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Evaluation of *Stevia rebaudiana* collection using chemical variables and SDS-protein electrophoresis under different ecotypes in Egypt

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Abstract

One of the most significant naturally occurring sweetening plants with few calories is stevia (*Stevia rebaudiana*, Bertoni). The current study was done to evaluate chemical differentiation and SDS-protein of stevia plants grown at various ecotypes (Sharkia, Cairo, Damietta and Kafrelshakh Governorates) in Egypt. The samples of stevia plants were collected during summer seasons of 2022 and 2023. The obtained results refer to that the highest pigments values (chlorophyll a, b, a+ b and carotenoids contents as fresh weight) were noticed in Cairo governorate region with significant difference with the other locations under study. The lowest values in leaf pigments recorded in Kafrelshakh Governorate region. Moreover, the highest values in nitrogen, phosphorus and protein percentages were recorded under Sharkia and Damietta Governorate regions compared to the other two ones under study. Also, Sharkia Governorate region recorded the highest values in potassium and total carbohydrates percentages. SDS-PAGE scored nearly the same polymorphism 36.99 and 37.5%, respectively.

Keywords: *Stevia rebaudiana*, Ecotypes, Chlorophyll, Carbohydrates, SDS-protein.

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1. Introduction

A member of the *Asteraceae* family, stevia (*Stevia rebaudiana*, Bertoni) is a significant plant that can be used as a natural low-calorie sweetener. As all-natural, low-calorie sweeteners, the glycosides that are derived from stevia leaves are neither poisonous nor mutagenic [1]. Antibacterial, antifungal (including anti-yeast), anti-inflammatory, antimicrobial, antiviral, cardiotonic, diuretic, and hypoglycemic effects have all been reported for stevia leaf extract. For people with diabetes, it is a blessing [2]. According to [3, 4], stevia production offers a great deal of potential for intensive agriculture and fits well for high return. In human nutrition and food technology applications, dried leaves of *Stevia* are commonly used with the sweet taste that are about 300 times sweeter than regular sucrose, but with the reduced caloric value of 2.7 kcal/g [5]. Increased sucrose consumption has led to deleterious health problems; therefore, the consumption of low-calorie sweeteners has been increasingly encouraged [6]. *Stevia* is used as a substitute for sucrose, for glycemic control in diabetes mellitus, obesity, hypertension, renal protective effects, and promotion of oral health [7].

Many plant species will not be able to adapt to the rapid changes in climate brought about by human activity. In fact, a significant amount of the world's biodiversity is expected to disappear by the end of this century [8]. The natural habitats of wild medicinal plant species have seen changes and tensions in recent years due to climate change and the effects of other variables as industrial development and human interference [9]. The ecosystems of wild medicinal plants in arid and semi-arid regions, which have developed into what they are today over many years, 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 [10].

The genetic resources of numerous aromatic and therapeutic plant species are abundant in Egypt. Protecting genetic resources begins with identifying wild and native variations in each place. In order to comprehend the ecotypes diversity of *Stevia rebaudiana* naturally growing in several Governorates of Egypt, the current study used leaf pigments, chemical contents and SDS-protein.

2. Materials and Methods

The current study was done to evaluate the chemical differentiation and SDS-protein of stevia plants grown at various ecotypes (Sharkia, Cairo, Damietta and Kafrelshakh Governorates) in Egypt. The samples of stevia plants were collected during the summer season of 2022 and 2023 years. Each sample contained 20 plants form each region to determine morphological traits, chemical constituents as well as SDS- protein.

2.1: Chemical constituents

2.1.1 Pigments content

Chlorophyll a, chlorophyll b, total chlorophyll (a+ b) and carotenoids contents (mg/ 100g as fresh weight) in fifth upper leaf were determined in stevia leaves according to [11].

2.1.2 Total nitrogen percentage

Total nitrogen percentage in dry herb of stevia was determined according to method reported by [12].

2.1.3 Total phosphorus percentage

Total phosphorus percentage in dry herb of stevia was determined according to the method adapted by [13].

2.1.4 Potassium percentage

Was determined in in dry herb of stevia by using flame photometer according to the method gave by [14].

2.1.5 Total protein percentage

Total protein percentage in dry herb of stevia was calculated by multiplying N % by the factor 6.25 to calculate the total protein percentage according to [15].

2.1.5 Total carbohydrates percentage

Total carbohydrate percentage in stevia dry herb was determined according to the method presented by [16].

2.2: SDS-protein electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of [17], as modified by [18]. Youngest–leaves samples were taken from Stevia plants located under Sharkia, Cairo, Damietta and Kafrelshakh Governorates.

2.3: 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 [19].

3. Results and Discussion

3.1: Chemical constituents

Results presented in Tables 1 and 2 shows that Stevia rebaudiana plants grown in Cairo Governorate recorded a significant increase in chlorophyll a, chlorophyll b and total chlorophyll a + b contents compared to the other locations under study in both seasons. All locations under study gave a significant increase in leaf pigments content compared to Kafrelshakh Governorate except that of chlorophyll b in the second season only. Whereas, the highest values of total nitrogen and total phosphorus percentages achieved under Sharkia and Damietta Governorates compared to the other two locations under study. Potassium and total protein percentages recorded significant increase under Sharkia Governorate location compared to the other locations under study during both seasons, in most cases. Furthermore, there are no significant difference between Sharkia and Damietta locations regard total carbohydrates percentage in both seasons.

Correction is made possible by gathering data on the genetic distance between individuals or ecotypes, understanding the relationships between the species in the breeding program, and having the ability to set up hereditary reserves, effectively sample genotypes, and make better use of program diversity [20]. The assessment of morphophysiological and phytochemical properties revealed that the phytochemical characteristics of the several ecotypes of *Salvia multicaulis* varied significantly [21]. Moreover, [22] showed that different ecotypes of *Amygdalus scoparia* plants had a wide variation regard all studied attributes.

3.2: SDS-protein pattern

The protein expression related to various ecotypes (Sharkia, Cairo, Damietta and Kafrelshakh Governorates) via number and density of disappeared and appeared protein bands by SDS-PAGE analysis (Table 3 and Fig.1). It was revealed 16 protein bands with different molecular weights ranged from 180 to 10 KDa included 4 specific band (polymorphic), 8 common band detected in all plants (monomorphic) and 4 genetic marker (unique) among stevia plants grown at different ecotypes. The total induced protein bands 45 among Sharkia Governorate revealed the highest induced 15 protein bands and 3 unique bands 65, 45 and 25 MW (genetic marker), Cairo Governorate revealed 11 protein bands and unique band 39 MW (genetic marker), Kafrelshakh Governorate revealed 9 induced protein bands and Damietta Governorate revealed 10 induced proteins bands. Furthermore, Electrophoretic protein banding pattern can be used to elucidate reliable biochemical genetic markers. It can also provide information about structural genes and their regulatory systems which control the biosynthetic pathways of that protein banding pattern [23]. In addition, [24] demonstrate the ecotype division and chemical diversity of *Cynomorium songaricum* in China from different geographical regions and provide a reference for the development of germplasm and directed plant breeding of endangered medicinal plants.

Table 1: The pigments variation of Stevia rebaudiana under different study locations during 2021 and 2022 seasons

Location	Chlorophyll a content (mg/100 g F.W.)		Chlorophyll b content (mg/100 g F.W.)		Chlorophyll a +b content (mg/100 g F.W.)		Carotenoids content (mg/100 g F.W.)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Sharkia	1.495 b	1.500 b	1.716 b	1.736 b	3.211 b	3.236 b	2.359 b	2.353 b
Cairo	1.515 a	1.518 a	1.836 a	1.839 a	3.351 a	3.357 a	2.479 a	2.484 a
Damietta	1.359 c	1.369 c	1.713 b	1.709 c	3.073 c	3.079 c	2.315 c	2.323 c
Kafrelshakh	1.303 d	1.302 d	1.704 c	1.706 c	3.001 d	3.001 d	2.286 d	2.297 d

Table 2: The N, P, K, protein and carbohydrates percentages variation of Stevia rebaudiana under different study locations during 2021 and 2022 seasons

Location	Total nitrogen %		Total phosphorus %		Potassium %		Total protein %		Total carbohydrates %	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Sharkia	1.447a	1.590a	0.820a	0.820a	2.200a	2.267a	9.041a	9.938a	18.230a	18.310a
Cairo	1.170c	1.250c	0.529b	0.529b	1.863d	1.930c	7.235c	7.813c	17.773bc	17.373b
Damietta	1.407a	1.390b	0.753a	0.753a	2.157b	2.197a	8.768a	8.688b	17.543c	17.477b
Kafrelshakh	1.260b	1.247c	0.741a	0.741a	2.060c	2.097b	7.874b	7.792c	18.037ab	17.957a

Table 3: Protein pattern analysis of Stevia rebaudiana plants treated with different locations under study using SDS-PAGE.

No.of bands	Bands (NW)	Primers				
		Damietta	**Sharkia**	**Cairo**	**Kafrelshakh**	**Polymorphism**
1	**180**	+	+	--	--	Polymorphic
2	**165**	+	+	+	+	Monomorphic
3	**130**	++	++	--	--	Polymorphic
4	**85**	++	++	++	+	Monomorphic
5	**75**	+	++	++	++	Monomorphic
6	**65**	--	+	--	--	Unique
7	**55**	+++	++++	++++	+++	Monomorphic
8	**48**	--	++	++	+	Polymorphic
9	**45**		++			Unique
10	**39**	--	--	++	--	Unique
11	**35**	+	++	++	+	Monomorphic
12	**30**	+	++	++	+	Monomorphic
13	**28**	+	++	++	+	Monomorphic
14	**25**		++	--	--	Unique
15	**20**		++	++	-	Polymorphic
16	**18**	+++	+++	+++	+++	Monomorphic
Total		**10**	**15**	**11**	**9**	**45**

TAB: total amplification band, PAB: polymorphic or specific band, MAB: monomorphic or common band, Unique: genetic marker, (**–**) Absent band, (+) Weak band, (++) Moderate band, (+++) Strong band, (++++) Very strong band.

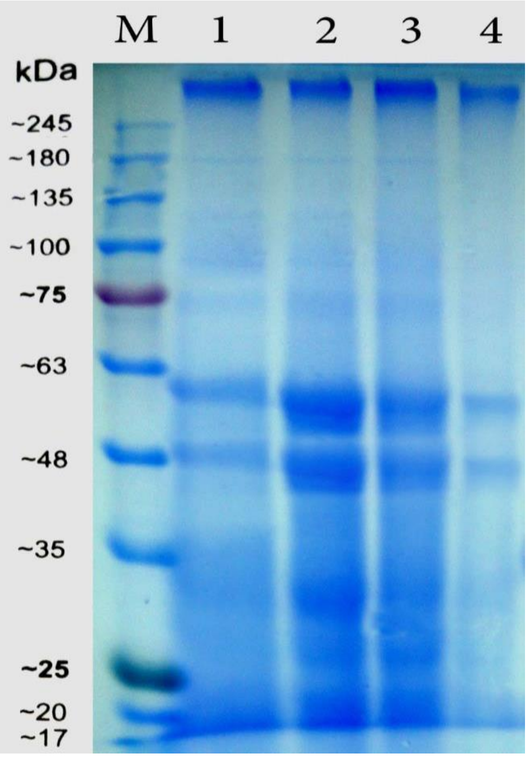

Fig. 1. Acrylamide gel 13 % electrophoreses showing protein pattern of *Stevia* plants grown under (2)- Sharkia, (3) - Cairo and (4)- Kafrelshakh and (1)-Damietta Governorates.

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