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Impact of Drought Stress and Foliar Application of Growth Regulators on Yield and Steviol Glycosides in Two Stevia Species

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ABSTRACT

The combined application of selenium (Se) and diverse plant growth regulators, particularly under drought stress, represents a crucial strategy for augmenting secondary metabolite synthesis in medicinal plants. This study used a split-split-plot factorial design within a randomized complete block, containing three replications in diverse climatic regions such as Varamin, Firuzabad, and Gorgan, each characterized by distinct climatic conditions. This study aimed to assess irrigation treatments (90 ± 5% and 25 ± 5% field capacity) as main plots and foliar applications involving Se and plant growth regulators (strigolactone: SLs and forchlorfenuron: CPPU) as well as various stevia genotypes (Indian and Chinese) as subplots. Severe drought stress reduced the yield of multiple plants at all sites, while increasing the proline content in the Chinese genotype. Foliar spray treatments, especially the combined application of Se, CPPU, and SLs, improved relative water content and mitigated drought-induced reductions in chlorophyll content. Superior leaf yield was observed by applying SLs under non-drought conditions in the Indian genotype across the regions, with averages of 1249.3, 2061.2, and 2982.2 kg ha^{-1}, respectively. The combined application of Se + SLs + CPPU under drought stress conditions enhanced stevia leaf yield in the Indian genotype significantly. In addition, such treatments positively impacted steviol glycosides (SvGls) content and yield, especially under drought stress conditions. The peak of SvGls content at ES-1 was related to applying SLs and Se under drought stress conditions in the Indian genotype (11.39 and 11.59%, respectively). However, employing SLs at ES-2 and ES-3 under non-stress conditions in the Chinese genotype showcased the highest SvGls content (11.13 and 14.08%, respectively). Comparing stevia genotypes indicated the superior performance of the Indian genotype in terms of relative water content, photosynthetic pigments, as well as SvGls content and yield. However, the Chinese genotype exhibited superior enzyme activity levels. Based on the results, Se application under drought stress conditions enhanced the activity of catalase and peroxidase, resulting in improved plant yield while mitigating the adverse effects of drought stress.

Introduction

Stevia (*Stevia rebaudiana* Bertoni), renowned for its sweet taste, belongs to the Asteraceae family (Aghighi Shahverdi et al., 2020). This herbaceous perennial, native to the mountainous regions of Brazil and Paraguay, is highly valued for its intensely sweet leaves. These leaves are a rich source of nutritional elements, containing carbohydrates (35.2–61.93%), proteins (9.8–20.4%), fats (1.9–4.34%), ash (6.3–13.1%), and dietary fiber (15.2–18.5%). They also provide various essential minerals, including potassium, calcium, sodium, magnesium, copper, manganese, iron, and zinc (Schiatti-Sisó et al., 2023).

The primary contributors to stevia's sweetness are steviol glycosides (SvGls), a group of tetracyclic diterpenes estimated to be 300 times sweeter than sucrose (Wang et al., 2023). Notably, the sweet taste is present in all green parts of the plant. Among the eight SvGls identified in stevia leaves, key components include stevioside (Stev: 4–13%), rebaudioside-A (Reb-A: 2–4%), rebaudioside-C (Reb-C: 1–2%), and dulcoside (0.4–0.7%) (Afshari et al., 2022).

Stevia cultivation is significantly influenced by environmental conditions and management practices, which determine whether the plant grows annually or exhibits mixed growth patterns (Aghighi Shahverdi et al., 2018). Effective agricultural management promotes vigorous growth and dense branching in stevia bushes (Aghighi Shahverdi et al., 2020; Schiatti-Sisó et al., 2023). However, water scarcity, particularly in semi-arid and arid regions such as Iran, poses a major challenge. Limited annual precipitation exacerbates agricultural complexities, which are further intensified by population growth and the resulting demand for increased food production (Afshari et al., 2022).

Addressing water scarcity through innovative strategies has become a critical priority in academic research, aiming to ensure sustainable agricultural productivity for growing populations (Bakhoum et al., 2023). Drought stress, which affects approximately 25% of the Earth's surface, leads to reduced germination, stunted growth, lower yields, and alterations in metabolite synthesis (El-Bassiouny et al., 2023). Employing ameliorating agents as agronomic tools is a recognized approach to mitigate environmental stresses. These agents, often applied through foliar nutrition, can enhance plant growth and the accumulation of active components (Mahajan et al., 2021). Afshari et al. (2022) observed that the optimal SvGls content in stevia occurred under partial water scarcity stress, whereas severe water scarcity significantly reduced secondary metabolite levels. Furthermore, foliar nutrition has been shown to substantially increase metabolite production compared to control conditions (Elewa et al., 2017).

Providing adequate nutrition during stressful conditions helps plants cope with various forms of stress (Wahab et al., 2022). Selenium (Se), a beneficial element with oxidative and anticancer properties vital for human and animal health, exists as a quasi-metal in the environment (Aghighi Shahverdi et al., 2018). Plants primarily absorb Se through their roots in the forms of selenates (SeO_4^{2-}) and selenites (SeO_3^{2-}) (Babajani et al., 2019). At low concentrations, Se benefits plant growth and enhances stress tolerance (Borbély et al., 2021). Foliar fertilization with Se, either alone or in combination with iron, has been shown to alleviate the adverse effects of salinity stress on stevia growth and physiological traits (Aghighi Shahverdi et al., 2018). Interestingly, Borbély et al. (2021) reported that applying Se as a foliar spray decreased stevioside (Stev) and rebaudioside-A (Reb-A) concentrations while significantly increasing stem length, leaf number, and Se content. Despite numerous studies investigating Se utilization in stevia plants (Aghighi Shahverdi et al., 2018; Borbély et al., 2021), uncertainties persist. Conflicting findings regarding Se's role and the effects of various Se sources indicate the need for further research to achieve conclusive results.

Strigolactones (SLs), a relatively new group of plant growth regulators, were first identified in the parasitic plant striga. SLs reduce the abscisic acid-to-gibberellin ratio, enhancing seed germination in plants (Bhoi et al., 2021). Research has highlighted several roles of SLs, including suppressing excessive shoot branching by inhibiting axillary bud growth, promoting symbiosis with arbuscular mycorrhizal fungi, and influencing root system architecture (Sedaghat et al., 2017). Sedaghat et al. (2021) further demonstrated that SL applications improve photosynthesis and plant performance under stress by combining drought tolerance mechanisms and escape strategies. Foliar application of SLs enhances drought tolerance by reducing membrane leakage and increasing relative water content, membrane stability index, and antioxidant enzyme activity (Sedaghat et al., 2017). These properties make SLs a promising tool for managing drought stress, particularly in wheat plants.

Forchlorfenuron (N-(2-chloro-4-pyridyl)-N′-phenylurea), commonly known as CPPU, is an artificial cytokinin that, when combined with auxin, stimulates cell division and subsequent growth (Roussos et al., 2021). This phenylurea cytokinin exhibits physiological activity, inducing seed formation, promoting shoot regeneration, stimulating seed germination and fruit growth, and delaying leaf senescence (Singh et al., 2019). Cytokinin applications through foliar sprays are known to enhance stevia growth, yield, physiological performance, and overall quality. Forchlorfenuron is widely used in horticultural and medicinal plants to boost secondary metabolite production. For instance, it has been shown to increase catalpol and verbascoside levels in *Siraitia grosvenorii* glands (Shi et al., 2019) and schisandrol content in *Schisandra chinensis* (Song et al., 2014).

This study investigates the effects of water scarcity, Se, and plant growth regulators on yield and biochemical responses in stevia plants. It examines the quantification of SvGls, leaf yield, physiological parameters, and biochemical compositions in Indian and Chinese stevia genotypes across various irrigation treatments and climatic conditions in Golestan, Tehran, and Fars provinces of Iran. The findings aim to optimize cultivation practices to improve productivity and quality in stevia. Given the high value of SvGls in the food and pharmaceutical industries, along with the significant costs associated with synthetic production, identifying methods to enhance the natural production of these compounds at minimal cost is of considerable importance.

Materials and Methods

Experimental design and plant materials

This study was conducted using a split-split-plot factorial design within the framework of a randomized complete block design (RCBD), with three replications in three distinct climatic regions of Iran. The regions included Varamin in Tehran (ES-1: 35.33° N, 51.65° E), Firuzabad in Fars (ES-2: 28.85° N, 52.59° E), and Gorgan in Golestan (ES-3: 36.85° N, 54.44° E), each characterized by unique climatic conditions under field conditions (Table 1).

The main plots consisted of irrigation treatments, while subplots included foliar applications of nutritional elements and plant growth regulators. These treatments were applied across two stevia genotypes during the 2021–2022 growing season. Irrigation treatments were administered at two levels: 100% field capacity, representing normal irrigation without stress, and 25 $\pm$ 5% of field capacity, representing severe drought stress (Afshari et al., 2022).

The foliar spray treatments encompassed selenium (Se) compounds and plant growth regulators at six levels: no foliar spray (Ctrl), CPPU (forchlorfenuron), strigolactones (SLs), selenium (Se), CPPU + SLs, and Se + CPPU + SLs. The two stevia genotypes, 'Chinese' and 'Indian,' were treated as sub-subplots. The overall experimental design included a total of 72 plots for each region.

Soil samples were taken from a depth of zero to 30 cm before sowing in the field to analyze their physicochemical properties (Table 2). The procedure proposed by Carter and Gregorich (2007) was utilized to ascertain the concentration of soil elements and various soil characteristics. All of the essential micro- and macro-nutrients were present at levels deemed adequate to support the growth of plants (NPK: 75, 45, 40 kg ha^{-1}) (Verma et al., 2020). The main plots, each measuring 20 × 14 m, were separated 2 m apart from each other. Each main plot was subdivided into 12 smaller ones, each measuring 135 cm × 90 cm, with distances between subplots set at 0.5 and 1 m.

The stevia seeds (both species) were sourced from the 'Zargiah Herbal Field' in Firuzabad, Iran. The average seed moisture content ranged between 8.0% and 8.40%, and the 1000-seed dry weight was approximately 25.0 $\pm$ 0.27 mg. For seed germination, the seeds were mixed with clay soil and evenly distributed in a substrate consisting of clay soil, decomposed manure, and coco peat in equal proportions. Irrigation was carried out using misting in the greenhouse to maintain humidity levels between 60% and 65% and temperatures ranging from 23 to 25 °C, with watering every 12 h.

Table 1. Geographical and climatic characteristics of the experimental sites in Iran.

Experimental sites	Latitude	Longitude	Elevation (m.a.s.l.)	Avg. Precipitation (mm)	Avg. Temperature (°C)
Varamin (ES-1)	35.33° N	51.65° E	905	220	16
Firuzabad (ES-2)	36.85° N	54.44° E	5	600	19
Gorgan (ES-3)	28.85° N	52.59° E	1387	250	17

Table 2. Physical and chemical characteristics of the soil at the experimental sites (Gorgan, Firuzabad, and Varamin, Iran) studied through samples collected from soils at a depth of 0-30 cm.

Characteristic	Varamin	Firuzabad	Gorgan
Texture	Sandy Loam	Loam	Sandy Loam
Sand (2-0.05 mm, %)	75	30.1	76
Silt (0.05-0.002 mm, %)	13	43.3	10
Clay (< 0.002 mm, %)	12	25.4	14
pH	7.27	7.44	7.51
Electrical conductivity in saturated extract (dS m^{-1})	1.06	1.54	1.58
Organic matter (%)	0.32	0.78	0.81
Nitrogen (ppm of soil)	1.47	1.88	1.86
Phosphorus extractable with sodium bicarbonate (ppm of soil)	9.05	8.71	9.11
Potassium extractable (ppm of soil)	302	318	315
Iron extractable with DTPA (ppm of soil)	1.42	1.87	1.95
Manganese extractable with DTPA (ppm of soil)	2.14	3.28	3.42
Copper extractable with DTPA (ppm of soil)	0.27	0.50	0.59
Zinc extractable with DTPA (ppm of soil)	0.59	0.49	0.67

After germination, the stevia seedlings were cultivated in sand beds and subsequently transplanted into the fields at three months of age during the four-leaf stage. Transplantation was performed manually, maintaining a spacing of 45 × 45 cm between plants to achieve a planting density of approximately 50,000 plants ha−1, in line with standard practices in stevia cultivation (Aghighi Shahverdi et al., 2018). Planting in ES-1 and ES-2 occurred in late March, while planting in ES-3 was conducted in early April.

A drip irrigation system utilizing irrigation drip tape was implemented for the study. To examine the irrigation treatments, soil samples were collected from the field and analyzed in the laboratory to determine the field capacity (FC) and permanent wilting point (PWP). The water requirements for each experimental plot under standard conditions (FC) were calculated using the formula for accessible water (Eq. 1). The calculated value was then multiplied by 90 and 25 to determine the water volume required for treatments at 90% and 25% FC, respectively, following the methodology described by Kharazmi et al. (2019). The formula for calculating readily available water (RAW) is expressed as follows.

$$RAW = (100\, FC - PWP\ \times p.b) \times D\ \times MAD$$
(Equation 1)

where RAW, FC, PWP, BD, D, and MAD represent readily available water, field capacity, permanent wilting point, soil bulk density, root development depth (mm), and maximum allowable depletion (set at 0.55 for stevia), respectively. The total water usage during the growing season for the 100% FC irrigation strategy amounted to 5400, 5600, and 6000 m^3 ha^{-1} for ES-2, ES-3, and ES-1, respectively. For the 25% FC irrigation strategy, the corresponding water usage was 2000, 2100, and 2200 m^3 ha^{-1} at the same experimental sites (Afshari et al., 2022).

Based on prior research on selenium (Se) application in various contexts and the lack of studies evaluating the combined effects of two Se compounds on stevia plants, sodium selenite and sodium selenate were selected for this study to identify the most effective Se source. Foliar treatments were applied during two growth stages: the branching stage (V3: 31–40 d after planting) and the regrowth stage (V4: 41–50 d after planting), corresponding to BBCH scale values of 30 and 40 (20 to 25 nodes on the main stem), respectively. Treatments were administered from early morning to afternoon (Aghighi Shahverdi et al., 2018; Afshari et al., 2022).

Selenium was supplied as a combination of sodium selenite and sodium selenate at a rate of 10 g ha^{-1}. CPPU was applied at a concentration of 20 mg L^{-1}, while strigolactones (SLs) were administered at 0.01 mg L^{-1}, based on optimized protocols from previous studies (Zeng et al., 2016; Aghighi Shahverdi et al., 2018; Luqman et al., 2023). All compounds used in the study were sourced from Merck, Germany. To enhance nutrient absorption through foliage, a surfactant (Break-Thru S 233, a patented non-ionic trisiloxane surfactant from Evonik Industries AG, Essen, Germany) was incorporated at 0.1%. The surfactant reduced the surface tension of the spray solution, improving its interaction with the plant foliage and optimizing absorption (Afshari et al., 2022).

Sampling and measuring biochemical and physiological traits

Young, developed leaves were collected from each plot after 68-73 d (55 on the BBCH scale) to analyze biochemical and physiological characteristics.

Photosynthetic pigments

The levels of photosynthetic pigments, including chlorophyll a and b, were determined using a method described by Lichtenthaler and Buschmann (2001). Fresh tissue samples (0.25 g) were extracted with 5 mL of 80% acetone and then centrifuged at 11,000 rpm for 10 min. The optical density (OD) of the resulting extract was measured using a Perkin Elmer Lambda 25 spectrophotometer (USA) at wavelengths of 645 nm and 663 nm to calculate the concentrations of the photosynthetic pigments. The following equations were then used to determine the pigment content.

$$Chlorophyll\ a\ (\mu g\ g^{-1}\ FW) = \frac{12.7\ (OD_{663}) - 2.69\ (OD_{645}) \times V}{W \times 1000}$$
(Equation 2)

$$Chlorophyll\ b\ (\mu g\ g^{-1}\ FW) = \frac{22.9\ (OD_{645}) - 4.68\ (OD_{663}) \times V}{W \times 1000}$$
(Equation 3)

Where W, V, and OD represent the fresh weight of the extracted tissue (g), final volume of the extract in 80% acetone, and optical density at a specific wavelength, respectively.

Proline content

Free proline content was determined by employing a method proposed by Bates et al. (1973). Thus, 0.5 mg of leaf material was homogenized in a sulfosalicylic acid solution, heated, and mixed with toluene. The resulting supernatant was used for measuring proline concentration via a spectrophotometer at 520 nm.

Sampling and measuring plant yield

The plants were harvested 75–79 d after planting (corresponding to 60 on the BBCH scale) to evaluate leaf yield. For this purpose, three plants were randomly selected from each experimental plot, and plant yield was assessed using fresh leaf weight as the primary yield criterion (Aghighi Shahverdi et al., 2018).

Steviol glycosides content and composition

A relevant method, with minor adjustments, was used for determining the SvGls content in stevia leaves (Aghighi Shahverdi et al., 2018; Afshari et al., 2022). Dried samples were processed in a hot air oven at 45 $\pm$ 3 °C for 48 h and then pulverized using a mill machine fitted with a 3 mm sieve. To measure the SvGls compositions, 100 mg of the powdered samples was extracted with 10 mL of water at 50 °C for 30 min. After centrifugation (7,000 rpm for 5 min) and filtration through a 0.2 µm membrane, 20 µL of the resulting solution was injected into a high-performance liquid chromatography (HPLC) system equipped with a C18 column (250 × 4.6 mm inner diameter, 5 µm particle size; Luna C18, Phenomenex, USA).

The SvGls were eluted using an isocratic phase of acetonitrile (ACN) 31% and water (pH 2.6, adjusted with formic acid) 69% at a flow rate of 1 mL min^{-1} for 30 min. The compositions of SvGls, including Stev and Reb-A, were detected at a wavelength of 200 nm. Stev and Reb-A standards were used for quantification. The results were expressed as a percentage of dry mass for total SvGls content and as a percentage of total SvGls for individual components. The SvGls yield was calculated by multiplying the plant leaf yield by the SvGls content.

Statistical analysis

SAS software (Statistical Analysis Software, version 9.2) was used for analyzing the data after assessing the normality of data distribution using the Kolmogorov-Smirnov and Shapiro-Wilk tests. A three-factor factorial ANOVA, based on a split-split-plot design, was performed separately for each experimental site to estimate the variance components related to drought stress, foliar nutrition, genotype effects, and their interactions. Differences among treatments were analyzed using Duncan's Multiple Range Test (DMRT) when the ANOVA F-test indicated statistical significance at the 0.05 level. Pearson correlation analysis between characteristics was conducted using Microsoft Excel 2013 and Minitab 18 software.

Results

Effect on photosynthetic pigments

The results revealed a significant reduction in photosynthetic pigments, including chlorophyll a and b, under drought stress across all experimental sites. However, various foliar spray treatments led to an increase in the mean values of these pigments compared to the control. As shown in Table 3, the Indian genotype consistently exhibited higher levels of chlorophyll a and b across the experimental sites when compared to the other stevia genotype.

The experimental site significantly influenced the effectiveness of foliar spray treatments. The highest chlorophyll a and b content in ES-1, ES-2, and ES-3 was observed in the Indian genotype when treated with an integrated application of selenium (Se), CPPU, and strigolactones (SLs).

Under non-stress conditions, this integrated treatment demonstrated high efficacy, leading to a notable increase in chlorophyll a content in the Indian genotype across all experimental sites. In contrast, the absence of foliar spray under non-stress conditions resulted in the lowest chlorophyll a content in the Chinese genotype, with values of 8.0, 5.76, and 5.52 μg g^{-1} FW in ES-1, ES-2, and ES-3, respectively (Fig. 1).

Table 3. Effect of drought stress and foliar nutrition on photosynthetic pigments and proline content of two stevia genotypes (Indian and Chinese) in experimental sites of Varamin (ES-1), Firuzabad (ES-2), and Gorgan (ES-3).

Treatments	Chlorophyll *a* (μg g^{-1} FW)			Chlorophyll *b* (μg g^{-1} FW)		
Genotypes (G)	ES-1	ES-2	ES-3	ES-1	ES-2	ES-3
Indian	16.02±3.29^{a}	18.65±5^{a}	22.53±7.31^{a}	6.45±2.62^{a}	7.58±3.35^{a}	12.07±6.39^{a}
Chinese	14.26±3.94^{b}	12.51±4.37^{b}	14.68±6.04^{b}	5.48±1.98^{b}	4.75±1.86^{b}	5.54±2.43^{b}
Drought stress (D)						
Non-stress	17.48±3.32^{a}	18.81±5.21^{a}	21.32±7.19^{a}	6.73±2.25^{a}	7.3±3.07^{a}	11.08±6.32^{a}
Stress	12.79±2.39^{b}	12.34±3.86^{b}	15.89±7.39^{b}	5.2±2.23^{b}	5.03±2.61^{b}	6.54±4.24^{b}
Foliar nutrition (FN)						
Ctrl	13.8±4.99^{c}	11.39±5.64^{d}	11.07±5.16^{d}	5.52±2.99^{c}	4.45±2.81^{c}	5.25±4.33^{d}
Forchlorfenuron (CPPU)	15.59±2.43^{ab}	15.73±4.23^{c}	17.74±5^{c}	6.02±2.32^{ab}	6.01±2.63^{b}	7.92±4.64^{c}
Strigolactone (SLs)	15.68±4.29^{ab}	17.43±5.56^{ab}	22.24±8.32^{b}	6.39±2.12^{ab}	7.18±2.87^{a}	10.66±5.49^{bc}
Selenium (Se)	14.99±4.31^{bc}	16.71±6.3^{b}	20.78±7.25^{b}	6.31±3.19^{ab}	7.19±4.59^{a}	10.82±8.82^{b}
CPPU + SLs	14.93±3.98^{bc}	16.96±6.98^{b}	22.19±8.34^{b}	5.99±1.95^{bc}	6.66±2.54^{ab}	10.57±5.24^{bc}
Se + CPPU + SLs	16.47±4.08^{a}	18.48±6.34^{a}	26.6±9.62^{a}	6.54±2.22^{a}	7.29±2.95^{a}	12.54±6.55^{a}
Interaction effects						
D × G	ns	**	**	**	**	**
D × FN	**	**	**	**	**	**
G × FN	**	**	**	*	**	**
D × G × FN	**	**	**	*	**	**

ns, *, and ** are non-significant, significant at the 5% probability level, and significant at the 1% probability level, respectively. The averages presented in each column, sharing similar letters, do not have a statistically significant difference based on Duncan's multiple range test at the 1% significance level.

Selenium application under non-drought stress conditions produced the highest chlorophyll b content in the Indian genotype, with averages of 10.95, 14.45, and 25.02 μg g^{-1} FW in ES-1, ES-2, and ES-3, respectively. However, under drought stress, the integrated foliar spray of Se, CPPU, and SLs resulted in a comparatively smaller reduction in chlorophyll b content in the Indian genotype than other treatments. The lowest chlorophyll b content under drought stress was observed in the no foliar spray treatment for the Indian genotype, with values of 1.53, 1.32, and 1.41 μg g^{-1} FW in ES-1, ES-2, and ES-3, respectively (Fig. 1).

Effect on proline content

Drought stress significantly increased proline levels compared to non-stress conditions, with the Chinese genotype consistently exhibiting higher proline content than the Indian genotype across all experimental sites. Foliar spray treatments notably influenced amino acid content, particularly at ES-2 and ES-3 (Table 4). Among the treatments, the combined application of selenium (Se), strigolactones (SLs), and CPPU under drought stress conditions in the Chinese genotype resulted in the highest proline content across all experimental sites. The lowest proline content (1.78 μmol g^{-1} FW) was recorded in ES-1, associated with selenium application under non-drought stress conditions in the Indian genotype. Additionally, the Indian genotype showed the lowest proline content under non-drought stress and no foliar spray conditions in ES-2 and ES-3, with averages of 1.60 and 1.15 μmol g^{-1} FW, respectively (Fig. 2A).

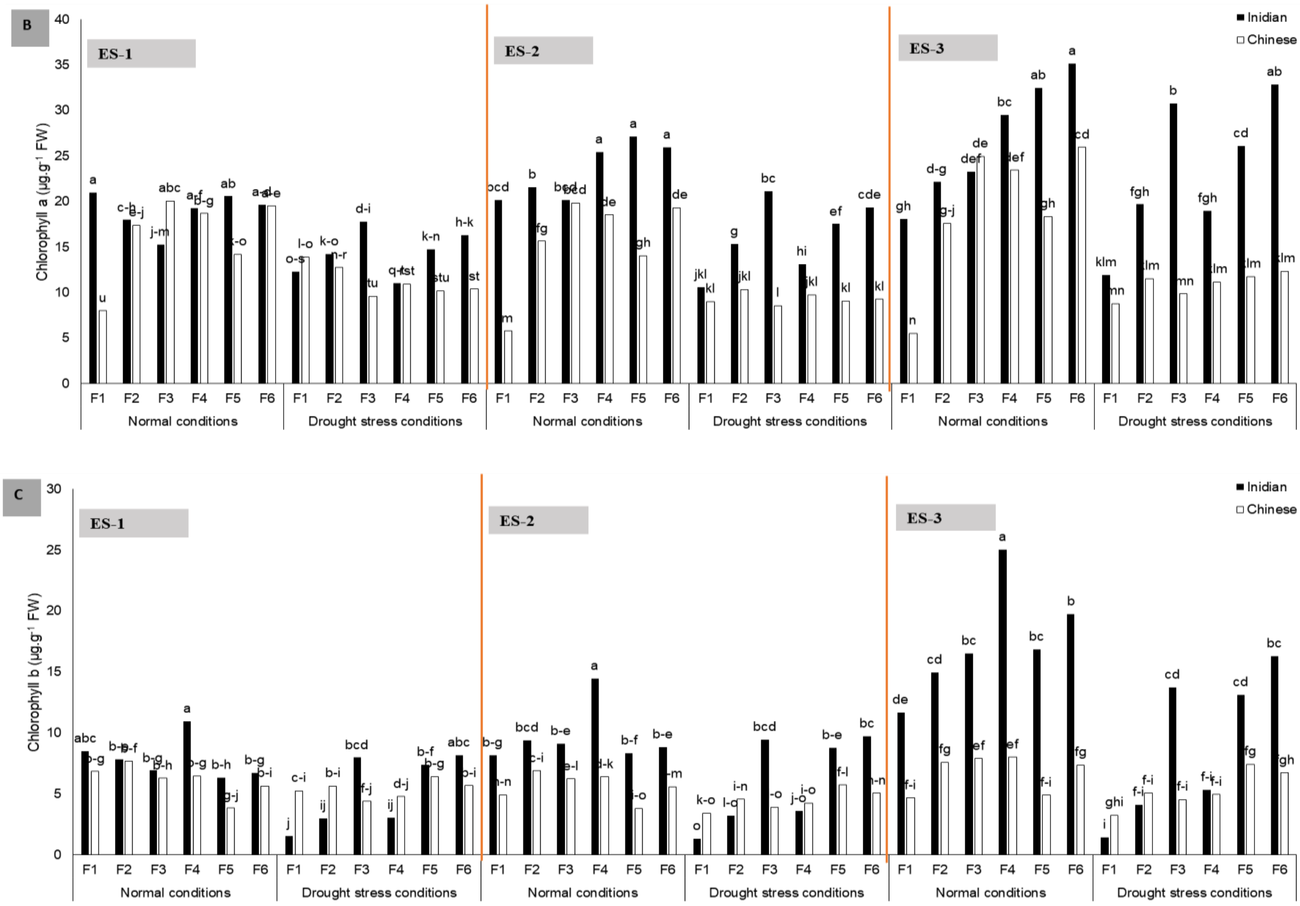

Fig. 1. Interaction effects of drought stress and foliar spray (F1-F6) with various compounds on chlorophyll *a* and chlorophyll *b* content in two stevia genotypes (Indian and Chinese) at the experimental sites in Varamin (ES-1), Firuzabad (ES-2), and Gorgan (ES-3), Iran. In each experimental site, the means (± SE) with similar letters do not have a significant difference at the 5% probability level according to Duncan's multiple range test (DMRT). F1: non-application (Ctrl), F2: forchlorfenuron (CPPU), F3: strigolactones (SLs), F4: selenium (Se), F5: forchlorfenuron (CPPU) + strigolactones (SLs), F6: selenium (Se) + forchlorfenuron (CPPU) + strigolactones (SLs).

Effect on leaf yield

Leaf weight is a crucial index for plant yield in stevia cultivation due to the economic importance of harvested leaves. The leaf yield was significantly influenced by drought stress, foliar spray treatments, genotype, and their interactions across the experimental sites. Drought stress consistently reduced leaf yield, whereas foliar spray treatments increased it compared to the control. Data analysis revealed that the Indian genotype consistently outperformed the Chinese genotype in leaf yield across all experimental sites.

Among the sites, the highest average leaf yield was recorded at ES-3, while ES-1 exhibited the lowest. The superior leaf yield was observed in the Indian genotype treated with SLs under non-drought conditions, with averages of 1249.3, 2061.2, and 2982.2 kg ha^{-1} in ES-1, ES-2, and ES-3, respectively. Additionally, the combined application of Se, SLs, and CPPU under drought stress significantly enhanced leaf yield in the Indian genotype. In contrast, the absence of foliar spray under drought stress conditions in the Chinese genotype resulted in the lowest leaf yield, with average values of 112.4, 100.2, and 164.8 kg ha^{-1} at ES-1, ES-2, and ES-3, respectively (Fig. 2B).

Effect on SvGls content and yield

The results revealed significant effects of drought stress at ES-1, genotype at ES-1 and ES-2, and foliar spray, as well as the interactive influence of drought stress, foliar spray, and genotype across the experimental sites on Reb-A content. Drought stress increased Reb-A content at ES-1, while causing a non-significant decrease at ES-2 and ES-3. The Indian genotype showed the highest average Reb-A content at ES-1, whereas the Chinese genotype exhibited this trait most prominently at ES-2. No significant difference in Reb-A content was observed between the two genotypes at ES-3.

Table 4. Effect of drought stress and foliar nutrition on proline content and plant yield of two stevia genotypes (Indian and Chinese) in experimental sites of Varamin (ES-1), Firuzabad (ES-2), and Gorgan (ES-3).

Treatments	Proline (μmol g^{-1} FW)			Plant yield (kg ha^{-1})		
Genotypes (G)	ES-1	ES-2	ES-3	ES-1	ES-2	ES-3
Indian	2.48±0.55^{b}	1.71±0.45^{b}	2.32±0.73^{b}	532.51±292.08^{a}	779.01±465.73^{a}	1078±713.13^{a}
Chinese	2.92±0.6^{a}	2.71±0.73^{a}	3.15±1^{a}	389.34±269.34^{b}	348.08±264.3^{b}	574.44±460.94^{b}
Drought stress (D)						
Non-stress	2.42±0.49^{b}	1.89±0.63^{b}	2.37±0.85^{b}	557.02±306.45^{a}	691.72±463.4^{a}	1028.3±689.04^{a}
Stress	2.97±0.61^{a}	2.53±0.8^{a}	3.1±0.94^{a}	364.84±235.75^{b}	435.37±364.88^{b}	624.15±540.56^{b}
Foliar nutrition (FN)						
Ctrl	2.55±0.57^{a}	1.63±0.49^{c}	1.67±0.4^{d}	389.43±227.28^{c}	389.6±311.94^{d}	457.53±339.37^{d}
Forchlorfenuron (CPPU)	2.64±0.42^{a}	2.12±0.6^{bc}	2.5±0.64^{c}	419.07±208.33^{b}	467.58±263.78^{c}	664.4±388.81^{cd}
Strigolactone (SLs)	2.71±0.71^{a}	2.41±0.81^{abc}	3.12±0.91^{b}	592.59±482.95^{a}	823.63±809.07^{a}	1248.8±1160.1^{a}
Selenium (Se)	2.83±0.85^{a}	2.57±1.13^{a}	3.28±1.16^{ab}	547.06±252.93^{ab}	720.81±383.74^{ab}	1115.61±589.0^{b}
CPPU + SLs	2.75±0.76^{a}	2.45±0.97^{ab}	3.32±1.05^{ab}	428.48±243.14^{ab}	564.15±378.75^{b}	893.6±558.86^{bc}
Se + CPPU + SLs	2.69±0.37^{a}	2.38±0.68^{abc}	3.5±0.62^{a}	547.06±252.93^{ab}	720.81±383.74^{ab}	1260.54±646.18^{a}
Interaction effects						
D × G	ns	*	ns	**	ns	*
D × FN	**	**	*	**	**	**
G × FN	**	**	**	*	**	**
D × G × FN	**	**	**	**	**	**

ns, *, and ** are non-significant, significant at the 5% probability level, and significant at the 1% probability level, respectively. The averages presented in each column, sharing similar letters, do not have a statistically significant difference based on Duncan's multiple range test at the 1% significance level.

Foliar spray treatments contributed to an elevated average Reb-A content compared to the control across all experimental sites (Table 5). The highest Reb-A content was recorded at ES-1 in the Indian genotype under drought stress conditions, associated with foliar spray treatments containing CPPU and Se, with averages of 2.91% and 2.98%, respectively. In contrast, foliar spray applications with SLs under non-stress conditions in the Chinese genotype resulted in Reb-A content averages of 2.82% at ES-2 and 3.59% at ES-3. The absence of foliar spray under drought stress conditions in the Indian genotype led to the lowest Reb-A content at ES-2 and ES-3, with values of 1.11% and 1.07%, respectively. Additionally, the minimum Reb-A content at ES-1 was observed in the Chinese genotype treated with Se under non-stress conditions, with an average of 1.48% (Fig. 3A).

Drought stress exhibited an augmentative effect on Stev content at ES-1 but a diminishing impact at ES-2 and ES-3. Stev levels were higher in the Indian genotype at ES-1 and ES-3, while the Chinese genotype showed greater levels at ES-2 (Table 5). The application of foliar sprays with SLs and Se, particularly under drought stress conditions, resulted in the highest Stev content (8.84% and 8.60%, respectively) in the Indian genotype at ES-1. Conversely, foliar sprays with SLs under non-stress conditions in the Chinese genotype produced the highest Stev content at ES-2 and ES-3, with values of 8.31% and 10.49%, respectively.

The absence of foliar spray under drought stress conditions in the Chinese genotype led to the lowest Stev content at ES-1 and ES-2, with values of 2.26% and 2.21%, respectively. The interactive effects highlighted that, irrespective of stress conditions, the Chinese genotype consistently outperformed the Indian genotype in Stev content (Fig. 3B).

The results demonstrated significant variations in SvGls content, including Stev and Reb-A, influenced by drought stress, foliar spraying, and genotype across the experimental sites. Drought stress reduced SvGls content at ES-1 but increased it at ES-2 and ES-3. The application of foliar sprays with various combinations enhanced the average SvGls content across all sites. At ES-1, the highest SvGls content was observed when SLs and Se were applied under drought stress conditions in the Indian genotype (11.39% and 11.59%, respectively). Conversely, at ES-2 and ES-3, the Chinese genotype exhibited the highest SvGls content under non-stress conditions when treated with SLs (11.13% and 14.08%, respectively).

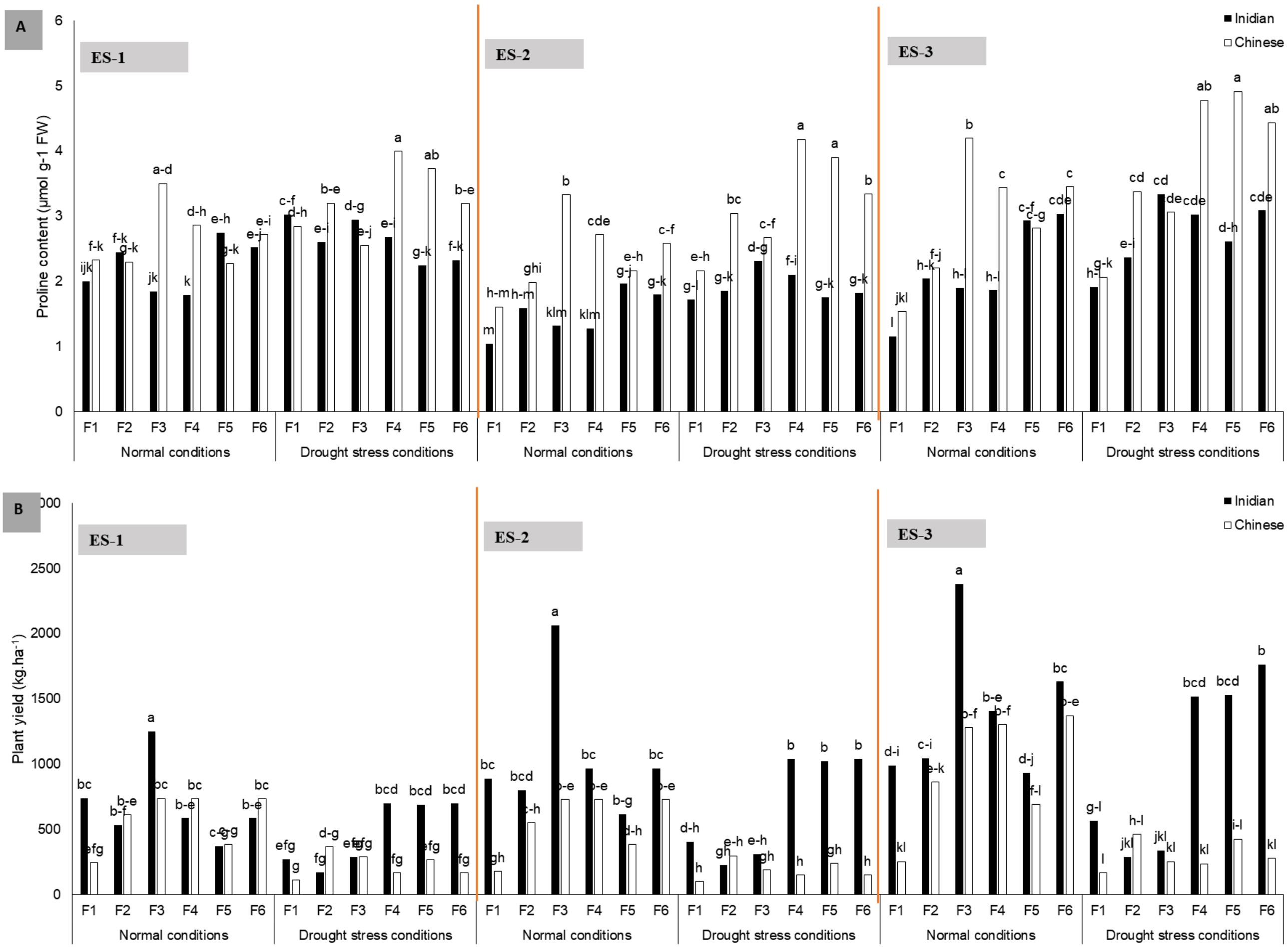

Fig. 2. Interaction effects of drought stress and foliar spray (F1-F6) with various compounds on proline content (A) and plant yield (B) in two stevia genotypes (Indian and Chinese) at the experimental sites in Varamin (ES-1), Firuzabad (ES-2), and Gorgan (ES-3), Iran. In each experimental site, the means (± SE) with similar letters do not have a significant difference at the 5% probability level according to Duncan's multiple range test (DMRT). F1: non-application (Ctrl), F2: forchlorfenuron (CPPU), F3: strigolactones (SLs), F4: selenium (Se), F5: CPPU + SLs, F6: Se + CPPU + SLs.

The lowest SvGls content was recorded in the Chinese genotype under drought stress conditions without foliar spray at all experimental sites (4.89%, 3.38%, and 3.29%, respectively) (Fig. 4A). The SvGls yield, calculated by multiplying plant yield and SvGls content, also showed significant variations driven by drought stress, foliar spraying, genotype, and their interactions across the sites. Drought stress reduced the SvGls yield, whereas foliar spraying significantly increased it (Table 5). The application of SLs under non-drought conditions yielded the highest SvGls levels in the Indian genotype across the experimental sites (97.9, 127.9, and 269.1 kg ha^{-1}, respectively). The Indian genotype generally outperformed the Chinese genotype in SvGls yield across most treatment combinations. Using Se on the Indian genotype under drought stress conditions proved more effective in enhancing SvGls yield compared to other foliar spray treatments. The lowest SvGls yield was observed in the Chinese genotype under drought stress conditions without foliar spraying at all sites (14.0, 6.27, and 8.26 kg ha^{-1}, respectively) (Fig. 4B).

Correlations between traits

Tables 6, 7, and 8 present the results of the simple correlation analysis among traits for ES-1, ES-2, and ES-3, respectively. A significant relationship was observed across the experimental sites between leaf yield and the content of photosynthetic pigments, such as chlorophyll *a* and *b*. Additionally, a positive correlation was identified between Stev and SvGls content and leaf yield at ES-1 and ES-3; however, this correlation was not significant at ES-2. Moreover, a negative correlation was reported between the activity of antioxidant enzymes and the level of the amino acid proline with both leaf yield and SvGls content across the experimental sites.

Table 5. Effect of drought stress and foliar nutrition on Steviol glycoside content and yield of two stevia genotypes (Indian and Chinese) in experimental sites of Varamin (ES-1), Firuzabad (ES-2), and Gorgan (ES-3).

Treatments	Rebaudioside-A content (%)			Stevioside content (%)			Steviol glycoside content (%)			Steviol glycoside yield (kg ha^{-1})		
Genotypes (G)	ES-1	ES-2	ES-3	ES-1	ES-2	ES-3	ES-1	ES-2	ES-3	ES-1	ES-2	ES-3
Indian	2.19 ± 0.41^{a}	1.51 ± 0.3^{b}	2.05 ± 0.59^{a}	6.46 ± 1.33^{a}	4.44 ± 0.94^{b}	6.05 ± 1.77^{a}	8.64 ± 1.71^{a}	5.95 ± 1.22^{b}	8.1 ± 2.34^{a}	45.8 ± 25.78^{a}	47.02 ± 30.33^{a}	93.54 ± 74.15^{a}
Chinese	1.92 ± 0.4^{b}	1.79 ± 0.48^{a}	2.09 ± 0.7^{a}	4.92 ± 1.75^{b}	4.61 ± 1.91^{a}	5.41 ± 2.54^{b}	6.84 ± 2.07^{b}	6.39 ± 2.33^{a}	7.5 ± 3.16^{b}	28.81 ± 26.04^{b}	25.4 ± 25.97^{b}	50.97 ± 56.22^{b}
Drought stress (D)												
Non-stress	1.98 ± 0.34^{b}	1.69 ± 0.48^{a}	2.12 ± 0.64^{a}	5.61 ± 1.39^{b}	4.78 ± 1.61^{a}	6 ± 2.11^{a}	7.59 ± 1.69^{b}	6.47 ± 2.05^{a}	8.12 ± 2.7^{a}	43.76 ± 27.76^{a}	45.14 ± 31.48^{a}	90.34 ± 71.83^{a}
Stress	2.13 ± 0.49^{a}	1.6 ± 0.36^{a}	2.02 ± 0.64^{a}	5.77 ± 2.02^{a}	4.27 ± 1.35^{b}	5.46 ± 2.28^{b}	7.89 ± 2.45^{a}	5.87 ± 1.63^{b}	7.48 ± 2.86^{b}	30.85 ± 25.18^{b}	27.29 ± 26.06^{b}	54.17 ± 61.29^{b}
Foliar nutrition (FN)												
Ctrl	1.8 ± 0.28^{d}	1.13 ± 0.13^{d}	1.18 ± 0.2^{e}	4.85 ± 1.62^{g}	2.97 ± 0.69^{g}	3.15 ± 1.02^{f}	6.65 ± 1.87^{f}	4.1 ± 0.75^{f}	4.33 ± 1.19^{f}	28.83 ± 24.03^{c}	17.95 ± 17.64^{e}	23.02 ± 23.63^{e}
Forchlorfenuron (CPPU)	2.26 ± 0.52^{a}	1.79 ± 0.45^{b}	2.12 ± 0.53^{c}	6.2 ± 1.55^{b}	4.89 ± 1.28^{b}	5.83 ± 1.6^{cd}	8.46 ± 2.03^{b}	6.68 ± 1.7^{b}	7.95 ± 2.11^{cd}	34.46 ± 18.99^{bc}	31.09 ± 18.39^{cd}	54.14 ± 37.53^{c}
Strigolactone (SLs)	2.26 ± 0.43^{a}	1.98 ± 0.53^{a}	2.63 ± 0.74^{a}	6.56 ± 2.05^{a}	5.69 ± 1.88^{a}	7.59 ± 2.59^{a}	8.82 ± 2.43^{a}	7.67 ± 2.36^{a}	10.22 ± 3.26^{a}	53.2 ± 40.2^{a}	61.81 ± 51.26^{a}	131.14 ± 109.18^{a}
Selenium (Se)	2.11 ± 0.67^{abc}	1.79 ± 0.42^{b}	2.39 ± 0.68^{ab}	5.37 ± 2.2^{e}	4.45 ± 1.13^{e}	6.02 ± 2.1^{c}	7.48 ± 2.76^{d}	6.23 ± 1.34^{d}	8.41 ± 2.59^{c}	42.69 ± 27.92^{ab}	47.39 ± 31.16^{b}	102.34 ± 71.94^{b}
CPPU + SLs	1.96 ± 0.29^{bcd}	1.71 ± 0.34^{b}	2.37 ± 0.39^{b}	5.58 ± 1.78^{d}	4.81 ± 1.65^{bc}	6.68 ± 2.09^{b}	7.54 ± 2.03^{d}	6.53 ± 1.9^{bc}	9.04 ± 2.38^{b}	31.08 ± 17.56^{bc}	33.36 ± 19.43^{c}	76 ± 45.31^{c}
Se + CPPU + SLs	1.95 ± 0.32^{bcd}	1.69 ± 0.28^{b}	2.53 ± 0.34^{ab}	5.64 ± 1.43^{d}	4.92 ± 1.45^{b}	7.38 ± 1.95^{a}	7.59 ± 1.69^{d}	6.61 ± 1.7^{b}	9.91 ± 2.26^{a}	44.71 ± 25.14^{ab}	49.09 ± 27.18^{b}	134.32 ± 76.21^{a}
Interaction effects												
D × G	**	**	**	**	**	**	**	**	**	**	**	**
D × FN	**	**	**	**	**	**	**	**	**	**	**	**
G × FN	**	**	*	**	**	**	ns	**	**	**	**	**
D × G × FN	**	**	**	**	**	**	**	**	**	**	**	**

ns, *, and ** are non-significant, significant at the 5% probability level, and significant at the 1% probability level, respectively. The averages presented in each column, sharing similar letters, do not have a statistically significant difference based on Duncan's multiple range test at the 1% significance level.

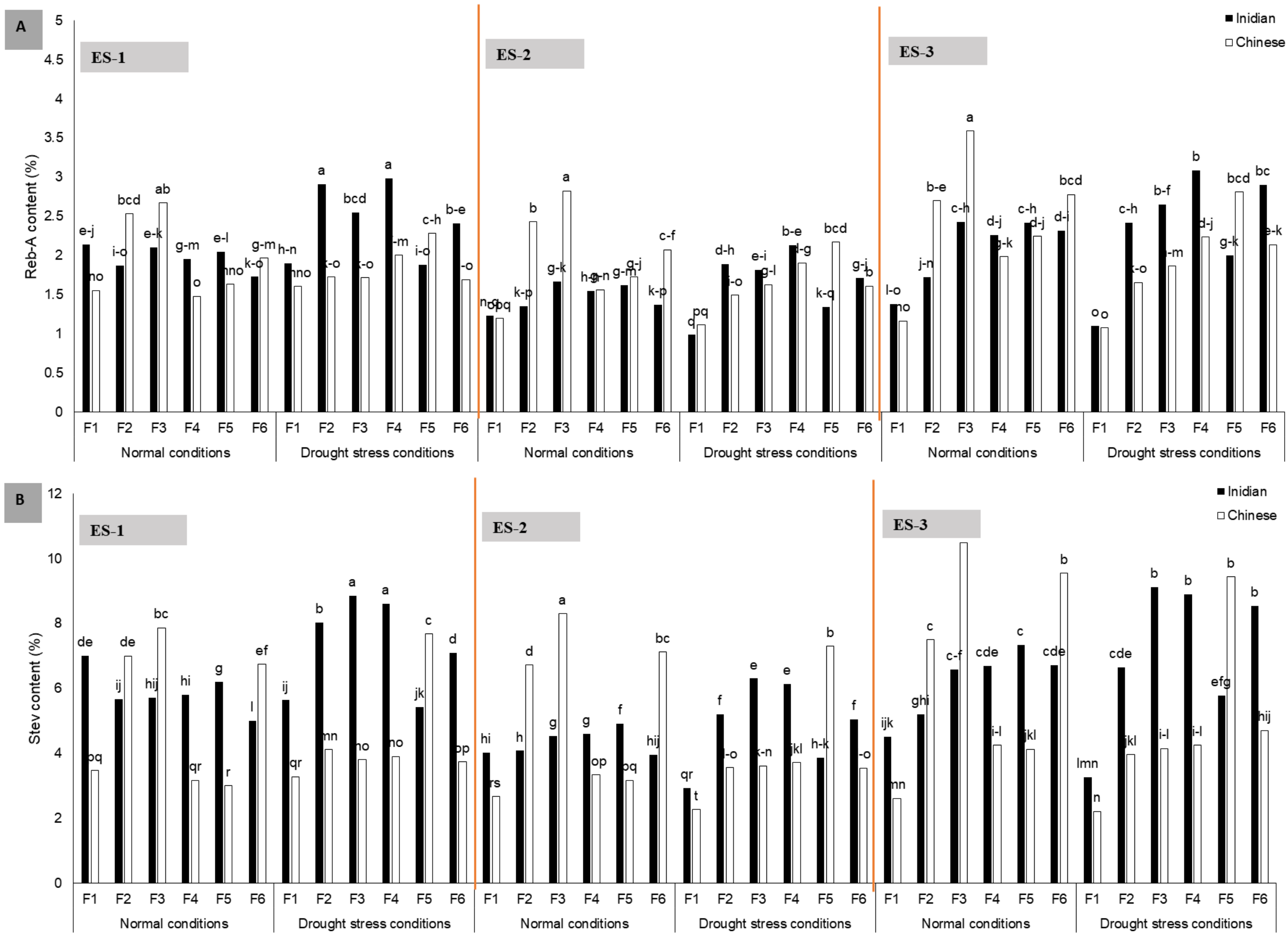

Fig. 3. Interaction effects of drought stress and foliar spray (F1-F6) with various compounds on Reb-A content (A) and Stev content (B) in stevia genotypes (Indian and Chinese) at the experimental sites in Varamin (ES-1), Firuzabad (ES-2), and Gorgan (ES-3), Iran. In each experimental site, the means (± SE) with similar letters do not have a significant difference at the 5% probability level according to Duncan's multiple range test (DMRT). F1: non-application (Ctrl), F2: forchlorfenuron (CPPU), F3: strigolactones (SLs), F4: selenium (Se), F5: forchlorfenuron (CPPU) + strigolactones (SLs), F6: selenium (Se) + forchlorfenuron (CPPU) + strigolactones (SLs).

Discussion

The results indicated that drought stress significantly reduced the mean values of leaf yield and photosynthetic pigment content across the experimental sites. Similar declines in yield parameters and photosynthetic pigment levels have been documented in various plant species under drought stress, including stevia (Afshari et al., 2022) and sesame (Mahdavi Khorami et al., 2020). This reduction in plant growth and yield under drought stress is primarily attributed to alterations in specific enzymes and plant growth regulators (Wahab et al., 2022). Furthermore, oxidative damage caused by the excessive production of reactive oxygen species (ROS) and a decrease in the RWC of plant tissues exacerbate these effects. Reduced tissue water content lowers cell turgor pressure, thereby impeding cell enlargement and division, which in turn restrains overall plant growth (Mahdavi Khorami et al., 2020).

Drought stress also caused a significant reduction in photosynthetic pigments, including chlorophyll *a* and *b*. This decline in physiological factors, particularly chlorophyll content and photosynthetic pigments, plays a crucial role in the observed reduction in plant yield under drought stress. Scholarly studies suggest that diminished photosynthetic pigment content, combined with increased antioxidant enzyme activity and elevated synthesis of osmoprotectants such as proline, collectively contribute to the physiological basis for reduced growth and yield in stevia (Afshari et al., 2022).

The combined application of foliar compounds was notably more effective than individual treatments in mitigating drought stress effects and enhancing plant yield.

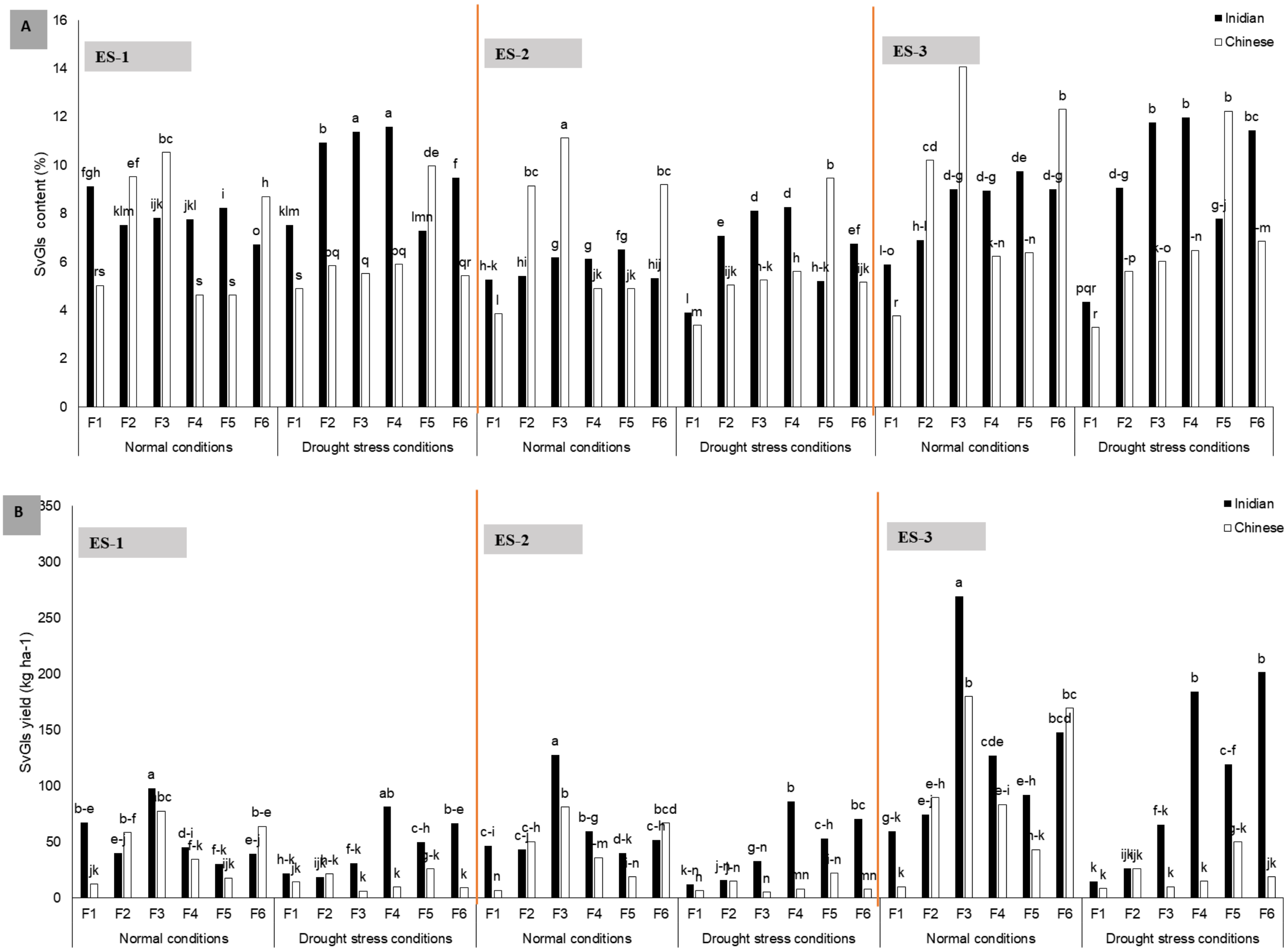

Fig. 4. Interaction effects of drought stress and foliar spray (F1-F6) with various compounds on SvGls content (A), and SvGls yield (B) in stevia genotypes (Indian and Chinese) at the experimental sites in Varamin (ES-1), Firuzabad (ES-2), and Gorgan (ES-3), Iran. In each experimental site, the means (± SE) with similar letters do not have a significant difference at the 5% probability level according to Duncan's multiple range test (DMRT). F1: non-application (Ctrl), F2: forchlorfenuron (CPPU), F3: strigolactones (SLs), F4: selenium (Se), F5: forchlorfenuron (CPPU) + strigolactones (SLs), F6: selenium (Se) + forchlorfenuron (CPPU) + strigolactones (SLs).

Table 6. Simple correlation between physiological, biochemical, and plant yield traits and quality of two stevia genotypes under drought stress and foliar spraying in Varamin (ES-1), Tehran, Iran.

	Plant yield	Chlorophyll *a*	Chlorphyll *b*	Proline	Rebaudioside-A	Stevioside	Steviol glycosides content
Chlorophyll *a*	0.52					(ES-1)	
Chlorphyll *b*	0.37	0.5					
Proline	-0.42	-0.41	-0.49				
Rebaudioside-A	0.24	0.06	-0.14	0.12			
Stevioside	0.33	0.25	0.02	0.01	0.91		
Steviol glycosides content	0.32	0.21	-0.01	0.03	0.94	0.99	
Steviol glycosides yield	0.93	0.46	0.28	-0.33	0.54	0.62	0.61

Coefficients higher than 0.50: Significant at the 1% probability level (**). Coefficients between 0.49-0.30: significant at the 5% probability level (*). Coefficients lower than 0.29: Non-significant (ns). Chlorophyll *a* (Chl-*a*), Chlorphyll *b* (Chl-*b*), Rebaudioside-A (Reb-A, Stevioside (Stev), Steviol glycosides (SvGls).

Table 7. Simple correlation between physiological, biochemical, and plant yield traits and quality of two stevia genotypes under drought stress and foliar spraying in Firuzabad (ES-2), Fars, Iran.

	Plant yield	Chl-*a*	Chl-*b*	Proline	Reb-A	Stev	SvGls content
Chlorphyll *a*	0.63						
Chlorphyll *b*	0.58	0.77				(ES-2)	
Proline	-0.49	-0.49	-0.47				
Rebaudioside-A	0.07	0.07	-0.09	0.46			
Stevioside	0.22	0.28	0.09	0.21	0.88		
Steviol glycosides content	0.19	0.24	0.05	0.27	0.92	0.99	
Steviol glycosides yield	0.93	0.59	0.47	-0.32	0.39	0.53	0.51

Coefficients higher than 0.50: significant at the 1% probability level (**). Coefficients between 0.49-0.30: significant at the 5% probability level (*). Coefficients lower than 0.29: non-significant (ns). Chlorophyll *a* (Chl-*a*), Chlorphyll *b* (Chl-*b*), rebaudioside-A (Reb-A, stevioside (Stev), steviol glycosides (SvGls).

Table 8. Simple correlation between physiological, biochemical, and plant yield traits and quality of two stevia genotypes under drought stress and foliar spraying in Gorgan (ES-3), Golestan, Iran.

	Plant yield	Chlorphyll *a*	Chlorphyll *b*	Proline	Rebaudioside-A	Stevioside	Steviol glycosides content
Chlorphyll *a*	0.69					(ES-3)	
Chlorphyll *b*	0.61	0.78					
Proline	-0.13	-0.01	-0.23				
Rebaudioside-A	0.45	0.54	0.20	0.54			
Stevioside	0.48	0.61	0.29	0.37	0.92		
Steviol glycosides content	0.48	0.60	0.28	0.41	0.95	0.99	
Steviol glycosides yield	0.95	0.71	0.53	0.03	0.66	0.68	0.69

Coefficients higher than 0.50: significant at the 1% probability level (**). Coefficients between 0.49-0.30: significant at the 5% probability level (*). Coefficients lower than 0.29: non-significant (ns). Chlorophyll *a* (Chl-*a*), Chlorphyll *b* (Chl-*b*), rebaudioside-A (Reb-A, stevioside (Stev), steviol glycosides (SvGls).

-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

Specifically, the simultaneous application of selenium (Se) in its various forms positively influenced leaf yield under drought stress. According to Aghighi Shahverdi et al. (2018), combined Se utilization significantly improved the growth and quality of stevia plants. Although high concentrations of Se can be toxic and act as a stress factor, studies have highlighted the beneficial effects of low Se concentrations. These benefits are associated with its ability to protect plants from abiotic stresses by activating mechanisms that alleviate oxidative damage (Lanza and Dos Reis, 2021). Selenium also prevents chlorophyll degradation, leading to enhanced photosynthesis (Rady et al., 2020).

Drought stress across the experimental sites led to a notable increase in proline content, unlike under non-stress conditions. Proline serves multiple roles beyond its function as an osmolyte in osmotic regulation and protection. It stabilizes cellular structures, including membranes, proteins, and DNA, while scavenging free oxygen radicals, quenching singlet oxygen, maintaining cellular redox potential (NADPH/NADP+), regulating cell pH, and safeguarding the photosynthetic apparatus (Zali et al., 2018; Afshari et al., 2022; Chen et al., 2022). Additionally, proline influences ethylene production, delays aging, and acts as a source of nitrogen, carbon, and energy during stress recovery (Aghighi Shahverdi et al., 2019; 2020; Zafari et al., 2020).

The Chinese stevia genotype exhibited higher proline content compared to its Indian counterpart under stress conditions. This disparity in proline levels may contribute to the reduced leaf yield observed in the Chinese genotype relative to the Indian one. Zali et al. (2018) reported similar findings, noting variations in proline levels among three *Foeniculum vulgare* genotypes under drought stress. Plants allocate a significant portion of their metabolic resources toward proline synthesis during stress, which limits resources available for growth and yield (Sadras and Calderini, 2009). This distinctive metabolic pattern was not observed in the Indian genotype. A significant negative correlation between proline content and leaf yield was identified at the experimental sites, supporting the assertion that increased proline synthesis is associated with reduced plant yield.

Drought stress in several plant species, including stevia (Afshari et al., 2022), safflower (Zafari et al., 2020), and sesame (Mahdavi Khorami et al., 2020), has been shown to elevate proline levels, resulting in decreased overall yield. However, the application of the plant hormone strigolactones (SLs) significantly enhanced the content and yield of SvGls under non-stress conditions compared to the control treatment and water spray. Plants employ sophisticated signaling systems and growth regulators to combat stress and enhance survival (Mubarik et al., 2021).

Strigolactones, a novel class of terpenoid lactones isolated from the root hairs of the parasitic plant striga (Sattar et al., 2022), play a critical role in regulating plant growth and development under stress (Rochange et al., 2019). These hormones have been shown to improve water relations, increase photosynthetic pigments, enhance gas exchange, and elevate antioxidant enzymatic activities, thereby promoting drought tolerance in maize seedlings (Sattar et al., 2022). Additionally, phytohormones such as gibberellins, methyl jasmonate, cytokinins, and auxin have been implicated in the synthesis of SvGls in stevia (Tavakoli et al., 2019). Further research into the role of SLs in the gene synthesis pathways of SvGls could provide valuable insights.

The findings highlight that plant growth regulators, particularly SLs, increased the synthesis of Reb-A and Stev, especially under drought stress. Growth and yield parameters, along with physiological traits such as chlorophyll a and b content and SvGls yield at the Gorgan site (ES-3), showed significantly higher averages compared to the other experimental sites, particularly in the Indian genotype. Environmental and climatic factors such as temperature, altitude, and precipitation substantially influenced the quantitative and qualitative characteristics of stevia. The elevated average temperature and precipitation levels at the Gorgan site likely contributed to its superior performance relative to other locations. Aghighi Shahverdi et al. (2018) underscored the pivotal role of temperature and precipitation as critical climatic parameters affecting the growth, yield, and biochemical traits of the stevia plant.

Conclusions

Key findings revealed that drought stress reduced chlorophyll levels and leaf yield across all locations, while significantly increasing proline levels, particularly in the Chinese genotype. Foliar spray treatments—especially those combining selenium with CPPU and strigolactones—enhanced chlorophyll content and mitigated the adverse effects of drought on yield. Both Se and plant growth regulators markedly increased SvGls content and yield, even under drought conditions. Selenium also boosted antioxidant enzyme activity during drought stress, while SLs application under non-stressed conditions stimulated SvGls production. The Indian genotype outperformed the Chinese genotype, exhibiting higher levels of photosynthetic pigments, and achieving superior SvGls yield and content. Additionally, the Gorgan site, characterized by higher temperatures and precipitation, demonstrated improved plant yield and secondary metabolite production compared to other experimental locations.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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