

EVALUATION OF GROWTH, ACTIVE INGREDIENTS AND GENETIC DISTANCES OF SOME ECOTYPES OF STEVIA (*Stevia rebaudiana* Bert.) PLANT

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ABSTRACT:

*In order to evaluate growth, active ingredients and genetic distances of some ecotypes of stevia (*Stevia rebaudiana* Bert.) plant, the samples were collected from Sharkia, Cairo, Damietta and Kafrelshakh Governorates during the two consecutive summer seasons of 2022 and 2023.*

*The collected samples of stevia plants from different regions under study pointed out that the highest values of plant height, leaf number per plant, as well as fresh and dry herb weights per plant were obtained from Damietta Governorate compared to the other ecotypes. Whereas, the highest values of root length were noticed under Sharkia Governorate region with significant difference with the other regions under study. Also, Sharkia and Cairo Governorate regions recorded the highest values of total carbohydrates percentage and total chlorophyll content, respectively. Two compounds of glycosides of *Stevia rebaudiana* plant were identified by HPLC. The main components were the stevioside which followed by rebaudioside. More increases in stevioside and rebaudioside contents were resulted under Sharkia and Kafrelshakh regions.*

The dendrogram shows that two groups of the populations separated very early from each other in past to form discrete populations. The ecotypes of Sharkia and Cairo regions were observed in a unique cluster, providing some common phylogenetic relationship among them.

***Conclusively,** through this study, it is preferable to conduct several studies on environmentally different medicinal and aromatic plants depending on the growth and production areas to determine the best agricultural regions for them in terms of growth, yield, and active substances.*

Keywords: *Stevia rebaudiana, Ecotypes, growth, genetic distances, stevioside, rebaudioside.*

INTRODUCTION:

A member of the Asteraceae family, stevia (*Stevia rebaudiana*, Bertoni) is a herbaceous perennial plant native to the northeastern Paraguay region. It grows naturally on sandy soils near streams on the margins of marshlands, acid infertile sand, or muck soils (Madan *et al.*, 2010). With a high concentration of steviol glycosides (SG), which may serve as a replacement for artificial sweeteners (Reis *et al.*, 2015) and are significantly sweeter than sugarcane and sugar beet but notably calorie-free (Cardello *et al.*, 1999), dry leaves are the valuable portion of the stevia plant (Ramesh *et al.*, 2006). The leaf powders or residue left over after the sweetening ingredients were extracted were shown to be suitable for use as food additives or dietary supplements. Giving rise to an intriguing plan for the creation of novel food items (Barba *et al.*, 2014).

Many plant species will not be able to adapt to the rapid changes in climate brought about by human activity (Bellard *et al.*, 2012). As a result, the ecosystems of medicinal plants in arid and semi-arid regions, which have developed into what they are today through time, are crucial to preserving the equilibrium of ecosystems, and the use of these resources should be predicated on a comprehensive examination of the features of their habitats (Zank *et al.*, 2015). In order to assess the ecotypes listed in gene banks and learn about their salient features, including cultural attributes, morphological features are required (Valois *et al.*, 2001). The utilizing of an HPLC column provided an excellent indicator of the active ingredient and had strong selectivity for all steviol glycosides as well as good repeatability (Woelwer-Rieck *et al.*, 2010).

Diverse plant cultivars may have distinct adaptive reactions to identical environmental circumstances. In fact, depending on the species or ecotype, the total photosynthetic capacity under stress may vary and may even be influenced by the degree of environmental stress. Annuals subjected to both high and low temperatures showed altered morphological and anatomical traits, increased carbohydrates, and up regulated photosynthesis (Muller *et al.*, 2014 and Stewart *et al.*, 2016). In addition, Izanloo *et al.* (2019) reported that under drought stress conditions ecotypes such as Mashhad, Nishabur, Khosf and Bojnoord had the highest performance and belong in one class, while Ilam and Roman genotypes were in middle classes and Gonabad ecotype stays on in lowest yield.

Little research has been done on the suitable habitat distribution, ecotype delimitation and quality assessment of *Stevia rebaudiana* Bertoni.

The purpose of this research was to evaluate the morphological, genetic distance and some active ingredients of different ecotypes of stevia and selection criteria for obtaining good type.

MATERIALS AND MEDTHODS:

In order to study morphological traits, genetic distance and some chemical constituents of different ecological areas of *Stevia rebaudiana* in the different Governorates of Egypt, four different natural habitats, including Sharkia, Cairo, Damietta and Kafrelshakh were selected and the desired sampling during the summer season of 2022 and 2023. Each sample contained 9 plants form each region. The mechanical and chemical properties of different regions under study are shown in Table 1 according to **Chapman and Pratt (1978)**. The collected plants utilized to determine the following data:

Morphological traits:

Growth parameters expressed as plant height (cm), Number of leaves per plant, fresh and dry weights of herb per plant (g) and root length (cm) were listed.

Chemical and active ingredients:

Total chlorophyll (a+ b) content (mg/ 100g as fresh weight) in fifth upper leaf were determined according to (**Mazumder and Majumder, 2003**). Also, total carbohydrate (%) in stevia dry herb was determined according to the method presented by **Dubois et al. (1956)**. Determination of stevioside and rebaudioside: Two compounds of glycosides (stevioside and rebaudioside) of *Stevia rebaudiana* plant were identified by HPLC as described by **Aranda-González et al. (2015)**.

Genetic distances:

It was explained by utilizing Dendrogram based on UPGMA using Gower general similarly coefficient between different regions under study during both seasons. This indicator was utilized for some morphological traits and total chlorophyll and total carbohydrates.

Statistical analysis

The obtained data was statistically analyzed and the means were compared using least significant difference (LSD) at 5% level; computer program Statistix Version 9 was used (**Analytical Software, 2008**).

Table 1. Physical and chemical properties of the experimental regions

Properties	Different regions			
	Sharkia	Kafrelshakh	Cairo	Damietta
<i>Physical properties</i>				
Sand %	51.61	53.65	53.22	54.34
Silt %	31.75	28.93	31.54	28.45
Clay %	16.64	17.42	15.24	17.21
Soil texture	Clay	Clay	Clay	Clay
<i>Chemical properties</i>				
pH	8.21	7.57	8.22	7.33
E.C. (dS/m)	4.13	5.23	8.13	9.05
O.M.	1.11	1.35	1.12	1.11
Available N (mg/kg soil)	29.48	30.01	29.54	27.82
Available P (mg/kg soil)	9.19	9.59	9.23	8.78
Available K (mg/kg soil)	102.05	112.17	100.01	111.03
Available Zn (mg/kg soil)	0.24	0.27	0.24	0.21
<i>Cations (meq/l)</i>				
Ca ⁺⁺	14.02	15.14	13.78	12.83
Mg ⁺⁺	11.19	10.87	10.75	11.16
Na ⁺	44.31	46.76	30.45	30.29
K ⁺	54.28	56.82	48.71	49.35
<i>Anions (meq/l)</i>				
CO ₃ ⁻	0.03	0.02	0.05	0.04
HCO ₃ ⁻	3.19	2.84	2.46	2.34
Cl ⁻	65.61	57.14	51.02	50.97
SO ₄ ⁻	51.41	48.73	38.74	38.57

RESULTS AND DISCUSSION:

Morphological traits:

Data presented in Table 2 reveal that plant height, number of leaves per plant and total fresh and dry weights of herb per stevia plant recorded significant increase under Damietta Governorate region compared to the other regions under study during both seasons. However, the highest root length values of stevia plant were obtained from Sharkia Governorate compared with the other regions under study during both seasons. In the

Table 2. The plant height, number of leaves per plant, fresh and dry weights of herb and root length variation of *Stevia rebaudiana* under different study locations during 2022 and 2023 seasons

Location	Plant height (cm)		Number of leaves/ plant		Herb fresh weight (g)		Herb dry weight (g)		Root length (cm)	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
Sharkia	49.33b	46.00b	135.0b	128.0b	10.30c	10.45c	4.23c	3.61c	23.00a	22.50a
Cairo	22.33c	22.00d	13.0c	13.7c	5.31d	5.00c	2.05d	1.97c	7.00c	6.67d
Damietta	112.3a	103.67a	648.0a	661.3a	59.00a	68.33a	19.33a	20.67a	18.33b	18.67b
Kafrelshakh	45.00b	42.00c	150.3b	144.7b	14.47b	16.13	5.98b	7.41b	17.67b	16.00c

a, b, c* Means having the same letter (s) within the same column are not significantly different according to LSD all-pairwise comparisons test at 5% level of probability.

same line, **Salehi *et al.* (2017)** found that showed that there was a considerable variation among various *Salvia multicaulis* ecotypes in respect of morphological parameters (plant height, inflorescence length and plant dry weight). In addition, **Rashvand *et al.* (2021)** observed that the ecotype of *Lallemantia* spp. "Azadshahr," with an estimated yield of 375 kg ha⁻¹, possessed the maximum plant height, total plant dry weight, and seed output. Also, **Shirzadi *et al.* (2023)** indicated that the ecotypes of Homag, Sekhuran and Bekhun of *Amygdalus scoparia* had the highest values of the serious breeding traits including (branch length, the secondary branches number, the sub-branch dry weight and the sub-branch dry weight/plant percentage).

Chemical and active ingredients:

In the two seasons, the total chlorophyll (a + b) content of *Stevia rebaudiana* plants growing in Cairo Governorate was much higher than that of the other study regions (Table 3). Additionally, there is no significant change in the percentage of total carbs between the Sharkia and Damietta ecotype during either season. Also, data represented effect of different regions under study (Sharkia, Cairo, Damietta and Kafrelshakh Governorates) on leaf tissue contents of stevioside and rebaudioside were recorded in Figure (1). It is clear that stevia plants grown under Sharkia and Kafrelshakh regions resulted in increases in stevioside and rebaudioside contents in leaves comparing to the other ecotypes under study. According to **Shaltouki *et al.* (2021)**, the ecotypes of Iranian *Ziziphora clinopodioides* (Evard and Sorkhgarive) possessed the greatest values of the crucial breeding characteristics, such as the largest essential oil yield, the highest ratio of leaf to vegetative body weight, and the highest total phenol content.

Table 3. The total chlorophyll content (a +b) and total carbohydrates (%) variation of *Stevia rebaudiana* under different study locations during 2022 and 2023 seasons

Location	Chlorophyll a +b content (mg/100 g F.W.)		Total carbohydrates %	
	1 st season	2 nd season	1 st season	2 nd season
Sharkia	3.211 b	3.236 b	18.230a	18.310a
Cairo	3.351 a	3.357 a	17.773bc	17.373b
Damietta	3.073 c	3.079 c	17.543c	17.477b
Kafrelshakh	3.001 d	3.001 d	18.037ab	17.957a

a,b, c Means having the same letter (s) within the same column are not significantly different according to LSD all-pairwise comparisons test at 5% level of probability.

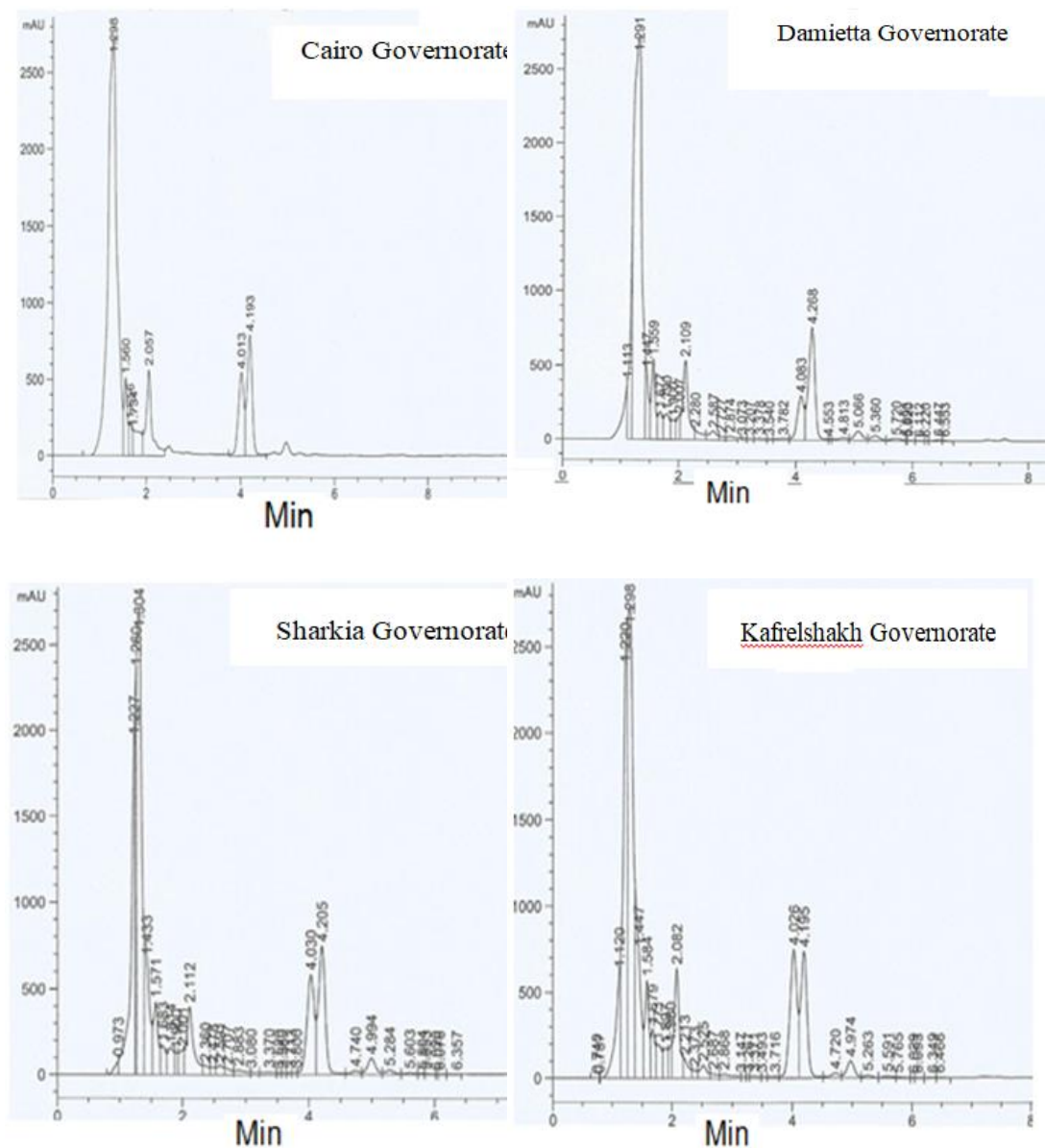


Figure. 1. Auto scaled chromatogram showing the peak concentrations of Stevioside and Rebaudioside production in stevia plant filtrated with different ecotypes under study growing during second season (2023) by HPLC technique.

Genetic distances:

The MVSP dendrogram based on genetic distances among some growth and chemical constituent cleared very clear pictures of different ecotypes evolutionary pattern in relationship and variability shown in Figs. 2, 3, 4 and 5. The dendrogram of plant height shows that three groups of the populations separated very early from each other in Damietta as alone cluster and Sharkia and Kafrelshakh regions together (Fig. 2). The ecotype of Damietta was observed in alone cluster again under herb dry weight, whenever, Sharkia and Cairo regions providing common phylogenetic relationship among them in this regard (Fig. 3). The dendrogram of total chlorophyll content shows that two groups of the populations separated very early from each other in Damietta and Kafrelshakh as well as Sharkia and Cairo regions (Fig. 4). Ecotypes of stevia grown in Damietta and Cairo appeared in the same cluster when total carbohydrates percentage was determined (Fig. 5). Similarly Sharkia and Kafrelshakh also grouped them in a separate group. Moreover, **Rashvand *et al.* (2021)** found that the ecotypes were divided into three separate clusters based on the cluster analysis and biplot diagram. The cluster1 ecotypes belonging to *Lallemantia iberica* had higher seed yield than the other two ones.

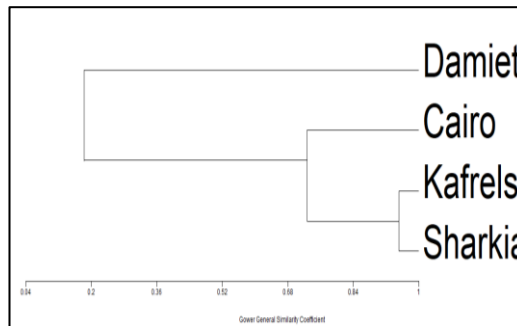


Figure. 2. Dendrogram for plant height of 4 *Stevia* Ecotypes

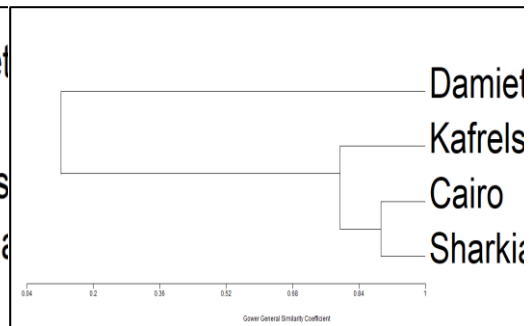


Figure. 3. Dendrogram for herb dry weight of 4 *Stevia* Ecotypes

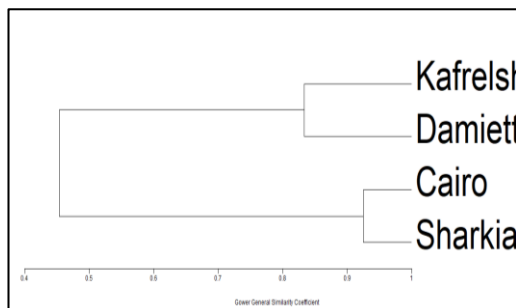


Figure. 4. Dendrogram for total chlorophyll content of 4 *Stevia* Ecotypes

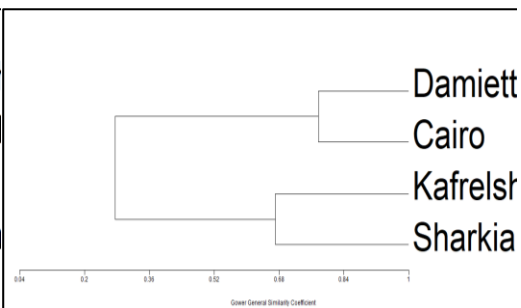


Figure. 5. Dendrogram for total carbohydrates percentage of 4 *Stevia* Ecotypes

Conclusively, From the above-mentioned results, it is clear that the best vegetative growth of stevia plants was obtained in Damietta Governorate, and that the highest percentages of stevioside and rebaudioside components were produced in Sharkia and Kafrelshakh Governorates. Ecotypes of stevia grown in Damietta and Cairo appeared in the same cluster when total carbohydrates percentage was determined.

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تقييم النمو والمواد الفعالة والتباين الوراثي لبعض الأنماط البيئية لنبات الإستيفيا

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اجريت التجربة لتقييم النمو والمواد الفعالة والتباين الوراثي لبعض الأنماط البيئية لنبات الإستيفيا، حيث تم جمع العينات من محافظات الشرقية والقاهرة ودمياط وكفر الشيخ خلال موسمي الصيف المتتاليين لأعوام ٢٠٢٢ و٢٠٢٣. أشارت عينات نباتات الإستيفيا من المناطق المختلفة محل الدراسة إلى أن أعلى قيم ارتفاع النبات وعدد الأوراق لكل نبات وكذلك أوزان الطازجة والجافة للعشب لكل نبات تم الحصول عليها من محافظة دمياط مقارنة بالأنماط البيئية الأخرى. بينما لوحظت أعلى قيم لطول

الجزور في منطقة محافظة الشرقية مع اختلاف معنوي عن المناطق الأخرى قيد الدراسة. كما سجلت منطقتا الشرقية والقاهرة أعلى قيم للنسبة المئوية من الكربوهيدرات الكلية والمحتوى من الكلوروفيل الكلي، على الترتيب. تم التعرف على مركبين من جليكوسيدات نبات الإستيفيا بواسطة التحليل الكروماتوجرافي. حيث كانت المكونات الرئيسية هي الإستيفيوسيد الذي يليه الريباوديوسيد. وقد نتجت زيادة أكبر في محتوى ستيفيوسيد وريباوديوزيد في منطقتي الشرقية وكفر الشيخ. يوضح مخطط الدندوجرام أن مجموعتين من المجموعات المتقاربة انفصلت في وقت مبكر جدًا عن بعضها البعض في الماضي لتشكل مجموعات منفصلة تحت معظم الصفات المدروسة. وقد لوحظت الأنماط البيئية في منطقتي الشرقية والقاهرة في مجموعة قريبة من بعضها، مما يوفر بعض العلاقات التطورية المشتركة فيما بينها.

التوصية: عموماً من خلال تلك الدراسة، يفضل إجراء العديد من الدراسات على النباتات الطبية والعطرية المختلفة بيئياً تبع مناطق النمو والانتاج لتحديد أفضل الأماكن الزراعية لها من حيث النمو والمحصول والمواد الفعالة.

الكلمات المفتاحية: الاستيفيا ، الأنماط البيئية ، النمو ، التباعد الوراثي ، الاستيفيوسيد ، الريبوسيد