stevioside yield in ratoon crop is much higher than plant crop. Such findings may be due to the larger root system of all propagating methods in the second year of plant life than the first year which gave larger leaves and stem mass which was reflected in stevioside production. In all cutting ages applying of 40 kg N/fed/cut gave the highest values of stevioside yield in all cuts through the two production years (Table 10). Maximum value of stevioside yield was obtained from plants received 40 kg nitrogen/fed/cut in the fifth cut (251.30 kg/fed) in plant crop and (277.70 kg/fed) in ratoon crop). By the end of the season, values of stevioside yield/fed became closer among the three nitrogen fertilization levels. The close values of the three fertilization levels may be due to the higher biomass and root system for all plants by the end of the second season which gave them the opportunity to use the nitrogen in the soil. First, second and fifth cuts (warm season cuts) extremely exceeded low temperature season cuts (third and fourth cuts) in stevioside yield (Table 10). The five cuts gave stevioside yield values of 124.60, 178.00, 79.70, 109.00 and 239.30 kg/fed in plant crop and 184.04, 210.80, 80.67, 85.11 and 264.53 kg stevioside/fed, respectively. The low stevioside yield values of low temperature weather show that stevia plant needs higher temperatures for good productivity and high quality.

Table 7. Effect of propagation method, nitrogen fertilization and their interaction on leaves fresh weight (t/fed) of plant crop (2002-2003) and ratoon crop (2003-2004) seasons during five respective cuts each year.

Treatment		Cutting age (days)														
		Plant crop (2002-2003)					Ratoon crop (2003-2004)									
		90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )
Propagation Method (M)	Seed	0.82	2.42	1.49	1.43	3.29	2.96	2.96	1.52	1.51	3.40	2.96	2.96	1.52	1.51	3.40
	Tissue culture	2.55	2.68	1.56	1.53	3.32	3.44	3.30	1.69	1.62	3.52	3.44	3.30	1.69	1.62	3.52
	Root cutting	2.75	2.99	1.55	1.58	3.27	3.29	3.44	1.71	1.63	3.53	3.29	3.44	1.71	1.63	3.53
L.S.D at 0.05		0.02	0.07	0.04	0.03	0.03	0.42	0.45	0.03	0.05	N.S	0.42	0.45	0.03	0.05	N.S
Nitrogen (Kg/fed./cut) (N)	0	1.81	2.47	1.51	1.45	3.20	3.19	2.93	1.61	1.53	3.44	3.19	2.93	1.61	1.53	3.44
	20	2.04	2.72	1.53	1.52	3.30	3.19	3.33	1.65	1.60	3.50	3.19	3.33	1.65	1.60	3.50
	40	2.28	2.91	1.56	1.57	3.38	3.32	3.35	1.67	1.63	3.52	3.32	3.35	1.67	1.63	3.52
L.S.D at 0.05		0.05	0.06	0.03	0.03	0.02	N.S	0.35	0.04	N.S	0.04	N.S	0.35	0.04	N.S	0.06
Interaction MxN																
Seed	0	0.80	2.38	1.46	1.34	3.20	2.90	2.80	1.51	1.40	3.35	2.90	2.80	1.51	1.40	3.35
	20	0.82	2.38	1.50	1.45	3.33	2.98	3.01	1.52	1.54	3.41	2.98	3.01	1.52	1.54	3.41
	40	0.85	2.50	1.52	1.51	3.34	3.02	3.06	1.54	1.60	3.44	3.02	3.06	1.54	1.60	3.44
Tissue culture	0	2.10	2.56	1.55	1.48	3.26	3.48	2.89	1.63	1.59	3.48	3.48	2.89	1.63	1.59	3.48
	20	2.71	2.67	1.56	1.53	3.30	3.39	3.40	1.71	1.63	3.53	3.39	3.40	1.71	1.63	3.53
	40	2.85	2.82	1.57	1.57	3.40	3.45	3.61	1.74	1.64	3.56	3.45	3.61	1.74	1.64	3.56
Root cutting	0	2.53	2.47	1.51	1.54	3.15	3.18	3.10	1.68	1.61	3.49	3.18	3.10	1.68	1.61	3.49
	20	2.60	3.19	1.54	1.58	3.28	3.21	3.58	1.72	1.62	3.55	3.21	3.58	1.72	1.62	3.55
	40	3.13	3.40	1.59	1.63	3.39	3.49	3.64	1.73	1.65	3.56	3.49	3.64	1.73	1.65	3.56
L.S.D at 0.05		0.08	0.11	N.S	N.S	0.03	N.S*	N.S	N.S	N.S	N.S	N.S*	N.S	N.S	N.S	N.S
Mean		2.04	2.71	1.53	1.51	3.29	3.23	3.20	1.64	1.59	3.49	3.23	3.20	1.64	1.59	3.49

Table 8. Effect of propagation method, nitrogen fertilization and their interaction on leaves air dried weight (t/fed) of plant crop (2002-2003) and ratoon crop (2003-2004) seasons during five respective cuts each year.

Treatment		Cutting age (days)																						
		Plant crop (2002-2003)					Ratoon crop (2003 - 2004)					Total												
		90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	Total	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	Total						
Propagation Method (M)	Seed	0.27	0.80	0.50	0.48	1.09	3.14	0.87	0.88	0.50	0.49	1.11	3.85	0.85	0.89	0.52	0.51	1.10	0.55	0.51	1.13	4.55		
	Tissue culture	0.91	0.99	0.52	0.53	1.09	4.04	1.05	1.14	0.56	0.52	1.14	4.42	0.01	0.03	0.01	0.01	0.10	0.15	0.05	N.S	1.15		
	Root cutting	0.60	0.82	0.50	0.48	1.06	3.46	0.94	0.94	0.53	0.50	1.11	4.02	0.68	0.90	0.51	0.65	1.09	1.07	0.54	0.51	1.13	4.24	
L.S.D at 0.05	Nitrogen (Kg/fed./cut) (N)	0.76	0.96	0.52	0.52	1.12	3.88	1.05	1.11	0.54	0.52	1.14	4.36	0.01	0.02	0.01	0.01	N.S	0.10	N.S	N.S	1.13		
	L.S.D at 0.05	0.01	0.02	0.01	0.01	N.S	0.05	N.S	0.10	N.S	N.S	1.13	4.21	0.01	0.01	0.01	0.01	N.S	0.10	N.S	N.S	1.13		
		Interaction MxN																						
	Seed	0.26	0.79	0.49	0.45	1.06	3.05	0.84	0.82	0.49	0.48	1.08	3.71	0.26	0.79	0.50	0.49	1.11	0.87	0.89	0.51	1.12	3.88	
	Tissue culture	0.28	0.83	0.50	0.50	1.11	3.22	0.90	0.93	0.50	0.51	1.13	3.97	0.69	0.85	0.52	0.49	1.08	0.98	0.96	0.54	0.50	1.12	4.10
	Root cutting	0.90	0.88	0.52	0.51	1.09	3.90	1.09	1.13	0.55	0.52	1.14	4.43	0.95	0.94	0.52	0.52	1.13	1.20	1.03	0.56	0.51	1.13	4.24
	L.S.D at 0.05	0.85	0.82	0.50	0.51	1.05	3.73	1.01	1.03	0.56	0.51	1.13	4.24	0.86	1.03	0.52	0.53	1.08	1.19	1.19	0.55	0.52	1.14	4.40
	Mean	1.04	1.13	0.53	0.54	1.13	4.37	1.13	1.21	0.57	0.52	1.15	4.58	0.03	0.04	N.S	0.01	0.01	N.S	N.S	N.S	N.S	1.15	
	Mean	0.68	0.90	0.51	0.69	1.09	3.87	0.99	1.04	0.54	0.51	1.13	4.21	0.68	0.90	0.51	0.69	1.09	1.04	1.04	0.54	0.51	1.13	4.21

Table 9. Effect of propagation method, nitrogen fertilization on stevioside percentage of plant crop (2002-2003) and ratoon crop (2003-2004) seasons during five respective cuts each year.

Treatment		Cutting age (days)														
		Plant crop (2002-2003)					Ratoon crop (2003 - 2004)									
		90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )
Propagation Method (M)	Seed	11.52	14.38	14.06	14.65	19.13	18.01	18.77	12.10	14.04	20.39					
	Tissue culture	18.93	20.77	15.76	15.65	22.25	17.97	20.52	16.33	17.82	22.27					
	Root cutting	21.12	22.25	16.42	16.21	24.17	19.70	21.53	16.40	18.20	27.57					
Nitrogen (Kg/fed./cut) (N)	0	16.66	18.20	14.93	15.07	20.96	18.01	19.82	14.10	15.76	22.57					
	20	17.43	19.68	15.30	15.51	22.14	18.69	19.80	14.00	17.01	23.30					
	40	17.51	19.79	16.01	15.92	22.44	19.07	21.20	21.00	17.29	24.36					
Interaction MxN																
Seed	0	11.75	13.07	13.60	14.91	18.31	17.54	17.80	11.10	12.00	19.30					
	20	12.84	15.46	15.66	15.65	18.96	17.60	18.50	10.00	14.91	21.40					
	40	12.57	15.32	15.78	15.83	19.65	18.90	20.00	15.20	15.20	20.48					
Tissue culture	0	19.43	21.22	15.24	15.36	22.30	17.80	20.66	15.50	17.76	21.40					
	20	20.73	21.43	15.45	15.40	22.08	18.50	20.20	16.00	17.71	21.30					
	40	21.30	22.34	15.80	15.86	23.75	17.60	20.71	17.50	18.00	24.10					
Root cutting	0	21.09	21.99	15.54	15.44	23.08	18.70	20.99	15.70	17.53	27.00					
	20	22.64	23.45	16.32	16.77	24.65	19.96	20.70	16.00	18.41	27.20					
	40	22.56	23.70	17.19	16.99	24.74	20.70	22.90	17.50	18.67	28.50					
Mean		18.32	19.78	15.62	15.80	21.95	18.59	20.27	14.94	16.69	23.41					

Table 10. Effect of propagation method, nitrogen fertilization on stevioside yield (kg/fed) plant crop (2002-2003) and ratoon crop (2003-2004) seasons during five respective cuts each year.

Treatment	Cutting age (days)																							
	Plant crop (2002-2003)					Ratoon crop (2003 - 2004)					Total													
	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	Total	90 (1 <sup>st</sup> )	180 (2 <sup>nd</sup> )	240 (3 <sup>rd</sup> )	300 (4 <sup>th</sup> )	360 (5 <sup>th</sup> )	Total							
Propagation Method (M)	31.10	115.00	70.30	70.30	208.50	495.30	156.68	165.17	60.50	68.79	226.32	677.48	160.90	184.90	82.00	79.80	244.80	752.30	190.48	225.72	89.81	90.88	251.65	848.55
Root cutting	192.20	220.30	85.40	85.90	263.50	847.20	208.82	245.44	91.84	94.64	314.29	955.04	12.12	7.73	4.01	0.64	6.48	30.41	11.48	10.08	5.63	1.49	5.48	28.42
L.S.D at 0.05	100.00	149.20	74.70	72.30	222.20	618.40	169.29	186.30	74.73	78.80	250.52	759.65	118.50	177.10	78.00	100.80	241.30	715.80	185.03	211.86	75.60	86.75	263.29	822.53
Nitrogen (kg/fed./cut) (N)	133.10	190.00	83.30	82.80	251.30	740.40	200.23	235.32	113.40	89.90	277.70	916.56	10.03	3.41	2.20	0.55	5.12	20.06	12.31	3.38	3.01	1.07	7.11	18.36
L.S.D at 0.05	30.60	103.30	66.60	67.10	194.10	461.60	147.33	145.96	54.39	57.60	208.44	613.72	33.40	122.10	78.30	76.70	210.50	521.00	153.12	164.65	51.00	73.05	239.68	681.50
Seed	35.20	127.20	78.90	79.20	218.10	538.50	170.10	186.00	76.00	77.52	231.42	741.04	134.10	180.40	79.20	75.30	240.80	709.80	174.44	198.33	83.70	88.80	239.68	784.95
Tissue culture	186.60	188.60	80.30	78.50	240.70	774.70	201.65	228.26	88.00	92.09	242.82	852.82	202.40	210.00	82.20	82.50	268.40	845.40	195.36	248.52	98.00	93.60	274.74	910.22
Root cutting	179.30	180.30	77.70	78.70	242.30	758.40	188.87	216.19	87.92	89.40	305.10	887.49	194.70	241.50	84.90	88.90	266.20	876.20	199.60	246.33	88.00	95.73	310.08	939.74
L.S.D at 0.05	234.60	267.80	91.10	91.70	279.60	964.80	233.91	277.09	99.75	97.08	327.75	1035.58	14.13	5.32	2.09	1.03	7.98	36.61	15.66	7.41	4.97	2.50	10.12	41.23
Mean	124.60	178.00	79.70	109.00	239.30	730.50	184.04	210.80	80.67	85.11	264.53	825.17												

Interaction MxN

### 9- Accumulated stevioside yield

In both plant and ratoon crops, root propagated plants gave maximum values of total stevioside yield (847.20 kg/fed) in plant crop and (955.04 kg/fed) in ratoon crop. This result shows that root cutting propagated plants were superior in stevioside production and/or leaves production until the end of the first ratoon. Plants that received 40 kg N/fed gave the maximum values of total stevioside yield/fed/year in both plant and ratoon crops which gave 740.40 kg/fed in plant crop and 916.56 kg/fed in ratoon crop.

### Effect of the interaction between propagation method and nitrogen fertilization of plant crop and ratoon crop

Results did not show any significant effect of the interaction between propagation method and nitrogen fertilization levels at any cut on all studied traits during ratoon crop season (Tables 4-10). Meanwhile, in plant crop season, the interaction exerted significant effects on plant height only in the last cut, flowering plants percentage only in the first cut, number of branches/plant only in the first cut, leaves fresh weight in the first, second and fifth cut, leaves dry weight in first second, fourth and fifth cuts and all cuts of stevioside yield. This result is expected since after the first year of plant life, plants propagated through the three propagation method had larger root system and so all plants could absorb nitrogen from soil efficiently. Therefore, nitrogen fertilization will not have a special effect on one propagation method rather than the other.

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## إستخدام أندول حامض البيورتريك على العقل الساقية للأستييفيا وإنتاجية وجودة محصول الغرس والخلفة

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أقيمت أربعة تجارب في محطة بحوث الجيزة - مركز البحوث الزراعية. تم إجراء تجربتين في الصوبة الزجاجية خلال الموسمين ٢٠٠١، ٢٠٠٢ لدراسة تأثير موقع العقل الساقية والمعاملة بإندول حمض البيورتريك والتفاعل بينهما علي نجاح إكثار نباتات الاستيفيا بالعقل الساقية. اشتملت الدراسة الثانية على عاملان، طريقة الإكثار والتسميد النيتروجيني والتفاعل بينهما علي محصول وجودة نبات الاستيفيا لمحصول الغرس (٢٠٠٢-٢٠٠٣) ولمحصول الخلفة (٢٠٠٣-٢٠٠٤). تم الحصول على خمس حشات لكل موسم زراعي. تفوقت العقل القاعدية في كل الصفات التي تمت دراستها والتي أعطت أعلى نسبة لبقاء العقل (٨٧,٥٦%) والوزن الرطب للأوراق (١,٢٣جم) والوزن الجاف للأوراق (١,٠٧جم) والوزن الرطب للسيقان (٢,٣١جم) والوزن الجاف للسيقان (٠,٧٠جم) والوزن الرطب للجذور (١,٨٠جم) والوزن الجاف للجذور (٠,٤٩جم). لم يكن لأندول حمض البيورتريك أي تأثير علي العقل فيما عدا صفة نسبة بقاء العقل والتي زادت بزيادة تركيز الاندول من صفر إلي ٥٠٠ و ١٠٠٠ جزء في المليون والتي أعطت ٧٤,٤٤ و ٨٢,٤٤ و ٨٦,٨٩ % علي الترتيب. تحققت أعلى قيم لنسبة نجاح العقل من العقل القاعدية والتي عوملت باندول حمض البيورتريك بتركيز ١٠٠٠ جزء في المليون (٩٥,٣٣%) في حين كان أقل نجاح من العقل القمية والتي لم تعامل باندول حمض البيورتريك (٧٠%). أوضحت النتائج أنه في كل من موسمي الغرس والخلفة تفوقت النباتات المكثرة بالعقل الجذرية علي كل من النباتات المكثرة بالبذرة أو بنواتج زراعة الأسجة بالنسبة لكل الصفات. كذلك تفوقت إضافة ٤٠ كجم نيتروجين/فدان/حشة علي كل من معاملة المقارنة (بدون تسميد) و ٢٠ كجم نيتروجين/فدان/حشة. أعطت النباتات المكثرة بالعقل الجذرية والتي تلقت ٤٠ كجم نيتروجين/فدان/حشة في الحشة الخامسة أعلى قيم للوزن الجاف للأوراق (١,١٣ طن/فدان بالنسبة لمحصول الغرس و ١,١٥ طن/فدان بالنسبة لمحصول الخلفة) والوزن الكلي للأوراق الجافة (٤,٣٩ طن/فدان/سنة بالنسبة لمحصول الغرس و ٤,٥٨ طن/فدان/سنة بالنسبة لمحصول الخلفة) والنسبة المئوية للاستيفيوزيد (٢٤,٦٠%) بالنسبة لمحصول الغرس و ٢٨,٥٠% بالنسبة لمحصول الخلفة) ومحصول الاستيفيوزيد (٢٧٩,٦٠ كجم/فدان بالنسبة لمحصول الغرس و ٣٢٧,٧٥ كجم/فدان بالنسبة لمحصول الخلفة) والمحصول الكلي للاستيفيوزيد (٩٦٢,٤٣ كجم/فدان/سنة بالنسبة لمحصول الغرس و ١٠٣٥ كجم/فدان/سنة بالنسبة لمحصول الخلفة).