EFFECT OF PROPAGATION METHOD AND NITROGEN FERTILIZATION ON STEVIA (*STEVIA REBAUDIANA.*, BERTONI) YIELD AND QUALITY IN EGYPT

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Abstract

Two field experiments were carried out in Giza Agricultural Station – Agricultural Research Center during the two successive seasons 2001/2002 and 2002/2003 to study the effect of propagation method (seed, tissue culture and root cuttings), nitrogen fertilization (0, 20 and 40 kg N/fed/cut) and their interaction on *Stevia rebaudiana* yield and quality. Seeds of stevia variety Spanti imported from Spain and locally regenerated vegetatively were used to produce plantlets, root cuttings and shoot apex tissue culture. Results showed that tissue culture and root cuttings propagated plants were significantly taller than seed propagated plants. Tissue culture and root cutting propagation methods exceeded seed propagated plants in No. of branches / plant in first, second and fifth cuts. Root cutting propagated plants gave the maximum values of leaves fresh weight (2.7, 3.12, 1.55 and 1.58 t/fed for the first, second, third and fourth cuts, respectively). Plants propagated through root cuttings exceeded plants propagated by seed and tissue culture in stevioside % in all cutting dates giving maximum value of 24.16% in the first season and 21.14% in the second season in the 5th cut respectively. Application of 40kg N/fed/cut gave significant maximum values of plant height in three cuts (54.06, 54.33 and 53.07cm. for first, second and fifth cuts, respectively). Nitrogen fertilization affected all cuts for the No. of branches/plant and the application of either 20 or 40 kg N/fed/cut significantly exceeded 0 kg N/fed/cut. Application of 40 kg N/fed/cut gave maximum values of fresh leaves weight in all cutting dates (2.27, 2.86, 1.56, 1.56 and 3.37 t/fed respectively), meanwhile dry leaves weight yield gave (0.87, 0.96, 0.52, 0.52 and 1.13 t/fed. respectively). Application of 20 and 40 kg N/fed/cut exceeded 0kg N/fed/cut in stevioside % in both seasons. Application of 40 kg N/fed/cut for stevia plants propagated by root cutting produced maximum yield of dry leaves weights of 1.06, 1.13 and 0.54 t/fed. for first, second and fourth cuts, respectively. Therefore, total stevioside yield reached its maximum value (962.43 kg/fed/year) when root cutting propagation method and application of 40 kg N/fed/cut were used.

INTRODUCTION

The worldwide demand for high potency sweeteners is expected to increase especially with the new practice of blending different sweeteners, as well as the demand of alternatives is expected to increase. The sweet herb of Paraguay, stevia is an herb with incredible sweetening power. Its ability to sweeten is approximately 300 times that of sugar. Typically, it has a mild Licorice-like taste and is completely natural in its biochemical profile. What makes stevia so intriguing is that unlike other natural sweetening agents, it is completely calorie-free, never initiates a rise in blood sugar, and does not provide suitable media for microorganisms like bacterias and yeasts. Stevia plant was introduced to Egypt in 1990. Stevia has a long history of use as a natural sweetener and medicinal aid. It is heat stable, non-caloric and can be used by diabetics. Today, because the demand for stevia is escalating, researchers and chemists are working to provide even better stevia supplements and may even end up teeming with governmental agencies to introduce stevia as economic crop in Egypt.

Linear and significant increase in fresh and dry leaves weights, fresh and dry yield of stem, growth of new tiller and lateral branches, fresh yield and stevioside content were associated with increasing nitrogen rates up to 30 kg/fed/cut (Allam *et al.*, 2001). High number of shoots could be obtained by repeating the cycle of stem tip propagation. These shoots could be rooted and the regenerated plants could be transplanted to soil. (Tamura *et al.*, 1984). Plants produced from seeds were less productive in the first year than those from cuttings. Both types of propagation (cuttings or seeds) could be used for growing *Stevia rebaudiana*, Bertoni. for obtaining stevioside for practical use even in the climatic conditions of the Central Europe (Nepovim *et al.*, 1998). Stevia had been successfully taken to locations of wide range of climatic conditions around the world and apparently grown successfully, although often by using vegetative propagation methods and seedling establishment in a greenhouse before planting in the field (Midmore and Rank 2002). Stevia vegetative growth is reduced when temperatures are below 20 degrees and when day length is less than 12 hours.Increasing day length to 16 hours and increasing light intensity can increase vegetative growth and stevioside levels (Yermakov and Kochetov, 1996).

Although stevia may be the most remarkable sweetener in the world, yet its recognition in Egypt remains relatively low, so the present work was carried out to investigate the effect of different propagation methods (seed, root division and tissue culture), nitrogen fertilizer rates and their interaction on stevia plant productivity and quality.

MATERIALS AND METHODS

Two factorial experiments were conducted during the two successive seasons 2001/2002 and 2002/2003 (plant crop / plantation of first year) in the Agricultural Research Center Experimental Station – Giza - Egypt 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/m3)	2.65
CaCO3 %	1.81
Available N (ppm)	11.00
Available P (ppm)	9.12
Available K (ppm)	35.86

The experimental treatments:

A- 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. The first dose was added before the first irrigation come after cut and the second dose was added before the next irrigation. Meanwhile, these 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).

B- Propagation method:

Three propagation methods were used in this study (root division, tissue culture, and seeds) during both experimental seasons (2001/2002and 2002/2003).

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 Institute (AGERI-ARC) through shoot apex culture technique (Tamura *et al.*,1984). Clonal propagation of *Stevia rebaudiana,* Bertoni. was achieved by culturing stem-tips with a few leaf primordia on an agar medium supplemented with a high concentration (10 mg/litre) of kinetin. High numbers of shoots could be obtained by repeating the cycle of multiple-shoot formation from a single stem-tip. Rooting of in vitro-derived shoots was made following subculture into auxin-containing MS medium. The composition of the basal medium used through this study containing MS salts in addition to the following ingredients, 100 mg/l myo-inosital, 3% sucrose, 1 ml MS vitamin mixture and 2 g/l phytagel, supplemented with 0.01 mg/l IBA and 10 mg/l GA_3 . Adaptation and acclimatization of *Stevia rebaudiana,* Bertoni. were carried out in the conventional greenhouse facilities. Plantlets (7-10 cm hight) obtained by in vitro teqnique were washed under running tap water and soaked in fungicide solution (2 g/l of Benlate) for 20 minutes. Plantlets were cultured in plastic pots (15 cm in diameter) contained various mixtures of beat moss and sand. To keep constant high humidity (up to 90%) the pots were covered with polyethylene bags, which gradually removed within one week, plantlets were transplanted in the open field after adaptation in the greenhouse.

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 (SI) and preserved in the refrigerator at 5 C^0, then it was planted in the glasshouse in January 15th 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 at 1st of April in both seasons (2001/2002 and 2002/2003) on rows.Plot area was $10.5m^2$ (6 ridges, 3.5m long and 50cm apart), spacing between hills was 30cm to reach a population of 28000 plants per feddan ($4200m^2$).

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 at 10 cm above soil surface on mentioned dates.

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

Cut No.	Cutting date	Plant age	Season	Day length
*1st	July 1st	90 days	Summer	13.53
*2nd	October 1st	180 days	Autumn	11.44
**3rd	December 1st	240 days	Autumn	10.19
**4th	February 1st	300 days	Winter	10.58
*5th	April 1st	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- No. 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 (ton/fed/cut).

Collected leaves were air dried in shade under natural conditions.

6-Accumulated leaves dry weight (ton/fed/year).

Leves dry weight (ton/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 1gram 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 lyophyilization (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_2O/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 90^0C 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 dry leaves yield of each cut by stevioside percentage determined for the same cut.

9- Total stevioside yield (kg/year).

Stevioside yield (kg/year) was calculated by summing the stevioside yield (kg/fed/cut) of the five cuts taken each 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. Since there was homogeneity between both seasons of plant crop, a combined data analysis was made (Snedecor and Cochran, 1969). For means comparison, LSD values were calculated at 5% level of significance following the method of Gomez and Gomez (1984).

RESULTS AND DISCUSSION

1- Plant height

Propagation method significantly affected plant height only in the second, fourth and fifth cuts. Plant length of tissue culture and root cutting propagation methods were significantly taller than those of seed propagation method, meanwhile, there was no significant difference between tissue culture and root cutting propagation methods, Table 3. The rapid growth rate of root cutting tissue culture propagation methods especially in the early days of planting where tiny poorly germinated seed takes more time to establish seedling and consequently vegetative growth might led to such result. Several researchers concluded the same result among whom, Molinas, (1988) and Lester, (1999) who showed that there was an advantage of using root cuttings and tissue culture propagation methods for stevia plantation lies in quick growing and fast entering into yield phase.

Nitrogen fertilization had significant effects on first, second and fifth cuts (warm and long day length season cuts), where plants supplied by 40 kg N/fed/cut exceeded those of 0 and 20 kg N/fed/cut. This result indicates the distinguished effect of nitrogen fertilization on meristemic activity and cell elongation which reflected in plant height. Meanwhile, this effect did not exist during low temperature season since plant growth was reduced. The same result was obtained by Chalapathi *et al.* (1999). A significant effect of the interaction is obvious only during the third and the fifth cuts. In the third cut, maximum value of plant height was obtained from tissue culture propagated plants received 0 or 20 kg N/fed/cut being 26.34 and 26.31 cm, respectively, while in the fifth cut maximum value of plant height was obtained from root cutting propagated plants received 20 or 40 kg N/fed/cut. Cuts taken from growth/regrowth of low temperature and less day length season (< 12 h.) (third and fourth cuts) had a very slow growth rate giving short plants (25.30 and 21.07 cm respectively). While first, second and fifth cuts (warm season cuts) showed rapid growth rate giving taller plants (52.45, 52.66 and 52.15 cm respectively). These data are in accordance with those obtained by Yermakov and Kochetov, (1996) who found that stevia vegetative growth is reduced when teperatures are below 20 degrees and day length less than 12 hours.

2- Number of branches /plant

Results showed significant effect of propagation method on stevia number of branches/plant at 90, 180 and 360 days from planting (first, second and fifth cuts), while it had no significant effect in the other two cuts (ie, 240 and 300 days from planting). However, tissue culture and root cutting propagated plants exceeded propagation by seed giving No. of branches of 16.77, 18.72 and 21.16 and 16.08, 17.38, and 20.06 for root cutting and tissue culture propagation methods determined at 1^{st}, 2^{nd} and 5^{th} cuts, respectively. Tissue culture and root cuttings propagated plants showed rapid branching if compared with remarkably slow growth of seedlings produced by tiny seeds causing such plants to perform less and slow branching capabilities. Similar results were obtained by Brandle *et al.* (2000) and Midmore and Rank (2002), who showed that since germination rates were poor and seedlings were very slow to establish, it is best to use vegetative transplanting for stevia plantation. For all cutting dates, the application of 40 kg N/fed/cut gave maximum values of number of branches/plant being 14.51, 17.23 , 14.32 , 14.22 , 21.61 for 90, 180, 240, 300 and 360 days from planting respectively. However, no significant difference was detected between application of 20 and 40 kg N/fed/cut for all cuts except for the third and fifth cuts. Previously it was found linear and significant increase in growth of new tillers associated with the increase in nitrogen rates up to 30 kg/fed/cut (Chalapathi, 1997 and Allam *et al.*, 2001).

Interaction between propagation method and nitrogen fertilization (Table 4) showed a significant effect on number of branches/plant only in the first two cutting dates (90 and 180 days from planting), maximum values were obtained from plants propagated by root cuttings and received 40 kg N/fed/cut

(19.31 and 21.26 branches/plant respectively). This result may be due to rapid and early formation of root system which promoted plant capability to absorb nutrients especially nitrogen, hence, such circumstances enhance vegetative growth including branching which increased number of branches/plant. Number of branches/plant fluctuated depending upon cutting date (Table 4). However, number of branches/plant increased in the first two cuttings being 13.30 and 16.58 branches/ plant of first and second cuts, respectively. Then it was decreased in the following two cuttings (cuts of plant growth/regrowth during low temperature and short day length season) being 13.18 and 13.35 respectively. In the last cut, number of branches /plant was increased again because of the suitable warm weather of the spring induced branching (Table 2). These data are in great accordance with those obtained by Allam *et al.* (2001) who found that vegetative traits and yield of stevia were affected by environmental conditions since growth of stevia greatly influenced by temperature and day length.

3- Flowering plants percentage

Propagation method affected flowering plants percentage in the first cut only where root cutting propagation method exceeded both seed and tissue culture propagation methods being 56.25%, while differences between root cuttings and tissue culture propagation methods did not reach the significant level of second, third and fourth cuts where all plants flowered (100.00 %), hence, environmental conditions during such period of the year affect flowering behaviour. Results in Table (5) show that flowering percentage in first and fifth cuts was affected by nitrogen fertilization. In the first cut, flowering percentage of plants applied either 20 or 40 kg N/fed/cut exceeded that of 0 kg nitrogen being 46.48 and 46.89%, respectively with no significant difference between both of them. The same trend was obtained in the fifth cut, whereas, maximum value of flowering plants percentage was obtained from stevia plants received 40 kg N/fed/cut (26.95%). Increasing flowering plants % by increasing nitrogen fertilization may be due to the favourable endogenous balance of nutrients realized by nitrogen fertilization. However, such effect was not detected in the other three cuts since all plants tended to flower as a result of short days conditions (Table 1). These results are in accordance with those obtained by Slamet and Taharat, (1988), who found that urea treatment did not affect the time of flowering of stevia plant. Results could not reveal any significant effect of the interaction between propagation methods and nitrogen fertilizer level on stevia flowering plants percentage (Table 5), meaning that both studied factors act independently.

4- Leaves fresh weight

Propagation method affected significantly the yield of fresh leaves in all cutting dates except the last cut Table 6, where, root cutting propagated plants exceeded both seed and tissue culture propagated plants in weight of fresh leaves, and gave mean values of 2.75, 3.12, 1.55, and 1.58 t/fed for first, second, third and fourth cuts, respectively. This result may be due to the basically higher capability of root cutting propagated plants to enhance vegetative growth/regrowth, such circumstances gave an opportunity to have more branching Table 4 and more growth rate, moreover, due to the basically higher weight of root cutting propagated plants which gave more branching, such differences among the three propagation methods were not obvious by the end of the season. Data in Table (6) indicate significant effect of N fertilization level on stevia fresh leaves weight in all cuts. Nitrogen fertilization level of 40 kg N/fed/cut gave maximum values of fresh leaves weight in all cutting dates being 2.27, 2.86, 1.56, 1.56 and 3.37 ton/fed/cut respectively in the respective five cuts. This result is in accordance with those obtained by Utumi *et al.* (1999) and Allam *et al.* (2001). Results showed significant effect of the interaction between propagation method and nitrogen fertilization on stevia fresh leaves weight in the first, second and fourth cuts only, whereas, root cutting propagated plants and application of 40 kg N/fed/cut significantly exceeded other treatments in fresh leaves weight giving maximum values of 3.12, 3.40 and 1.62 ton/fed in the first, second and fourth cuts respectively. Growth/regrowth of stevia plant during the warm season (first and second cuts) showed higher values of fresh leaves weight (2.03 and 2.73 ton/fed respectively) exceeding values of low temperature season, (third and fourth cuts) (1.53 and 1.51 ton/fed, respectively). Meanwhile, spring cut had the greatest value of fresh leaves weight (3.29 ton/fed), these differences indicate the effect of the weather on leaf production and growth rate. This result is in accordance with those obtained by Allam *et al.* (2001).

5- Leaves dry weight

Results Table 7 indicate the significant effect of propagation method on leaves dry weight in all cuts except the last cut. Root cutting propagated plants significantly exceeded both seed and tissue culture propagated plants in dry leaves weight (0.94, 1.04 and 0.53 in the first, second and fourth cuts, respectively). While in the third cut both tissue culture and root cutting propagated plants gave the same value of dry leaves weight (0.52 ton/fed) but significantly exceeded seed propagated plants. The superiority of root cutting propagated plants in dry leaves weight may be due to its more growth rate and larger root system after transplanting. This result is in the same line with those obtained by Brandle *et al.* (2000) and Midmore and Rank, (2002). In all cutting dates, application of 40 kg N/fed/cut gave maximum values of leaves dry weight being 0.78, 0.96, 0.52, 0.52 and 1.13 ton/fed respectively for the respective five cuts. This result indicates the considrable effect of nitrogen fertilization on capacity of holding leaves since such application (40 kg N/fed/cut) increased branching, enhanced meristimic activities and accumulated more dry matter in leaves. Several researchers found that rates near to 40 kg N/fed/cut were the best for maximum stevia yield, among whom, Utumi *et al.* (1999), Chalapathi *et al.*(1999) and Allam *et al.* (2001).

Results showed significant effect of the interaction between propagation method and nitrogen fertilization on stevia dry leaves weight in first, second and fourth cuts where root cutting propagated plants received 40 kg N/fed/cut gave maximum values of dry leaves weight being 1.06, 1.13 and 0.54 ton/fed in the first, second and fourth cuts, respectively while no significant effect of the interaction could be detected on this trait during third and fifth cuts. This result shows that increasing in fertilization level to 40 kg N/fed/cut was more effective on root cutting propagated plants in the first, second and fourth cuts than other methods. It is obvious that there is difference between warm and low temperature seasons cuts where third and fourth cuts gave the lowest yield of dry leaves weight (0.51 ton/fed) while maximum dry leaves yield (1.10 ton/fed) was obtained from (spring cut) last cut (Table 7). This result shows the difference between dry leaves yield of warm and low temperature seasons cuts, also the increase in dry leaves yield towards the end of the first year cut.

6- Accumulated leaves dry weight

The maximum dry leaves yield/year was superior in response of using root cutting propagation method (4.12 ton/fed) while maximum dry leaves yield/year was obtained by applying 40 kg N/fed/cut (3.91 ton/fed). However, no significant effect of the interaction between propagation method and nitrogen fertilization was found (Table 7).

7- Stevioside percentage

Stevioside percentage of plants propagated through root cuttings exceeded the other two propagation methods (seed and tissue culture) in stevioside % with all cutting dates giving 22.13, 23.05, 16.35, 16.40 and 24.16 % in the first season and 21.12, 22.25, 16.42, 16.21 and 24.17 % in the second season, respectively during the five respective cuts Table 8. Remarkably biomass produced by root cutting propagated plants Table 4 and 5 may be due to the considrable photosynthates production including stevioside. Applying 40 kg N/fed/cut gave maximum values of stevioside percentage in both seasons (18.81, 20.45, 16.26, 16.23 and 22.71 % in the first season and 17.51, 19.79, 16.01, 15.92 and 22.44 % in the second season) in all cutting dates respectively. Root cutting propagated plants received 40 kg N/fed/cut gave maximum values of stevia stevioside % in all cuts (21.84, 23.29, 17.30, 16.88 and 24.60 % in the first season and 22.56, 23.70, 17.19, 16.99 and 24.74 % in the second season, respectively for the respective five cuts). The response of stevioside content to the increase of nitrogen application may be due to the role of nitrogen in the induction of photosynthesis and therefore, stevioside production. Differences among the five different cuts of the year meaning change in stevioside content as a response to weather conditions (increase in stevioside percentage with increasing temperature) is obvious (17.20, 19.22, 15.41, 15.50 and 21.85 % in the first season and 18.32, 19.78, 15.62, 15.80 and 21.95% in the second season, respectively).

8- Stevioside yield (kg/fed/cut)

During all five cuts, root cutting propagated plants exceeded both seed and tissue culture propagated plants in stevioside yield. It is realized from the first cut that seed propagated plants gave lowest (33.45 kg) stevioside yield/fed while tissue culture and root cutting propagated plants gave 169.46 and 203.28 kg stevioside/fed, respectively recording total yield of the five cuts of 504.37, 768.62 and 873.85, respectively. Maximum value of stevioside yield was obtained from root cutting propagated plants in the fifth cut (263.39 kg stevioside/fed). This result indicates that both tissue culture and root cutting propagated plants had more stevioside assimilation and accumulation than seed propagation method. Plants received 40 kg N/cut/fed exceeded control treatment (0 kg N/cut/fed) Table (9), whereas, the lowest value of stevioside yield was obtained from plants received 0 kg N/cut/fed in the third cut (74.30 kg stevioside/fed) and maximum value was obtained from plants received 40 kg N/cut/fed in the fifth cut (255.10 kg stevioside/fed). The total yield of the five cuts was superior with 40 kg/cut/fed followed by 20 and 0 kg N/cut/fed (757.39, 709.33 and 636.96g kg stevioside/fed, respectively). This result may be due to the effect of nitrogen on promoting photosynthate assimilation and hence yield of dry leaves and stevioside content. The values of the interaction between propagation methods and N fertilization Table (9) greatly varied from 30.03 kg stevioside/fed (seed propagated plants received no nitrogen fertilization in the first cut) to 278.77 kg stevioside/fed (root cutting propagated plants received 40 kg N/cut/fed in the fifth cut). The differences among cuts Table (9) were detectable between the warm season (first, second and fifth cuts) and low temperature season (third and fourth cuts). The warm season gave maximum yields (122.51, 177.00 and 240.85 kg stevioside/fed, for 1^{st}, 2^{nd} and 5^{th} cuts respectively). This result shows the effect of changes in temperatures around the year seasons on stevioside production due to the higher photosynthesis and stevioside production under high temperatures since stevia is a tropical plant that performs better under higher temperatures. From the mentioned results, it can be conclude that root cutting propagated plants with nitrogen fertilization of 40 kg/ fed/ cut may give maximum leaves yield and stevioside content of *stevia rebaudiana,* Bertoni. under Egyptian conditions.

9- Total stevioside yield (kg/fed/year)

Root cuttings propagtion method significantly exceeded both seed and tissue culture propagation method in total stevioside yield (873.85 kg/fed/year). Meanwhile, 40 kg N/fed/cut was superior in total stevioside yield giving 757.39 kg stevioside/year. The total yield of the five cuts gave its maximum yield from root cutting propagated plants received 40kg N/cut/fed (962.43 kgstevioside/fed) Table (9).

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Table (3) Effect of propagation method (M), nitrogen fertilization (N) and their interaction on plant length (cm.) at different cuts (combined data of two seasons).

Treatment		Cutting age (days)				
		90 (1st)	180 (2nd)	240 (3rd)	300 (4th)	360 (5th)
Propagation Method	Seed	51.79	50.60	24.46	20.78	49.70
	Tissue Culture	52.23	52.17	25.77	21.00	53.02
	Root Cutting	53.32	55.21	25.69	21.44	53.73
L.S.D		**N.S**	**0.59**	**N.S**	**0.52**	**0.97**
Nitrogen (kg/fed)	0	51.08	51.30	25.29	20.44	50.25
	20	52.21	52.36	25.64	21.47	52.51
	40	54.06	54.33	24.98	21.31	53.70
L.S.D		**2.08**	**1.07**	**N.S**	**N.S**	**0.90**
Interaction MxN						
Seed	0	49.18	48.84	23.39	19.98	49.37
	20	51.88	50.27	24.70	21.19	49.48
	40	54.31	52.69	25.30	21.18	50.26
Tissue Culture	0	50.57	51.35	26.34	21.06	51.15
	20	52.00	51.12	26.31	20.88	53.73
	40	54.14	54.10	24.66	21.05	54.20
Root Cutting	0	53.48	53.71	26.14	20.28	50.22
	20	52.76	55.69	25.92	22.34	54.30
	40	53.73	56.23	25.00	21.70	56.65
L.S.D		**N.S**	**N.S**	**1.76**	**N.S**	**1.55**
Total Mean		**52.45**	**52.66**	**25.30**	**21.07**	**52.15**

Table (4) Effect of propagation method (M), nitrogen fertilization (N) and their interaction on number of branches / plant at different cuts (combined data of two seasons).

Treatment		Cutting age (days)				
		90 (1st)	180 (2nd)	240 (3rd)	300 (4th)	360 (5th)
Propagation Method	Seed	7.06	13.65	12.99	13.18	19.30
	Tissue Culture	16.08	17.38	13.07	13.30	20.06
	Root Cutting	16.77	18.72	13.49	13.57	21.16
L.S.D		**1.19**	**2.41**	**N.S**	**N.S**	**0.90**
Nitrogen (kg/fed)	0	11.83	15.62	11.66	12.35	19.05
	20	13.57	16.90	13.57	13.48	19.87
	40	14.51	17.23	14.32	14.22	21.61
L.S.D		**1.51**	**1.05**	**0.70**	**0.80**	**1.21**
Interaction MxN						
Seed	0	5.54	13.23	11.25	11.58	19.10
	'20	6.71	13.81	13.41	13.53	18.22
	40	8.92	13.90	14.32	14.42	20.60
Tissue Culture	0	15.78	17.17	11.53	12.13	18.32
	20	17.17	18.45	13.30	13.65	20.08
	40	15.29	16.53	14.39	14.12	21.79
Root Cutting	0	14.18	16.47	12.21	13.34	19.75
	20	16.82	18.45	13.99	13.25	21.30
	40	19.31	21.26	14.26	14.12	22.43
L.S.D		**2.61**	**1.83**	**N.S**	**N.S**	**N.S**
Total Mean		**13.30**	**16.58**	**13.18**	**13.35**	**20.18**

Table (5) Effect of propagation method (M), nitrogen fertilization (N) and their interaction on flowering plants percentage at different cuts (combined data of two seasons).

Treatment		Cutting age (days)				
		90 (1st)	180 (2nd)	240 (3rd)	300 (4th)	360 (5th)
Propagation Method	Seed	39.96	100.00	100.00	100.00	25.82
	Tissue Culture	39.83	100.00	100.00	100.00	25.46
	Root Cutting	56.25	100.00	100.00	100.00	25.47
L.S.D		**5.40**	**N.S**	**N.S**	**N.S**	**N.S**
Nitrogen (kg/fed)	0	42.73	100.00	100.00	100.00	24.57
	20	46.48	100.00	100.00	100.00	25.23
	40	46.89	100.00	100.00	100.00	26.95
L.S.D		**3.08**	**N.S**	**N.S**	**N.S**	**1.65**
Interaction MxN						
Seed	0	39.45	100.00	100.00	100.00	23.97
	20	39.98	100.00	100.00	100.00	25.66
	40	40.46	100.00	100.00	100.00	27.83
Tissue Culture	0	37.94	100.00	100.00	100.00	25.24
	20	40.43	100.00	100.00	100.00	24.63
	40	41.25	100.00	100.00	100.00	26.51
Root Cutting	0	50.79	100.00	100.00	100.00	24.50
	20	59.01	100.00	100.00	100.00	25.40
	40	58.95	100.00	100.00	100.00	26.51
L.S.D		**N.S**	**N.S**	**N.S**	**N.S**	**N.S**
Total Mean		**45.36**	**100.00**	**100.00**	**100.00**	**25.58**

Table (6) Effect of propagation method (M), nitrogen fertilization (N) and their interaction on fresh leaves weight (t/f) in different cuts (combined data of two seasons).

Treatment		Cutting age (days)				
		90 (1st)	180 (2nd)	240 (3rd)	300 (4th)	360 (5th)
Propagation Method	Seed	0.82	2.42	1.48	1.43	3.29
	Tissue Culture	2.53	2.65	1.56	1.53	3.32
	Root Cutting	2.75	3.12	1.55	1.58	3.27
L.S.D		**0.15**	**0.01**	**0.03**	**0.02**	**N.S**
Nitrogen (kg/fed)	0	1.77	2.59	1.50	1.46	3.20
	20	2.04	2.74	1.53	1.52	3.30
	40	2.27	2.86	1.56	1.56	3.37
L.S.D		**0.11**	**0.07**	**0.02**	**0.02**	**0.05**
Interaction MxN						
Seed	0	0.77	2.35	1.44	1.35	3.20
	20	0.82	2.39	1.49	1.44	3.33
	40	0.86	2.52	1.51	1.50	3.34
Tissue Culture	0	2.05	2.60	1.55	1.49	3.26
	20	2.68	2.68	1.55	1.54	3.29
	40	2.85	2.67	1.57	1.56	3.40
Root Cutting	0	2.50	2.82	1.51	1.54	3.16
	20	2.63	3.16	1.54	1.57	3.27
	40	3.12	3.40	1.59	1.62	3.37
L.S.D		**0.19**	**0.12**	**N.S**	**0.03**	**N.S**
Total Mean		**2.03**	**2.73**	**1.53**	**1.51**	**3.29**

Table (7) Effect of propagation method (M), nitrogen fertilization (N) and their interaction on dry leaves weight (t/f) at different cuts (combined data of both seasons).

Treatment		Cutting age (days)					
		90 (1st)	180 (2nd)	240 (3rd)	300 (4th)	360 (5th)	Total
Propagation Method	Seed	0.28	0.80	0.50	0.48	1.10	**3.16**
	Tissue Culture	0.86	0.89	0.52	0.51	1.11	**3.89**
	Root Cutting	0.94	1.04	0.52	0.53	1.09	**4.12**
L.S.D		**0.05**	**0.03**	**0.01**	**0.01**	**N.S**	**0.22**
Nitrogen (kg/fed)	0	0.60	0.86	0.50	0.50	1.07	**3.53**
	20	0.70	0.91	0.51	0.51	1.10	**3.73**
	40	0.78	0.96	0.52	0.52	1.13	**3.91**
L.S.D		**0.04**	**0.01**	**0.01**	**0.01**	**0.02**	**0.18**
Interaction MxN							
Seed	0	0.26	0.78	0.48	0.46	1.07	**3.05**
	20	0.28	0.80	0.50	0.49	1.11	**3.18**
	40	0.30	0.84	0.51	0.51	1.11	**3.27**
Tissue Culture	0	0.69	0.87	0.52	0.50	1.09	**3.67**
	20	0.91	0.89	0.52	0.51	1.10	**3.93**
	40	0.97	0.92	0.53	0.52	1.13	**4.07**
Root Cutting	0	0.86	0.94	0.50	0.52	1.05	**3.87**
	20	0.90	1.05	0.52	0.53	1.09	**4.09**
	40	1.06	1.13	0.53	0.54	1.13	**4.39**
L.S.D		**0.07**	**0.02**	**N.S**	**0.01**	**N.S**	**N.S**
Total Mean		**0.69**	**0.91**	**0.51**	**0.51**	**1.10**	**3.72**

Table (8): Effect of propagation method (M) and nitrogen application (N) on stevioside percentage in dry leaves at different cuts (2001-2002 and 2002-2003 seasons).

Treatment		Cutting age (days)									
		Season 2001-2002					Season 2002-2003				
		90 (1st)	180 (2nd)	240 (3rd)	300 (4th)	360 (5th)	90 (1st)	180 (2nd)	240 (3rd)	300 (4th)	360 (5th)
Propagation Method	Seed	12.37	14.62	15.01	15.64	18.97	11.52	14.38	14.06	14.65	19.13
	Tissue Culture	20.48	21.66	15.50	15.54	22.71	18.93	20.77	15.76	15.65	22.25
	Root Cutting	22.13	23.05	16.35	16.40	24.16	21.12	22.25	16.42	16.21	24.17
Nitrogen (kg/fed/cut)	0	17.42	18.76	14.79	15.24	21.23	16.66	18.20	14.93	15.07	20.96
	20	18.74	20.11	15.81	15.94	21.89	17.43	19.68	15.30	15.51	22.14
	40	18.81	20.45	16.26	16.23	22.71	17.51	19.79	16.01	15.92	22.44
Interaction MxN											
Seed	0	11.35	13.10	13.30	14.21	17.93	11.75	13.07	13.60	14.91	18.31
	20	11.88	14.92	14.00	14.66	19.28	12.84	15.46	15.66	15.65	18.96
	40	11.41	15.11	14.87	15.08	20.17	12.57	15.32	15.78	15.83	19.65
Tissue Culture	0	18.36	20.62	15.68	15.32	21.65	19.43	21.22	15.24	15.36	22.30
	20	19.15	20.73	15.73	15.83	22.53	20.73	21.43	15.45	15.40	22.08
	40	19.29	20.96	15.87	15.80	22.56	21.30	22.34	15.80	15.86	23.75
Root Cutting	0	20.25	20.89	15.80	15.70	23.29	21.09	21.99	15.54	15.44	23.08
	20	21.27	23.38	16.17	16.04	24.62	22.64	23.45	16.32	16.77	24.65
	40	21.84	23.29	17.30	16.88	24.60	22.56	23.70	17.19	16.99	24.74
Mean		**17.20**	**19.22**	**15.41**	**15.50**	**21.85**	**18.32**	**19.78**	**15.62**	**15.80**	**21.95**

Table (9) Effect of propagation method (M), nitrogen fertilization (N) and their interaction on stevioside yield (kg/fed.) at different cuts (combined data of both seasons).

Treatment		Cutting age (days)					
		90 (1st)	180 (2nd)	240 (3rd)	300 (4th)	360 (5th)	Total
Propagation Method	Seed	33.45	116.00	72.68	72.70	209.55	**504.37**
	Tissue Culture	169.46	188.81	81.28	79.54	249.53	**768.62**
	Root Cutting	203.28	235.56	85.20	86.42	263.40	**873.85**
	L.S.D	**12.4**	**6.81**	**2.41**	**0.65**	**6.97**	**28.34**
Nitrogen (kg/fed)	0	102.24	158.93	74.30	75.78	225.72	**636.96**
	20	126.60	181.05	79.33	80.20	242.17	**709.33**
	40	141.65	193.15	83.90	83.59	255.10	**757.39**
L.S.D		**8.80**	**2.55**	**1.23**	**0.38**	**4.36**	**17.52**
Interaction MxN							
Seed	0	30.03	102.06	64.56	66.98	193.88	**457.51**
	20	34.61	121.52	74.15	74.26	212.23	**516.77**
	40	35.97	127.81	78.16	78.82	221.00	**541.76**
Tissue Culture	0	130.38	182.00	80.39	76.70	239.53	**709.00**
	20	181.45	187.61	81.07	79.64	245.36	**775.13**
	40	196.86	199.18	83.93	82.32	261.65	**823.94**
Root Cutting	0	177.76	201.54	78.35	80.96	243.44	**782.06**
	20	197.60	245.86	84.47	86.95	268.52	**883.40**
	40	235.32	265.49	91.40	91.45	278.77	**962.43**
L.S.D		**15.30**	**4.41**	**2.13**	**1.00**	**7.55**	**31.07**
	Total Mean	**122.51**	**177.00**	**79.13**	**79.82**	**240.85**	**699.29**

تأثير طريقة الإكثار والتسميد النيتروجيني على محصول وجودة نبات الاستيفيا في مصر

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أقيمت تجربتان حقليتان في محطة البحوث الزراعية بالجيزة مركز البحوث الزراعية خلال الموسمين المتتاليين ٢٠٠١/٢٠٠٢، ٢٠٠٢/٢٠٠٣ لدراسة تأثير طريقة الإكثار (البذرة وزراعة الأنسجة والعقل الجذرية) والتسميد النيتروجيني (صفر–٢٠–٤٠ كجم نيتروجين/فدان/حشة) والتفاعل بينهما علي محصول وجودة نبات الاستيفيا. تم استخدام بذور الصنف Spanti المستوردة من أسبانيا والتي تم إكثارها خضرياً في مصر لإنتاج البادرات والعقل الجذرية ونواتج زراعة الأنسجة للقمم المرستيمية. أظهرت النتائج أن النباتات المكثرة بزراعة الأنسجة والعقل الجذرية كانت أطول بشكل معنوي عن النباتات المكثرة بالبذرة، تفوقت النباتات المكثرة بالعقل الجذرية ونواتج زراعة الأنسجة على النباتات المكثرة بالبذرة في إنتاج عدد الأفرع/نبات في الحشة الأولي والثانية والخامسة. أعطت النباتات المكثرة بالعقل الجذرية أعلي القيم بالنسبة للوزن الرطب للأوراق (٢,٧–٣,١٢ ١،٥٥ ١،٥٨ طن/فدان) خلال الحشات الأولي والثانية والثالثة والرابعة علي الترتيب. كما تفوقت النباتات الناتجة من العقل الجذرية في محتواها من الستيفيوزايد لكل مواعيد الحش حيث أعطت الحشة الخامسة أعلي النسب في كلا الموسمين (٢٤,١٦% – ٢١,١٤ % على التوالي). أدت إضافة ٤٠ كجم نيتروجين للفدان في الحشة إلى الحصول على أعلى القيم بالنسبة لطول النبات في ثلاث حشات (٥٤,٠٦ ٥٤،٣ ٥٣،٠٧) سم بالنسبة للحشات الأولي والثانية والخامسة علي الترتيب. تفوقت إضافة إما ٢٠ أو ٤٠ كجم نيتروجين/فدان/حشة على معاملة المقارنة لصفة عدد الأفرع/نبات خلال كل الحشات. أدت إضافة النيتروجين بمعدل ٤٠ كجم/فدان/حشة إلي الحصول على أعلي قيم الوزن الرطب للأوراق في كل مواعيد الحش (٢,٢٧–٢,٨٦– ١,٥٦– ١,٥٦– ٣,٣٧ طن/فدان علي الترتيب). وكذلك بالنسبة لمحصول الأوراق الجافة (٠,٨٧– ٠,٩٦– ٠,٥٢– ٠,٥٢– ١,١٣ طن/فدان علي الترتيب). أعطت النباتات التي تلقت إما ٢٠ أو ٤٠ كجم نيتروجين/للفدان/حشة نسبة أعلي من محتوى الاستيفيوزايد في الأوراق عن تلك التي لم تتلقى أي تسميد نيتروجيني في كلا الموسمين. أدت إضافة ٤٠ كجم نيتروجين/فدان/حشة لنباتات الاستيفيا التي تم إكثارها بالعقل الجذرية إلي تفوق محصول الأوراق الجافة والذي كان مقداره ١,٠٦– ١,١٣– ٠,٥٤ طن/فدان/حشة خلال الحشة الأولي والثانية والرابعة علي الترتيب. وعلي ذلك فقد وصل محصول الاستيفيوزيد إلي أعلي قيمة (٩٦٢,٤٣ كجم/فدان/عام) عندما تم استخدام طريقة الإكثار بالعقل الجذرية مع إضافة ٤٠ كجم نيتروجين للحشة.