



**Study The Effect of Microgreen Algae on *Stevia rebaudiana*  
*In Vitro* and *Ex Vitro***

**By**

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**B.Sc. Faculty of Agriculture - Damanhuor, Alexandria University (2000)  
M.Sc. in Horticulture, Faculty of Agriculture, Tanta University (2011)**

**Thesis**

**Submitted in Partial Fulfillment of the  
Requirements for the degree of**

**Doctor of Philosophy**

**In**

**Genetic Engineering and Biotechnology  
Department of Plant Biotechnology  
(Biotechnology of Horticulture Crops)**

**Genetic Engineering and Biotechnology  
Research Institute (GEBRI)  
University of Sadat City**

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## 5- SUMMARY

This study was carried out in plant tissue culture laboratories, Plant Biotechnology Department, Microbial Biotechnology Department Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City, University. Medicinal and Aromatic plant department, Horticultural Research Institute, Agriculture of Research Center, Ministry of Agriculture and reclamation land.

This work aimed to:

- 1- Efficiency of used algae medium in *Stevia rebaudiana* micropropagation.
- 2- Studying the effect of modified MS medium by the filtrate algae medium on the multiplication of *Stevia rebaudiana*
- 3- Employment the various algae type as biofertilizers in different doses.

The investigation included three parts as the following

### **Part I: Efficiency of used algae medium on growth parameters of *Stevia rebaudiana*;**

Media containing various algae types (*Scenedesmus obliquus*, *Ankistrodesmus falacatus*, *Chlorella kessleri* and *Chlorella vulgaris*) were used as partially alternative media instead of MS medium.( The treatments depended on partially or totally substituted of MS medium[0.0 (MS without substitution), 20, 40, 60, 80 and 100% MS substituted with various algae types.MS substituted with Algae types affected the growth parameters of *Stevia rebaudiana*;i.e., number of shoot, shoot length, number of node and number of leaves after 3<sup>rd</sup> subculture.

The best results were:

MS substituted with 40% *Chlorella vulgaris* significantly increased stevia number of shoots (3.33shoots /jar). The highest shoots length (10.00cm) was recorded when MS substituted with 40% *Ankistrodesmus falacatus* algae.The highest number of nodes was recorded when MS substituted with 60, 80 or 100% *Ankistrodesmus falacatus* algae (8.83, 8.50 and 8.00 node/ explant).Full MS substitution (100% algae) with *Scenedesmus*



*obliquus* medium resulted in the highest number of leaves (12.67 leaves/shoot).

### **Rooting stage**

Full MS substitution with *Ankistrodesmus falacatus* was the most effective in increasing the numbers of roots (10.00 roots/explant ) MS substituted with 60%, *Chlorella vulgaris* or MS substituted with 40% *Ankistrodesmus falacatus* recorded the highest root length (12.00 cm).

### **Acclimatization stage**

The highest survival percentage (66%) was observed with MS substituted with either 60 or 80% *Chlorella vulgaris*. Also, full MS substituted with *Chlorella vulgaris* ,*Scenedesmus obliquus* or *Chlorella kessleri* (100% algae medium). Plantlet length observation proved that MS substitution with 60,80 or 100% *Chlorella kessleri* or *Chlorella vulgaris* possessed the highest plantlet length (10.0. , 10.0 and 9.33 cm, respectively).

### **Chemical analysis**

#### **Total phenolic content (TPC)**

The highest value of total phenol contents (625.40µg/g) was obtained with MS substituted with 60% *Scenedesmus obliquus*.

#### **Antioxidant activity percent**

The highest antioxidant (81.37%) was recorded with full MS substituted with *Scenedesmus obliquus* .

#### **Macro and microelements contents**

Macroelements; N, P, K, Ca and Mg contents(65.00, 4248.00, 1150.00, 89.00, 38,30 ppm respectively) were enhanced from MS substituted with 40% of either *Scenedesmus obliquus* or *Chlorella kessleri* algae, MS substituted with 40, 60, 80 or 100% *Chlorella vulgaris* , MS substituted with 40 or 60% *Chlorella kessleri* , full MS substitution with 100% *Chlorella kessleri* and MS substituted with 60% *Ankistrodesmus falacatus* , respectively. Plant content of microelements; Fe, Zn and Mn (176.30, 43.67 and 15,67 ppb respectively) were affected by treatments. (full MS (control) , MS substituted with 20% *Ankistrodesmus falacatus* .

and MS substituted with 40% *Ankistrodesmus falacatus*). Heavy metals; Cu and Pb, as well as Na contents (10.10, 9.57 and 18.67 ppb, respectively) were affected by MS substituted with 40% *Chlorella kessleri*, MS substituted with 40% *Ankistrodesmus falacatus* and MS substituted with 40% *Ankistrodesmus falacatus*.

### **Reccomendation**

In this study *Chlorella vulgaris* at 40% substituted percent of MS resulted in the maximum number of shoots of stevia plantlet. *Ankistrodesmus falacatus* at 40, 60, 80 and 100% substituted of MS increased shoot length and number of leaves.

### **Part II: Studying the effect of modified MS medium by the filtrate algae medium on the multiplication of *Stevia rebaudiana***

In this investigation, algae media filtrate of various algae types (*Scenedesmus obliquus*, *Ankistrodesmus falacatus*, *Chlorella kessleri* and *Chlorella vulgaris*) were used for partially or totally media substituted of MS medium. [0.0 (full MS) as control, 20, 40, 60, 80 MS substituted with algae filtrate and full MS substituted with algae filtrate (100%)]. MS substituted with Algae types filtrate affected the growth parameters of *Stevia rebaudiana*; number of shoots, shoot lengths, number of nodes and number of leaves after 3<sup>rd</sup> subculture.

The best results were:

The highest value of number of shoot (3.33 shoots/jar) was recorded with MS substituted with 20 or 40% *Chlorella kessleri*. The highest shoot length (8.50 cm) was achieved with MS substituted with 40% *Chlorella kessleri* filtrate. Number of Nodes (8.67 nodes/explant) and number of leaves (17.33 leaves/explant) were possessed the highest values with MS substituted with 20% *Scenedesmus obliquus* filtrate.

### **Rooting stage**

MS substituted with 40% *Scenedesmus obliquus* filtrate significantly increased the number of roots on explants (9.33 root/explant).

The highest roots length (5.50 cm) was obtained with MS substituted with 80% *Chlorella vulgaris* filtrate.

### **Acclimatization stage**

The highest survival value (50%) was obtained with either MS substituted with 20 or 60% of all algae types filtrate. Full MS substituted with *Chlorella vulgaris*, *Scenedesmus obliquus* or *Chlorella kessleri* filtrate at 100% (complete algae medium) gave the highest survival percentage (50%). The highest plantlets length (8.50 cm) was observed with MS substituted with 40% *Chlorella kessleri* filtrate

### **Chemical analysis**

#### **Total phenolic contents (TPC)**

The highest total phenol contents (TPC) (699.70µg/g) was obtained with MS substituted with 40% *Ankistrodesmus falacatus* filtration

#### **Antioxidant activity**

The highest antioxidant (95.33%) was recorded with MS substituted with 20% *Chlorella vulgaris* filtration.

### **Recommendation**

The highest values of shoot length and number of shoots resulted from MS substituted with 20, 40 % from *Chlorella kessleri*. The highest number of leaves were possessed the highest values with MS substituted with 20% *Sendesmus obliquus* filtrate.

### **Part III: Utilization of some microgreen algae as biofertilizer on *Stevia rebaudiana in vivo*:**

Four types of algae (*Scenedesmus obliquus*, *Ankistrodesmus falacatus*, *Chlorella kessleri* and *Chlorella vulgaris*) were cultivated and utilized as biofertilizer for *S. rebaudiana*. Homogenous algae media including one of the algae types was used as a source of biofertilizer in different doses {T1 (without fertilization), T2 (250 ml algae), T3 (150 ml algae) as well as and T4 (Recommended dose of NPK as mineral fertilization)}. Each dose was reached to 250 ml with water and was added weakly to each pot for two months. Biofertilizer doses affected the growth parameters of *Stevia rebaudiana*; branches number, plant length, number of nodes, number of leaves, fresh and dry weight after two monthes.

The best results were:

**Utilization of some microgreen algae as biofertilizer on growth parameters of *Stevia rebaudiana*;**

*Scenedesmus obliquus* at high dose significantly increased plant length and number of leaves of stevia (46.67 cm and 46.67 leaves/plant, respectively). *Ankistrodesmus falacatus* at high dose and *Chlorella kessleri* at (T3) gave the highest number of branches (4.33 branch/plant for each). *Ankistrodesmus falacatus* at high dose gave the highest number of nodes (17.33 node/plant). The higher fresh weight was recorded when biofertilizer were *Ankistrodesmus falacatus* or *Scenedesmus obliquus* at high dose (10.33 and 9.67 g for each), with no significant difference between them. The higher dry weight values (2.40 and 2.00g) were observed with either *Scenedesmus obliquus* or *Ankistrodesmus falacatus*, respectively at high dose.

**Macro and Micro elements content**

*Scenedesmus obliquus* at high dose gave the highest nitrogen content (75.00 ppm), which was also higher than any biofertilizer doses treatment. *Chlorella kessleri* at high dose (T2) gave the higher phosphorus, Pb and Cu content (1599 ppm, 15.67 and 23.67ppb) compare to other treatments. *Chlorella vulgares* algae high dose (T2) maximized the K, Na, Mn, Zn, Fe, Ca and Mg content (2532 ppm, 28.33, 25.33, 21.33, 550.30 ppb, 329.30 and 248.30 ppm respectively,)

**Reccomendation**

Finally it could be recommended that *Sendesmus obliquus* at high dose produced the highest number of leaves. When *Ankistrodesmus falacatus* and *Sendesmus obliquus* recorded the highest value of fresh and dry weight