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PLANT NUTRITION | ORIGINAL ARTICLE

Assessment of Morpho-physiological and Biochemical Profiles of *Stevia rebaudiana* Bertoni in Different Climatic Regions of Iran

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ABSTRACT

Cultivating stevia is imperative because it is a natural sweetener, providing a healthier and low-calorie substitute for sugar. A comprehensive study was undertaken in 2021-2022, employing a randomized complete block design with three replicates, to evaluate the growth, yield, physiological, and biochemical characteristics of stevia across diverse Iranian climates (Arak, Rasht, Gorgan, Hamedan, Isfahan, Kermanshah, Shiraz, Tabriz, Tehran, and Urmia). The findings unveiled notable variations in growth, yield, physiological, biochemical traits, and steviol glycoside content among different cultivation sites. Notably, Shiraz, Isfahan, Tehran, and Arak exhibited the highest leaf yield, recording values of 119.89, 91.25, 97.07, and 86.24 g m^{-2}, respectively. Conversely, Rasht and Gorgan showcased the lowest growth and yield parameters. Of particular interest, Shiraz samples displayed the highest photosynthetic pigment content (chlorophyll) and the lowest proline levels. Rasht samples demonstrated the highest average content of rebaudioside-A, stevioside, and total steviol glycosides (3.01%, 8.66%, and 11.67%, respectively). Furthermore, Shiraz samples yielded the most steviol glycosides at 122.6 mg m^{-2}. Correlation analysis between climatic data and yield, along with biochemical traits, indicated positive associations of average temperature, rainfall, and relative humidity with leaf yield and steviol glycoside content. In contrast, altitude exhibited a negative correlation with steviol glycoside content. In conclusion, Shiraz is recommended for stevia cultivation for optimal growth and leaf yield, while Rasht and Gorgan are preferable for steviol glycoside production.

1. Introduction

Stevia rebaudiana (Bert.), an herbaceous plant native to Paraguay and a member of the Asteraceae family is celebrated for its innate capacity to accumulate a non-caloric and safe sweetener (Shahverdi et al., 2020; Ahmad et al., 2023). Stevia has a storied history of being cultivated by indigenous communities who greatly appreciated its extraordinary sweetness. Its diterpenic glycosides, roughly 300 times sweeter than sucrose, establish it as a potent natural sweetener with low-caloric content and an extensive range of medicinal and therapeutic benefits (Aghighi Shahverdi et al., 2018). This distinctive plant offers diverse health advantages, encompassing anti-inflammatory and anti-hypertensive properties, the ability to hinder tumor cell proliferation, aphrodisiac effects for diabetic individuals, and characteristics that reduce lipid levels and blood sugar. Furthermore, it showcases antioxidant, antimicrobial, and antifungal properties (Ali et al., 2022; Mlambo et al., 2022). Steviol glycosides (SVglys) are recognized for their primary accumulation within stevia leaves, with their levels being influenced by the plant's genotype and cultivation conditions (Rodriguez-Paez et al., 2023). Therefore, accurate measurement of these glycosides holds significant importance. More than 60 SVglys have been identified in stevia. Yet, only a handful, including stevioside (Stev), rebaudioside-A (Reb-A), B, C, D, E, F, dulcoside-A, and steviolbioside, exhibit notable amounts and economic relevance (Shahverdi et al., 2018). Stevioside and Reb-A are the dominant compounds widely utilized in various industries (Wang et al., 2023). Globally, there are over 144 registered stevia genotypes, with the Morita II genotype being the most widely cultivated (Rodriguez-Paez et al., 2023). Stevia is a versatile perennial plant that originally comes from Paraguay but has shown adaptability to a wide range of environments. It can thrive in temperatures ranging from -6 to 43 °C and requires about 12-13 h of daylight. While it naturally grows in full sun conditions, it has been

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successfully cultivated in numerous countries worldwide. In tropical climates, it tends to perform well in highlands between 700 to 1500 m above sea level (altitude), where temperatures typically fall between 20 and 30 °C (Shahverdi et al., 2018; Dyduch-Siemińska et al., 2020; Rodriguez-Paez et al., 2023; Wang et al., 2023).

In recent years, stevia, a plant with sweetening, medicine, pharmaceuticals, and animal feed applications, has gained significant attention and witnessed a surge in global market demand. Its cultivation has increased annually to keep pace with the growing market requirements (Mlambo et al., 2022). Enhancing leaf yield and sweetener production is a prominent goal in stevia farming, where profitability is influenced by genetic factors, farming practices, and environmental conditions (Khiraoui et al., 2021).

In a research study, stevia plants' growth, yield, and biochemical characteristics were compared in two different environmental conditions in Iran, specifically Firuzabad and Anzali. Firuzabad displayed superior average growth parameters, biomass yield, and SVglys yield, while the Anzali region had a higher SVglys content. This suggests that SVglys levels vary notably depending on environmental conditions, with higher levels in regions resembling the plant's native habitat (Aghighi Shahverdi et al., 2018). Another study investigated the influence of Moroccan regions and varieties on stevia's dry leaf yield and SVglys content. The results showed variations influenced by both factors, highlighting the potential for successful commercial cultivation of stevia as a source of natural sweeteners in Moroccan conditions. However, there were significant differences in yields and SVglys contents among different regions and varieties (Khiraoui et al., 2021). Recently, the cultivation of this medicinal plant has seen an upward trend both worldwide and in Iran, with it being grown in most regions. Considering this, the present study aims to evaluate the growth, yield, physiological, and biochemical traits of the stevia plant in diverse climatic conditions across Iran.

2. Materials and Methods

2.1. Plant materials and experimental design

To evaluate the variations in growth, yield, physiological, and biochemical characteristics of the medicinal plant stevia in different climates within Iran, an experiment was conducted using a randomized complete block design with three repetitions during 2021-2022. In this study, ten farms located in cities such as Arak, Rasht, Gorgan, Hamedan, Isfahan, Kermanshah, Shiraz, Tabriz, Tehran, and Urmia were chosen, and random sampling was conducted from each of these farms. All the climatic and geographic data for the tested areas have been presented in Table 1. Furthermore, all selected farms were two years old and employed drip irrigation for watering. The soil of all selected farms was analyzed for nitrogen, phosphorus, and potassium elements throughout the growth period under favorable nutritional conditions.

Stevia seedlings (*Stevia rebaudiana* Bertoni) were raised in sand beds from seeds, and three-month-old seedlings were subsequently transferred to the fields. The manual transplantation process was carried out with a spacing of 45 cm × 45 cm to attain an approximate planting density of 50,000 plants per ha, aligning with the typical practice observed in Iranian stevia fields (Aghighi Shahverdi et al., 2018). The harvest from all fields was carried out about 60 to 70 days (depending on the specific location) after the start of regrowth in the second year.

2.2. Assessment of morphological and yield attributes

To assess various growth parameters such as plant height, number of branches per plant, leaf yield, biological (total biomass) yield, and leaf mass ratio (LMR), a random selection of three plants was made from each experimental plot, and these traits were subsequently measured. LMR, which represents the ratio of the entire plant's biomass to the leaf yield, expressed as a percentage, was also calculated. This reflects the harvest index (Aghighi Shahverdi et al., 2018). The leaves constituted the above-ground biomass of each plant, and the stems were manually separated and subjected to drying in an oven at 45 ± 3 °C for 48 h, following the method described by Barbet-Massin et al. (2015).

2.3. Assessment of photosynthetic pigments

Photosynthetic pigments were assessed previously described by Lichtenthaler and Wellburn (1983). In this procedure, 0.25 g of fresh tissue was subjected to extraction using 5 mL of 80% acetone. Following extraction, the mixture underwent centrifugation at 11,000 rpm for 10 min. The optical density (O.D.) of the resulting extract was then determined at wavelengths of 646.8 and 663.2 nm, allowing us to estimate the concentrations of chlorophyll-a (Chl-a) and chlorophyll-b (Chl-b) using a spectrophotometer (Perkin Elmer Lambda 25, USA). The quantity of pigment present in each sample was calculated using the following formulas:

$$\text{Chlorophyll a} = 12.7\,(\text{O.D of } 663) - 2.69\,(\text{O.D of } 645) \times \frac{v}{w \times 1000}$$

$$\text{Chlorophyll b} = 22.9\,(\text{O.D of } 645) - 4.68\,(\text{O.D of } 663) \times \frac{v}{w \times 1000}$$

$$\text{Total chlorophyll} = 20.2\,(\text{O.D of } 645) + 8.02\,(\text{O.D of } 663) \times \frac{v}{w \times 1000}$$

In these equations, W represents the fresh weight of the extracted tissue in g, V signifies the final volume of the 80% acetone extract, and O.D. corresponds to the optical density at a specific wavelength.

Table 1. Details of environmental characteristics of different experimental locations of Iran

Locations	Province	Longitude (E)	Latitude (N)	Altitude (m.a.s.l)	Relative humidity (%)	Average annual temperature (°C)	Average annual rainfall (mm)
Arak	Markazi	49° 46'	34° 06'	1708	46.0	13.9	341.7
Rasht	Gillan	49° 36'	37° 16'	-7	83.0	15.8	1506
Gorgan	Golestan	54° 25'	36° 50'	176	69.1	17.8	583.8
Hamadan	Hamadan	48° 30'	34° 53'	1913	56.1	11.3	317.7
Isfahan	Isfahan	52° 35'	31° 41'	1571	38.5	16.4	126.9
Kermanshah	Kermanshah	47° 04'	34° 19'	1374	45.4	14.5	437.0
Shiraz	Fars	52° 36'	29° 36'	1519	48.5	18	337.8
Tabriz	East Azerbaijan	46° 25'	38° 02'	1345	40.2	11.9	288.9
Tehran	Tehran	51° 17'	35° 36'	1543	46.2	17.0	229.9
Urmia	South Azerbaijan	45° 04'	37° 33'	1363	49.9	11.5	341

2.4. Assessment of total sugar

Fresh leaf samples weighing 0.2 g were first homogenized in 70% ethanol. After homogenization, the samples were filtered, and the pigments were extracted using benzene. Subsequently, 0.2 mL of the leaf extract was mixed with 1.0 mL of a 0.2% anthrone solution, and this mixture was allowed to react in a water bath for 10 min at a temperature of 100 °C. Following the reaction, the test tube was promptly cooled in an ice bath, and the absorbance was measured at 620 nm, following the method outlined by Yemm and Folkes (1953).

2.5. Assessment of proline

Proline content was assessed following the procedure outlined by Bates et al. (1973). Approximately, 0.25 g of fresh leaf material was homogenized in 10 mL of a 3% aqueous sulfosalicylic acid solution. Subsequently, this aqueous solution was filtered through Whatman's paper No. 2, and 2 mL of the filtered solution was combined with 2 mL of acid-ninhydrin and 2 mL of glacial acetic acid within a test tube. The mixture was then subjected to a water bath at 100 °C for 1 h. Afterward, the reaction mixture was extracted with 4 mL of toluene and allowed to cool to room temperature, and its absorbance was measured at 520 nm using a spectrometer.

2.6. Assessment of SVglys

The determination of SVglys content followed established procedures per previous studies (Aghighi Shahverdi et al., 2019). Initially, 0.1 g of powdered dried leaves was placed into 15 mL tubes, adding 3 mL of distilled water. This mixture was subjected to a water bath at 80 °C for 30 min. The resulting solution underwent centrifugation at 12,000 g for 5 min, with the supernatant collected, and this process was repeated three times. The final supernatant was diluted precisely to 10 mL using distilled water and filtered through a 0.45 µm nylon filter attached to a syringe. To purify SVglys, a C18 cartridge was employed. 0.5 mL of the filtered supernatant was loaded into the C18 cartridge and washed with an acetonitrile/water mixture (20:80, v/v). Two reverse-phase C18 columns were connected in series for chromatographic analysis of diterpene glycosides, and a UV-Vis detector set at 202 nm was utilized. A solvent gradient was created using acetonitrile and water as mobile phases, with a flow rate of 0.5 mL min^{-1}. The acetonitrile ratio was incrementally increased during specific time intervals: 50, 65, 80, 80, and 50% for 0-10, 10-18, 18-22, 22-24, and 24-30 min, respectively. To quantify SVglys, 40 mL of the purified extract was injected into the HPLC pump. Chromstar 7.0 software was used to calculate the HPLC peak area, and the results for SVglys content were expressed as a percentage of leaf dry weight (W/W). Calibration curves were generated from the relationship between external standards (ppm) and their corresponding HPLC peak area.

2.7. Statistical analysis

Upon assessing the normality of data distribution with Kolmogorov-Smirnov and Shapiro-Wilk tests, statistical analysis of the intended traits was conducted using Statistical Analysis System software (SAS Institute, Cary, NC, USA, version 9.2). Mean values were compared utilizing the least significant difference (LSD) test at a significance level of $P < 0.05$. Additionally, the Pearson correlation between the attributes was analyzed using SAS 9.2 software. Minitab version 18 software has been used for cluster and principal component analyses (PCA).

3. Results

3.1. Morphological and yield attributes

The plant height in the tested samples ranged from 18.87 to 38.83 cm. The results indicated that the tallest plants were observed in samples from Arak, followed by Shiraz, Kermanshah, Isfahan, Tehran, Urmia, and Hamadan. In contrast, samples from the cities of Rasht, Gorgan, and Tabriz had the lowest average values of this trait (Table 2).

According to the data analysis (Table 2), the samples from Shiraz and Tehran exhibited the highest number of branches, with 4.67 and 4 branches per plant, respectively. Conversely, samples from Rasht demonstrated the lowest mean for this characteristic, with 1.67 branches per plant.

The number of leaves per plant ranged from 69 to 242.3. Rasht had the lowest number of leaves, while Kermanshah, Tehran, Isfahan, and Shiraz had the highest number of leaves per plant (Table 2).

As shown in Table 2, the leaf yield ranged from 24.05 to 119.89 g m^{-2} in the harvested samples and varied significantly. Shiraz had the highest leaf yield, while Rasht had the lowest.

Table 2. Morphological variations and yield attributes of stevia in different climatic regions of Iran

Locations	Plant height (cm)	Number of branches / plant	Number of leaves / plant	Leaf yield (g m^{-2})	Biological yield (g m^{-2})	LMR (%)
Arak	38.83±7.2a	3±1bc	152.33±11.24ab	86.24±18.96ab	260.53±58.67b	33.13±0.66a
Rasht	21.27±3.72b	1.67±0.58c	69±22.72b	24.05±6.56d	89.33±15.75c	26.63±2.53a
Gorgan	20.1±1.57b	3.33±1.53ab	80.33±36.91b	36.91±18.72cd	119.31±45.27c	30.01±3.68a
Hamadan	33.87±4.84a	3±1bc	152.67±3.21ab	82.45±4.89b	254.45±3.32b	32.42±2.33a
Isfahan	37.4±5.1a	3±1bc	194.33±24.54a	91.25±7.16ab	279.68±32ab	32.75±1.95a
Kermanshah	37.47±7.12a	3±1bc	242.33±108.64a	92.03±2.28ab	275.47±10.19ab	33.42±0.91a
Shiraz	38.73±2.4a	4.67±0.58a	195.67±52.84a	119.89±39.08a	355.09±107.9a	33.63±0.68a
Tabriz	18.87±8.22b	2.67±1.15bc	75.67±60.62b	31.79±27.48d	111.79±71.02c	23.9±11.84a
Tehran	35.67±4.8a	4±1ab	199.33±100.98a	97.07±21.82ab	296.48±55.42ab	32.59±1.23a
Urmia	35.1±6.8a	3±0bc	84.33±17.93b	69.15±21.63bc	215.73±60.35b	31.87±1.08a
LSD (0.05)	9.07	1.65	100.99	34.93	93.85	10.43

Values are means ± SD of three replications followed by similar letters in the same column are not significantly different based on the LSD test at 5% probability level; LMR: leaf mass ratio

Table 3. Variations in physiological and biochemical characteristics of stevia under different climatic regions of Iran

Locations	Total chlorophyll (μg g^{-1} FW)	Chlorophyll a (μg g^{-1} FW)	Chlorophyll b (μg g^{-1} FW)	Total sugar (mg g^{-1} FW)	Proline (μmol g^{-1} FW)
Arak	29.75±1.49cd	20.15±1.33d	9.61±2.43bc	3.23±1.63a-d	2.08±0.58bc
Rasht	13.71±1.9f	10.65±2.12e	3.07±0.55e	2.5±0.89b-e	2.31±0.67abc
Gorgan	16.56±0.72f	12.04±0.41e	4.52±1.14e	1.62±0.01e	2.75±0.59ab
Hamadan	31.06±2.19bcd	19.6±0.61d	11.47±1.8b	2.14±0.61de	3.17±0.63a
Isfahan	28.48±3.15de	20.72±2.81cd	7.77±0.59cd	3.99±1.17a	2.26±0.1bc
Kermanshah	34.06±4.4bc	22.77±1.45bc	11.3±3.04b	3.6±0.76ab	2.44±0.4ab
Shiraz	43.44±2.15a	27.35±1.18a	16.1±1.58a	3.07±0.74a-d	1.44±0.53c
Tabriz	24.01±2.07e	18.82±0.65d	5.2±1.43de	4.08±0.14a	2.94±0.24ab
Tehran	34.95±3.35b	23.51±1.98b	11.45±1.53b	3.34±0.44abc	2.21±0.47bc
Urmia	16.25±2.71f	11.74±2.03e	4.51±0.77e	2.36±0.25cde	2.31±0.39abc
LSD (0.05)	4.67	2.57	2.86	1.20	0.86

Values are means ± SD of three replications followed by similar letters in the same column are not significantly different based on the LSD test at 5% probability level.

According to the results of the data analysis, the biological yield (stem + leaf + root) ranged from 89.33 g m^{-2} (associated with Rasht) to 355.09 g m^{-2} (associated with Shiraz) and showed significant variability (Table 2).

According to the analysis of variance, LMR did not show statistically significant differences in the harvested samples and ranged from 23.9% to 33.63%. The highest LMR values were observed in the samples from Tabriz and Shiraz, respectively (Table 2).

3.2. Chlorophyll-a, -b, and total content

The results of the data analysis revealed significant differences in the content of photosynthetic pigments, including chlorophyll a, b, and total, among different growth habitats. The total chlorophyll content ranged from 13.71 to 43.44 μg g^{-1} FW and showed variability (Table 2). The highest content of total chlorophyll, chlorophyll a, and chlorophyll b were found in the samples from Shiraz, with average values of 43.44, 27.35, and 16.1 μg g^{-1} FW, respectively. In contrast, the samples from Rasht exhibited the lowest average values for these traits (13.71, 10.65, and 3.07 μg g^{-1} FW) (Table 3).

3.3. Total sugar content

As shown in Table 3, the results indicated significant differences in the total sugar content among samples collected from different growth habitats. The highest total sugar content was observed in Isfahan (3.99 mg g^{-1} FW) and Tabriz (4.08 mg g^{-1} FW). Conversely, the lowest total sugar content was found in the samples from Gorgan (1.62 mg g^{-1} FW).

3.4. Proline content

The experimental findings demonstrated significant differences in proline content among samples collected from different regions. As shown in Table 3, the sample from Shiraz had the lowest proline content (1.44 μmol g^{-1} FW), whereas samples from Hamadan exhibited the highest proline content (3.17, 2.75 μmol g^{-1} FW).

3.5. Steviol glycosides compounds

The primary compounds, Reb-A and Stev, as well as the total of these compounds and their ratio, along with the SVglys yield, exhibited notable variances among samples collected from different cities. Specifically, samples from Rasht, located in Gilan Province, showcased the highest Reb-A and Stev content, along with the total SVglys (3.01%, 8.66%, and 11.67%, respectively).

Moreover, samples from Gorgan also demonstrated the highest average Stev content and total SVglys. Conversely, samples from Tabriz had the lowest average Reb-A, Stev, and total SVglys content (2.16%, 5.44%, and 7.6%, respectively).

As shown in Table 4, the highest Reb-A to Stev ratio was observed in samples collected from Tabriz (0.398), while the lowest ratio was found in samples collected from Gorgan (0.305). Steviol glycoside yield ranged from 24.8 to 122.6 mg m^{-2}. Samples from Shiraz and Tehran had the highest SVglys yield, whereas samples from Tabriz and Rasht had the lowest SVglys yield (24.8 and 28.0 mg m^{-2}, respectively).

Table 4. Variations in steviol glycosides (SVglys) compounds of stevia under different climatic regions of Iran

Locations	Reb-A (%)	Stev (%)	Total SVglys (%)	Reb-A/ Stev ratio	SVglys yield (mg m^{-2})
Arak	2.45±0.14b-e	7.25±0.21c	9.7±0.35bc	0.337±0.009bcd	83.6±18.2bc
Rasht	3.01±0.22a	8.66±0.1a	11.67±0.12a	0.347±0.03bc	28±7.4ef
Gorgan	2.67±0.15b	8.75±0.19a	11.42±0.34a	0.305±0.01d	42.21±21.4def
Hamadan	2.32±0.19cde	6.53±0.11d	8.86±0.29de	0.356±0.022bc	72.9±2.5bcd
Isfahan	2.34±0.11cde	7.08±0.48c	9.42±0.59cd	0.331±0.007cd	86.1±11.1bc
Kermanshah	2.24±0.23de	6.34±0.29d	8.58±0.52e	0.353±0.02bc	78.9±2.8bc
Shiraz	2.56±0.21bcd	7.75±0.2b	10.3±0.41b	0.329±0.018cd	122.6±35.3a
Tabriz	2.16±0.14e	5.44±0.42e	7.6±0.5f	0.398±0.027a	24.8±12.4f
Tehran	2.63±0.22bc	7.15±0.12c	9.79±0.1bc	0.368±0.037ab	95±21.4ab
Urmia	2.13±0.09e	6.25±0.22d	8.38±0.31e	0.341±0.003bc	58.3±20.1cde
LSD (0.05)	0.31	0.36	0.61	0.036	32.5

Values are means ± SD of three replications followed by similar letters in the same column are not significantly different based on the LSD test at 5% probability level.

3.6. Correlation, cluster, and PCA analyses

The outcomes of a basic correlation analysis revealed significant positive and negative associations, as well as some associations that were not statistically significant, among the measured characteristics. Leaf yield displayed significantly positive correlations with morphological attributes including plant height, number of branch, number of leaves, total biomass, and LMR, along with total chlorophyll and chlorophyll-a levels. Conversely, a significant negative correlation was observed with proline content. The results indicated that the total SVglys content had non-significant correlations with growth and yield traits. However, it showed significant negative correlations with chlorophyll-a, total sugar, and proline (Table 5).

Table 5. Simple correlation among the morpho-physiological, yield, and steviol glycosides (SVglys) of stevia under different climatic regions of Iran

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	0.51**														
3	0.79**	0.54**													
4	0.93**	0.75**	0.87**												
5	0.93**	0.74**	0.87**	0.99**											
6	0.90**	0.59**	0.76**	0.87**	0.87**										
7	0.69**	0.74**	0.85**	0.87**	0.87**	0.60**									
8	0.64**	0.70**	0.84**	0.83**	0.83**	0.51**	0.98**								
9	0.72**	0.76**	0.82**	0.89**	0.89**	0.69**	0.97**	0.92**							
10	0.26ns	0.05ns	0.46*	0.29*	0.30*	-0.06ns	0.45*	0.59**	0.24ns						
11	-0.53**	-0.50**	-0.39*	-0.57**	-0.56**	-0.45*	-0.43*	-0.41*	-0.44*	-0.22ns					
12	-0.32*	-0.11ns	-0.18ns	-0.26ns	-0.26ns	-0.15ns	-0.19ns	-0.23ns	-0.12ns	-0.38*	-0.27ns				
13	-0.22ns	0.02ns	-0.17ns	-0.17ns	-0.18ns	0.06ns	-0.22ns	-0.29*	-0.12ns	-0.54**	-0.33*	0.89**			
14	-0.24ns	-0.01ns	-0.18ns	-0.19ns	-0.20ns	0.02ns	-0.22ns	-0.29*	-0.12ns	-0.52**	-0.32*	0.93**	0.99**		
15	-0.18ns	-0.22ns	-0.03ns	-0.15ns	-0.13ns	-0.49*	0.12ns	0.21ns	-0.01ns	0.55**	0.35*	-0.34*	-0.72**	-0.65**	
16	0.88**	0.80**	0.83**	0.98**	0.98**	0.86**	0.87**	0.82**	0.90**	0.23ns	-0.66**	-0.09ns	0.01ns	-0.01ns	-0.25ns

-1 -0.9 -0.8 -0.7 -0.6 -0.5 -0.4 -0.3 -0.2 -0.1 0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

High negative correlation — Non-correlation — High positive correlation

1. Plant height, 2. Number of branches per plant, 3. Number of leaves per plant, 4. Leaf yield, 5. Biological yield, 6. LMR, 7. Total chlorophyll, 8. Chlorophyll a, 9. Chlorophyll b, 10. Total sugar, 11. Proline, 12. Reb-A, 13. Stev, 14. Total SVglys, 15. Reb-A/Stev, 16. SVglys yield

The cluster analysis findings, as shown in Table 6, categorized the gathered samples into three distinct clusters. The first cluster, comprising Gorgan and Rasht, exhibited excellence in Reb-A, Stev, and total SVglys content. The second cluster, consisting of Tabriz, Hamedan, and Urmia samples, demonstrated superiority in proline content and the ratio of Reb-A to Stev. The third cluster, which showcased excellence in growth, yield, and photosynthetic pigment content, encompassed samples from cities such as Kermanshah, Isfahan, Tehran, Arak, and Shiraz (Fig. 1).

The principal component analysis (PCA) outcomes are depicted in Fig. 2, where the primary component accounted for 55.5% of the variance, and the secondary component clarified 24.4% of the variance. Collectively, these two components represented 79.9% of the trait variability. Within the primary component, leaf yield and biological yield emerged as the most influential characteristics, justifying its selection as the yield component.

In light of the findings from the correlation analysis between climatic and geographical factors and certain vital yield and biochemical traits, it was observed that leaf yield exhibited a positive correlation with both altitude and the mean annual rainfall. Conversely, altitude negatively correlated with the Reb-A, Stev, and the total SVglys. The findings demonstrated that parameters such as relative humidity, average annual temperature, and average annual rainfall exhibited stronger positive correlations with the biochemical content of compounds in the stevia plant (Table 7).

Table 6. Cluster analysis and grouping of studied variables of stevia under different climatic regions of Iran

Variable	Cluster-1	Cluster-2	Cluster-3	Grand centroid
Plant height	20.683	29.278	**37.620**	31.730
Number of branches per plant	2.500	2.889	**3.533**	3.133
Number of leaves per plant	74.667	104.222	**196.800**	144.600
Leaf yield	30.480	61.129	**97.296**	73.083
Biological yield	104.320	193.991	**293.451**	225.787
LMR	28.318	29.395	**33.104**	31.034
Total chlorophyll	15.136	23.775	**34.137**	27.228
Chlorophyll a	11.346	16.719	**22.901**	18.736
Chlorophyll b	3.794	7.062	**11.245**	8.500
Total sugar	2.062	2.860	**3.445**	2.993
Proline	2.533	**2.805**	2.087	2.391
Reb-A	**2.838**	2.205	2.444	2.451
Stev	**8.708**	6.074	7.114	7.121
Total SVglys	**11.546**	8.279	9.558	9.572
Reb-A/Stev ratio	0.326	**0.365**	0.344	0.347
SVglys yield	3.506	5.200	**9.325**	6.924
Number of locations	2	3	5	-

The bold and underlined numbers indicate the highest averages for each trait compared to other clusters.

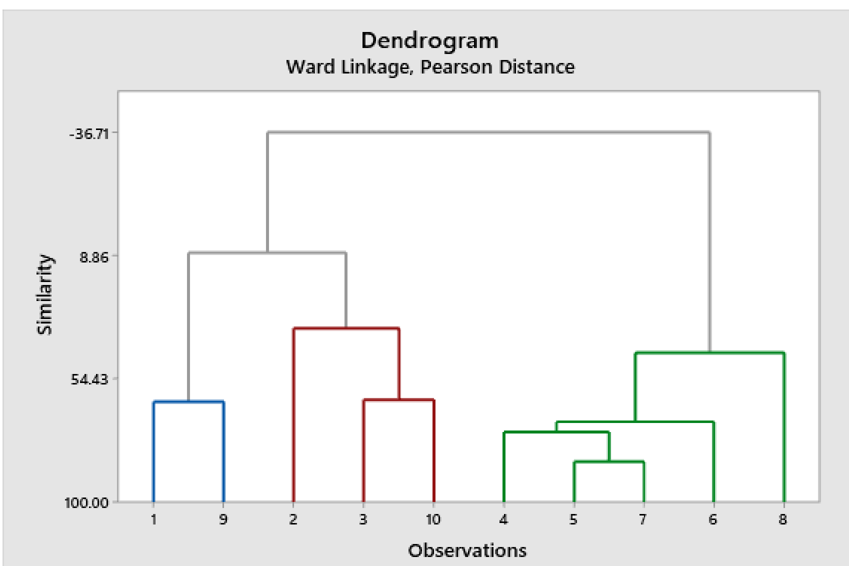

Figure 1. Cluster dendrogram of 10 stevia samples collected from different cities in Iran. (1. Gorgan; 2. Tabriz; 3. Hamedan; 4. Kermanshah; 5. Isfahan; 6. Tehran; 7. Arak; 8. Shiraz; 9. Rasht; 10. Urmia)

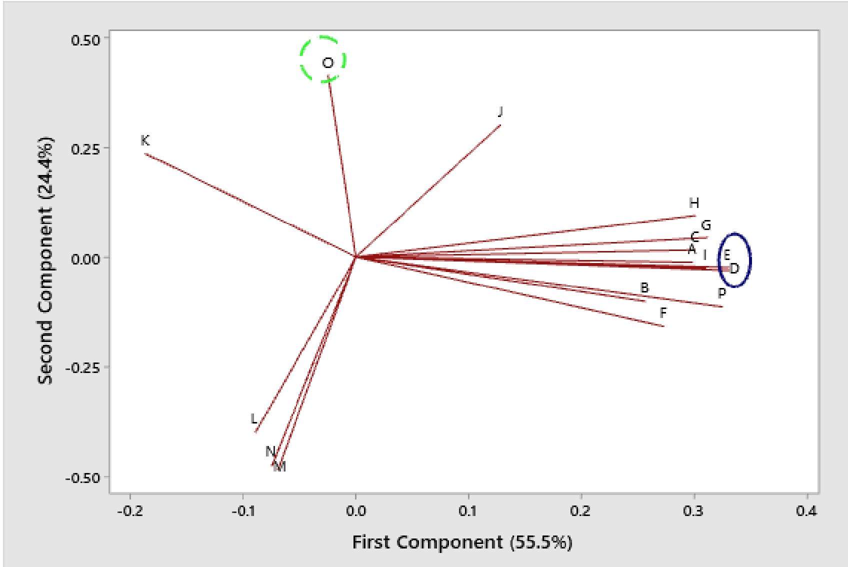

Figure 2.Principal Component Analysis (PCA) of the Stevia samples collected from 10 different agro-climatic regions of Iran. **A**. Plant height, **B**. Number of branches per plant, **C**. Number of leaves per plant, **D**. Leaf yield, **E**. Biological yield, **F**. LMR, **G**. Total chlorophyll, **H**. Chlorophyll a, **I**. Chlorophyll b, **J**. Total sugar, **K**. Proline, **L**. Reb-A, **M**. Stev, **N**. Total SVglys, **O**. RebA/Stev, **P**. SVglys yield

Table 7. Pearson correlation coefficient between environmental and traits with SVglys content and yield in different sites of Iran

	Leaf yield	Reb-A	Stev	Total SVglys	SVglys yield
Longitude	0.18	0.59	0.75	0.73	0.34
Latitude	0.15	0.01	-0.15	-0.12	-0.87
Altitude	0.74	-0.68	-0.69	-0.70	0.63
Relative humidity	0.28	0.77	0.74	0.76	0.12
Average annual temperature	0.21	0.66	0.75	0.74	0.37
Average annual rainfall	0.45	0.74	0.60	0.64	0.34

-1	-0.9	-0.8	-0.7	-0.6	-0.5	-0.4	-0.3	-0.2	-0.1	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1

High negative correlation Non-correlation High positive correlation

4. Discussion

The primary objective of this study was to evaluate a range of growth, yield, physiological, and biochemical parameters in stevia, a medicinal plant, across various climatic conditions in Iran. Ten experimental fields were selected from diverse locations with distinct climate profiles to achieve this aim, as detailed in Table 1. The findings of our investigation highlighted substantial variations in growth and yield attributes, such as plant height, branch number, leaf number, leaf yield, and biological yield. Additionally, we observed noteworthy distinctions in physiological and biochemical features, encompassing the levels of photosynthetic pigments, total sugar content, proline concentration, and the presence of SVglys (specifically, Stev and Reb-A) among the different cultivation sites that were analyzed.

Based on the results, Isfahan, Kermanshah, Shiraz, and Tehran outperformed in terms of overall growth and yield traits such as plant height, branch number, leaf number, leaf yield, and biological yield. In contrast, Rasht and Gorgan showed the lowest average values for these traits (Table 2). These results can be attributed to various environmental factors and climatic conditions unique to each of these cities. With its distinct climate, Isfahan likely provided favorable conditions for stevia's growth and development, resulting in higher plant height and increased yields. Kermanshah, Shiraz, and Tehran also demonstrated superior performance in these growth and yield parameters, possibly due to their climatic conditions, soil quality, or other region-specific factors conducive to stevia cultivation. On the other hand, Rasht and Gorgan presented the lowest average values for these traits. This outcome suggests that these regions' environmental factors or climatic conditions might not be as conducive to stevia's growth and yield. Factors such as temperature, humidity, and soil composition could limit the plant's performance in these areas (Aghighi Shahverdi et al., 2018; Farrokhi et al., 2021).

Aghighi Shahverdi et al. (2018) findings align with the current research, demonstrating that Firuzabad exhibited more favorable growth conditions than Anzali, particularly concerning growth and yield attributes. This underscores the significant influence of environmental conditions, including soil quality and climate, on the growth and yield attributes of stevia during its cultivation and growth phases. Notably, stevia plants cultivated in Firuzabad displayed the highest values for plant height, branch number, leaf yield, and biological yield. In contrast, the lowest mean values for these traits were observed in the Anzali location. These outcomes further corroborate the substantial impact of environmental conditions on stevia's growth and yield parameters, underscoring the importance of selecting suitable cultivation sites and optimizing ecological conditions to enhance stevia production.

The highest leaf yield was obtained in Shiraz. This city's climatic and geographical characteristics, characterized by its relatively low geographical latitude compared to other regions and its high annual average temperature, contributed to this result. These two climate parameters positively correlated with leaf yield (Table 6). The discussion primarily focuses on how temperature profoundly influences the stevia plant's growth, development, and biochemical processes. Pal et al. (2015) reported that environmental conditions significantly influence the growth and accumulation of dry matter in stevia during planting and vegetative growth. The best results were obtained under CSIR-IHBT conditions, while the lowest performance was observed under PAU conditions. This difference is likely due to the unfavorable temperature conditions, particularly the high temperatures exceeding 42 °C during the establishment and vegetative growth stages at PAU. These extreme temperatures, along with low relative humidity, may have negatively impacted photosynthesis and ultimately reduced the yield at PAU.

The higher growth and yield parameters in the Shiraz samples can be attributed to the increased content of photosynthetic pigments, including chlorophyll. The significant correlation between yield, growth characteristics, and chlorophyll content supports this. In this regard, Afshari et al. (2022) reported that the higher chlorophyll content increases the stevia's growth and yield. The chlorophyll content in leaves is not only determined by genetic factors and plant species but is also highly influenced by environmental and climatic conditions (Rastogi et al., 2020).

The results indicated that the samples from Hamadan had the highest proline content. The lower average temperature in this city has induced cold stress in the plant. Since stevia is sensitive to cold, an increase in proline content was observed in samples from this city. Proline plays a crucial role in enhancing the cold tolerance of plants through several mechanisms. Firstly, it helps maintain osmotic balance by preventing water freezing inside plant cells during cold conditions (Raza et al., 2023). Secondly, it preserves the structural integrity of plant cells by reducing ice crystal formation, which can lead to cell damage (Jahed et al., 2023). Lastly, proline regulates enzyme activity, influencing various metabolic processes that aid the plant's response to cold stress (Ghosh et al., 2022). Collectively, these mechanisms enable plants to better withstand and survive in cold environments.

Temperature plays a pivotal role in shaping the plant's overall yield, impacting factors like leaf number, size, sugar content, and the production of SVglys. Additionally, it highlights the plant's sensitivity to extreme cold temperatures, which can lead to damage (Soufi et al., 2016). The discussion underscores the significance of temperature as a critical determinant of stevia's growth, yield, and biochemical characteristics, emphasizing the need for further research to comprehend its effects comprehensively. It is a recognized fact that the leaf production and SVglys content in stevia leaves can be impacted by genetic traits and the surrounding environmental factors (Tavarini and Angelini 2013).

Steviol glycosides were discovered to accumulate in stevia leaves' chloroplasts, constituting approximately 10 to 20% of the leaf's dry weight. The predominant glycoside profile in stevia leaves consists of roughly 0.3% dulcoside, 0.6% Reb-C, 3–4% Reb-A, and 7–9% Stev (Shahverdi et al., 2019).

The present study's findings illustrate significant variations in SVglys among different growth locations, resulting in considerable differences in their levels and yields. Specifically, the highest SVglys content was observed in samples from Rasht and Gorgan, while the highest SVglys yield was found in samples from Shiraz. Steviol glycoside yield is calculated as the product of SVglys content and leaf yield. As a result, the environmental conditions for growth and yield in Shiraz significantly exceeded those in Rasht and Gorgan, leading to higher SVglys yields in Shiraz. However, regarding SVglys content, the similarity in climatic conditions between Rasht and Gorgan, the native habitats of the stevia plant, significantly contributed to the increased synthesis of these compounds in these samples. These findings align with previous studies on stevia plants conducted by other researchers (Aghighi Shahverdi et al., 2018). In a 2-year-old crop, the Stev content was significantly influenced by the experimental site and the interaction between harvest time and site. The plants grown in Jesi had the highest Stev content.

Moreover, there were more noticeable differences between the two sites in plants harvested at the end of July and mid-September, with Jesi showing higher Stev contents than Pieve Cesato. In contrast, the Reb-A content increased consistently from early July to September, peaking at the third harvest in the first year of growth (Tavarini and Angelini 2013). The differences in Stev content among leaves were more significant with location variations than Reb-A. This implies that the accumulation of Stev is notably affected by environmental and soil factors. Previous research has also highlighted that Stev levels can fluctuate depending on the cultivation conditions and the specific genotype of the plant (Pal et al., 2015; Aghighi Shahverdi et al., 2018).

5. Conclusion

In conclusion, this research underscores the significance of local environmental conditions in influencing the growth and yield of stevia plants. The variations observed among different cities emphasize the need for region-specific cultivation practices and the selection of suitable cultivars to optimize stevia production in various geographic locations within Iran. The findings suggest that if the objective of stevia cultivation is leaf production, Shiraz, Tehran, and Arak are suitable regions for cultivation. However, if the goal is to extract SVglys, it is advisable to cultivate them in the Rasht and Gorgan regions. Further studies examining the precise environmental factors contributing to these variations could provide valuable insights for enhancing stevia cultivation and increasing yield across different regions.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

Afshari F, Nakhaei F, Mosavi S, Seghatoleslami M. 2022. Physiological and biochemical responses of *Stevia rebaudiana* Bertoni to nutri-priming and foliar nutrition under water supply restrictions. Industrial Crops and Products 176: 114399. https://doi.org/10.1016/j.indcrop.2021.114399

Aghighi SM, Omidi H, Tabatabaei SJ. 2018. Plant growth and steviol glycosides as affected by foliar application of selenium, boron, and iron under NaCl stress in *Stevia rebaudiana* Bertoni. Industrial Crops and Products 125: 408-415. https://doi.org/10.1016/j.indcrop.2018.09.029

Ahmad MA, Chaudary S, Deng X, Cheema M, Javed R. 2023. Nano-stevia interaction: Past, present, and future. Plant Physiology and Biochemistry: 107807. https://doi.org/10.1016/j.plaphy.2023.107807

Ali A, Shahu R, Balyan P, Kumari S, Ghodmare R, Jobby R, Jha P. 2022. Antioxidation and antiglycation properties of a natural sweetener: *Stevia rebaudiana*. Sugar Tech 24: 563-575. https://doi.org/10.1007/s12355-021-01023-0

Barbet-Massin C, Giuliano S, Alletto L, Daydé J, Berger M. 2015. Nitrogen limitation alters biomass production but enhances steviol glycoside concentration in *Stevia rebaudiana* Bertoni. PLoS One, 10: e0133067. https://doi.org/10.1371/journal.pone.0133067

Bates LS, Waldren RP, Teare I. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil, 39: 205-207. https://doi.org/10.1007/BF00018060

Dyduch-Siemińska M, Najda A, Gawroński J, Balant S, Świca K, Żaba A. 2020. *Stevia rebaudiana* Bertoni, a source of high-potency natural sweetener-biochemical and genetic characterization. Molecules, 25: 767. https://doi.org/10.3390/molecules25040767

Farrokhi H, Asgharzadeh A, Samadi MK. 2021. Yield and qualitative and biochemical characteristics of saffron (*Crocus sativus* L.) cultivated in different soil, water, and climate conditions. Italian Journal of Agrometeorology. 2: 43-55. https://doi.org/10.36253/ijam-1216

Ghosh U, Islam M, Siddiqui M, Cao X, Khan M. 2022. Proline, a multifaceted signalling molecule in plant responses to abiotic stress: understanding the physiological mechanisms. Plant Biology, 24: 227-239. https://doi.org/10.1111/plb.13363

Jahed KR, Saini AK, Sherif SM. 2023. Coping with the cold: unveiling cryoprotectants, molecular signaling pathways, and strategies for cold stress resilience. Frontiers in Plant Science, 14:1246093. https://doi.org/10.3389/fpls.2023.1246093

Khiraoui A, Bakha M, Boulli A, Hasib A, Al Faiz C. 2021. The productivity of *Stevia rebaudiana* (Bertoni) on dry leaves and steviol glycosides of four varieties grown in six regions of Morocco. Biocatalysis and Agricultural Biotechnology, 37: 102151. https://doi.org/10.1016/j.bcab.2021.102151

Lichtenthaler HK, Wellburn AR. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. In.: Portland Press Ltd. Biochemical Society Transactions. 11(5): 591-592. https://doi.org/10.1042/bst0110591

Mlambo R, Wang J, Chen C. 2022. *Stevia rebaudiana*, a versatile food ingredient: The chemical composition and medicinal properties. Journal of Nanomaterials, 2022. https://doi.org/10.1155/2022/3573005

Pal PK, Kumar R, Guleria V, Mahajan M, Prasad R, Pathania V, Gill BS, Singh D, Chand G, Singh B. 2015. Crop-ecology and nutritional variability influence growth and secondary metabolites of *Stevia rebaudiana* Bertoni. BMC Plant Biology, 15: 67. https://doi.org/10.1186/s12870-015-0457-x

Rastogi A, Antala M, Gąbka M, Rosadziński S, Stróżecki M, Brestic M, Juszczak R. 2020. Impact of warming and reduced precipitation on morphology and chlorophyll concentration in peat mosses (*Sphagnum angustifolium* and *S. fallax*). Scientific reports, 10: 8592. https://doi.org/10.1038/s41598-020-65032-x

Raza A, Charagh S, Abbas S, Hassan MU, Saeed F, Haider S, Sharif R, Anand A, Corpas FJ, Jin W. 2023. Assessment of proline function in higher plants under extreme temperatures. Plant Biology, 25: 379-395. https://doi.org/10.1111/plb.13510

Rodriguez-Paez LA, Jimenez-Ramirez AM, Pompelli MF, Pineda-Rodriguez YY, Jarma-Orozco A, de Dios Jaraba-Navas J, Aramendiz-Tatis H, Combatt-Caballero E, Oloriz-Ortega MI, Rodríguez NV. 2023. Physiological and enzymatic evaluation of selected genotypes of *Stevia rebaudiana* Bertoni. Agronomy, 13: 403. https://doi.org/10.3390/agronomy13020403

Shahverdi MA, Omidi H, Damalas CA. 2020. Foliar fertilization with micronutrients improves *Stevia rebaudiana* tolerance to salinity stress by improving root characteristics. Brazilian Journal of Botany. 43: 55-65. https://doi.org/10.1007/s40415-020-00588-6

Shahverdi MA, Omidi H, Tabatabaei SJ. 2019. Stevia (*Stevia rebaudiana* Bertoni) responses to NaCl stress: Growth, photosynthetic pigments, diterpene glycosides and ion content in root and shoot. Journal of the Saudi Society of Agricultural Sciences, 18: 355-360. https://doi.org/10.1016/j.jssas.2017.12.001

Soufi S, D'Urso G, Pizza C, Rezgui S, Bettaieb T, Montoro P. 2016. Steviol glycosides targeted analysis in leaves of *Stevia rebaudiana* (Bertoni) from plants cultivated under chilling stress conditions. Food Chemistry, 190: 572-580. https://doi.org/10.1016/j.foodchem.2015.05.116

Tavarini S, Angelini LG. 2013. *Stevia rebaudiana* Bertoni as a source of bioactive compounds: the effect of harvest time, experimental site and crop age on steviol glycoside content and antioxidant properties. Journal of the Science of Food and Agriculture, 93: 2121-2129. https://doi.org/10.1002/jsfa.6016

Wang Y, Luo X, Chen L, Mustapha AT, Yu X, Zhou C, Okonkwo CE. 2023. Natural and low-caloric rebaudioside A as a substitute for dietary sugars: A comprehensive review. Comprehensive Reviews in Food Science and Food Safety, 22: 615-642. https://doi.org/10.1111/1541-4337.13084

Yemm E, Folkes B. 1953. The amino acids of cytoplasmic and chloroplastic proteins of barley. Biochemical Journal, 55: 700-707. https://doi.org/10.1042/bj0550700