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Research Article

The role of potassium foliar spray in improving the effects of drought stress in *Stevia rebaudiana*: Quantitative and qualitative yield, high-consumption elements, and some physiological traits

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Abstract

Drought stress (DS) is a significant constraint on agricultural productivity. Managing nutritional elements, such as potassium (K), can reduce the effects of drought stress. To investigate the impact of DS and K foliar spraying on the yield and some leaf compounds of *Stevia rebaudiana* B., a field experiment was performed as a factorial form in a randomized complete block design with three replications in 2017-2018. The first factor consists of three levels of DS: Irrigation after the evaporation of 40 mm, 70 mm, and 100 mm from a Class A evaporation pan. The second factor includes three levels of foliar potassium (K) spray, applied at concentrations of 0%, 1%, and 1.5%, using potassium sulfate as the source. After preparing the land, seedlings were planted by hand at intervals of 50 cm by 75 cm in planting rows, resulting in a density of 2.5 plants per square meter on March 6. The results showed that both the zero-stress treatment and the foliar spray of 1.5% potassium (K) resulted in the highest yields of dry matter (1280.72 kg/ha) and leaf yield (846.47 kg/ha). This was not significantly different from the zero-stress treatment with a foliar spray of 1% potassium (1279.88 kg/ha). Additionally, the highest percentage of stevioside was observed under severe stress conditions, where the application of potassium foliar spray did not influence the stevioside percentage. Considering the positive effects of potassium foliar spray on both dry matter yield and leaf yield, as well as its ability to reduce potential damage caused by drought, it is recommended to use potassium foliar spray to achieve high yields in stevia plants under drought conditions.

Keywords: Foliar nutrition, Leaf yield, Macro elements, Proline, Stevioside

Introduction

Due to the higher public attention to health and fitness, there have been numerous studies on sweeteners and their alternatives. Many new resources have been used in these studies for obtaining these nutritious additives, among which one of the most important ones is stevia (*Stevia rebaudiana* Bertoni). Stevia is a perennial, shrubby, short-stemmed species of the Asteraceae (Compositae) family. It is native to the mountainous region along the border of Brazil and Paraguay (Jaroslov *et al*., 2007).

Stevioside glycoside is a zero-calorie sweetener that does not ferment inside the body. The tendency to consume steviol glycosides has increased in various countries instead of regular sugar or sucrose (Orellana-Paucar, 2023; Samuel *et al.,* 2018; Singh and Rao, 2005).

The steviol glycosides found in the leaves of stevia are steviol monoside, roboside, steviol bioside, stevioside, rhabdomyoside A, rhabdomyoside B, rhabdomyoside F, rhabdomyoside C, and dalkoside. Stevioside and rebaudioside A are produced more in stevia leaves than other steviol glycosides (Yadav *et al.*, 2011).

Abiotic stressors are currently the major threat to plant growth and productivity .The most crucial stress affecting the crop yield is drought stress. Therefore, drought stress has become an indispensable part of

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farming throughout the world; under precipitation shortage and drought stress, the intake of nutrients from the root decreases due to the lower volume of water and the improper distribution of the nutrients inside the soil (Karimi *et al.*, 2013). Various studies have shown that drought stress may increase electrolyte leakage and reduce the height and the leaf area of stevia (Srivastava and Srivastava, 2014; Barrios *et al.*, 2005). In another study on two cultivars of stevia, it was shown that drought stress could reduce stomatal conductance, photosynthesis, and transpiration rate. Finally, all of these effects led to a reduction in the dry matter of the plant. On the other hand, the stevioside and riboside A increased due to drought stress (Benhmimou *et al.*, 2018). The reduced yield of the fresh biomass, as well as that of the dry leaves, was found to be due to the reduced growth rate of the leaves and the height of the plant, reduced intake, transfer, and distribution of the nutrients (Rouphael *et al.*, 2012), as well as the reduced photosynthesis and shading in the plant (Bhatt and Rao, 2005; Ghanbari *et al.*, 2013). In their study on stevia, Benhmimou *et al.* (2018) indicated that drought stress could increase the steviol glycosides content. Increased contents of steviol glycosides could be a pro-drought stress parameter.

Fertilization promotes plant yield and plant metabolism and improves nutrient reserves to cope with stress conditions (Oliet *et al*., 2004). Potassium (K) is a very important macro element. Although it is not an integral part of the plant structure, it is key to the internal reactions of the plant (Balabanli *et al.*, 2010). Various studies on the use of these elements have shown the increased growth and quantitative and qualitative yield of stevia in the case of the use of K. In an examination of stevia, Inugraha *et al.* (2014) found that K had a notable impact on the growth of stevia plants. In this examination, the role of potassium (K) in physiological processes of plants, such as photosynthesis, transpiration, and stomata conductance, is highlighted (Widaryanto, 2008). The highest amount of the total chlorophyll was obtained under the influence of potassium. Through increasing photosynthetic transfer of substances and root development under drought stress, potassium could reduce the adverse effects of stress and increase water consumption efficiency. On the other hand, the turgor pressure of guard cells and the opening and closure of stomata are controlled by using potassium and other anions (Neseim *et al.*, 2014). In a study on the sugar beet, Gohari *et al.* (1999) indicated that potassium treatment could increase the sugar yield under mild drought stress. However, under severe stress conditions, the effects of potassium decreased significantly. Another study found that potassium consumption and foliar spraying improved the chlorophyll concentration of sugar beet under drought stress conditions (Mehrandish *et al.*, 2012). Also, foliar spray of potassium on leaves could meet K spray and increase economic yield in arid regions (Marschner, 1995). Potassium foliar spray effectively forms secondary metabolites through its influence on photosynthesis and the metabolism of carbon compounds (Haukioja *et al.*, 1998).

To maintain human health, the use of zero-calorie sugars in the production of food products is expected to increase in the future. On the other hand, due to the increased effects of drought, studying the nutrient management methods will be of high importance. Therefore, this study aims to reduce the adverse effects of drought stress on the yield and some leaf compounds of stevia by foliar spray of K.

Materials and methods

Experimental design, treatments, and growth conditions: This experiment was performed in the 2017-2018 crop year at the research farm of Agricultural Sciences and Natural Resources University of Khuzestan, 35 km northeast of Ahvaz, in the Mollasani town (Bavi city) on the eastern bank of the Karun River with a latitude of 31° 35” and a longitude of 48° 53” with 22 altitudes. According to long-term meteorological statistics, Mollasani has an average annual precipitation of about 269 mm, an average temperature of 23°C, and average maximum and minimum temperatures of 36 and 9.5°C. So, it is considered an arid/semi-arid region. The average meteorological parameters of the region during the plant growth period are presented in Table 1.

Before the experiment, five different sections of the soil were sampled at a depth of 0 to 60 cm to determine their physical and chemical properties. The obtained mixture of the samples was evaluated in the laboratory for physical and chemical properties, the results of which are presented in Table 2.

This research was performed as a factorial experiment in a randomized complete block design with three replications. The first factor includes three levels of moisture stress: irrigation after 40 mm evaporation from a Class A evaporation pan (no stress), irrigation after 70 mm evaporation (mild stress), and irrigation after 100 mm evaporation (severe stress). The second factor includes three levels of K foliar application (0, 1, and 1.5%) from the potassium sulfate reservoir. In order to prevent the rain from entering the plots, rain shelters (transparent plastic) were designed, and then the seedlings were planted. The dimensions of each plot were 3 × 4 m, and the distance between planting rows was 75 cm. Also, the distance between plants in the row was 50 cm. Seedlings of stevia were prepared from the Darvash Giah Khazar medicinal plants complex in Rasht. Seedlings were planted by hand on March 6 with a density of 2.5 plants per square meter (Gomes *et al.,* 2018). 25-day-old seedlings were transplanted to the field when they reached the 5-6 leaf stage.

As fertilizer, 40 $kg.ha^{-1}$ of phosphorus was applied to the soil from triple superphosphate resources before planting. However, 150 $kg.ha^{-1}$ of nitrogen fertilizer was added to the soil in two stages from the urea resources to optimize nitrogen. To achieve this, 50% of the

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Table 1. Meteorological statistics of ahvaz station in the year 2017-2018

Month	Evaporation (mm)	Precipitation (mm)	Maximum Temperature (°C)	Minimum Temperature (°C)
March	117.5	54.15	27.8	10.71
April	119.5	49.33	32.54	16.12
May	240.8	36.32	40.50	21
June	290.1	25.45	45.21	24.35
July	320.2	36.80	48.17	26.58

Table 2. Soil properties of the experimental field

EC (dS/m)	Total nitrogen (%)	P ava. (mg.kg^{-1})	K ava. (mg.kg^{-1})	Organic matter (%)	Sand (%)	Clay (%)	Silt (%)	Soil texture	Depth (cm)
7.42	0.043	16.21	143	0.72	14	51	34	Silt Clay	0-30
7.78	0.024	15.45	156	0.88	19	44	37	Silt Clay	30-60

nitrogen fertilizer was applied to the field around 15 days after planting the seedlings, with the remaining amount applied about 45 days after planting. Weed control was performed manually.

The water required for each irrigation treatment was calculated using the following equation. The water is distributed evenly using a pump and meter (Alizedeh, 2004).

$V = [(FC- €m) \times Pb \times D_{root} \times A] / Ei$ (1)

V = Volume of irrigation water (m^3), FC = Moisture percentage of soil by weight at point FC, €m = Moisture percentage of soil by weight before irrigation, Pb = Apparent specific weight of soil 2 ($g.cm^{-3}$), A = Irrigated area (m^2), D_{root} = Root development depth (m), Ei = irrigation efficiency

Physiological indicators: To determine the percentage of relative water content (RWC) of the leaf, at the beginning of the flowering, the Ritchie *et al.* (1990) method was used. To specify the electrolyte leakage (EL) rate from the leaf tissues, 15 leaf discs were transferred to tubes containing 10 ml of distilled water and placed at room temperature with dim light for 24 hours. The EL of distilled water (EC1) containing the sample was measured by an EC meter (APERA EC 800). The test tubes were then placed in a boiling water bath (95°C) for 15 minutes, and after cooling, they were remeasured, and finally, the EL was calculated with the following equation (Sairam and Srivastava, 2001).

$EL\% = [EC_1/EC_2] \times 100$ (2)

To measure the amount of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and leaf carotenoids (LC), the methodology provided by Arnon (1949) was used in the vegetative stage of the plant and close to harvesting time (85 days after planting of the seedling). A spectrometer measured plant phosphorus content (Olsen and Sommers, 1982). The plant K content was measured by the method provided by Rayan *et al.* (2001) using a flame photometer. The Kjeldahl was used to measure the nitrogen content (Bremner, 1996). Proline content was also determined by the Bates *et al.* (1973) method.

Height, yield, and some leaf compounds: After the growth and development of the plant and just before the flowering stage, the plants were harvested by hand at a height of 20 cm from the soil surface, 110 days after planting on June 23, from the middle lines with a half-meter margin from both sides. To determine the amount of leaf dry matter, total dry matter, and leaf harvest index, the samples were placed in an oven at 70°C for 48 hours. The harvest index is calculated using the following formula (3).

Harvest Index (HI) = (Dry weight of leaf / Total dry plant biomass) x 100% (3)

To measure the percentage of stevioside, the samples were pulverized. 1.5 g of stevia leaf powder was placed in 50 mL of 70% ethanol. The percentage of stevioside and riboside A was measured after calibration of the HPLC device (Unicam Crystal 200 200). Some specifications of this device are as follows:

Device specifications:
UV detector = k-2501
Hplc pump =k-1001
Dynamic mixing chamber AO285
Model 2003 degasser
Solrem organizer k-1500
Column specifications:
Column: NH_2 250×4.6mm
Nucleodur 100-5; NH_2 5qm
Mobile phase: 80/20 acetonmiril/water=isocratic
Flew=1 ml/min
Run time = 10 min
Temp=29° C
Wavelength=260 nm

Statistical analysis: For the statistical analysis, the data were first evaluated for normality. After the normality of data (Kolmogorov-Smirnov test) was proven, analysis of variance was performed. The means were compared using the least significant difference (LSD) method at the error probability level of 5%. Statistical analyses were performed using SAS 9.1 and Minitab 17 software.

Results

Leaf photosynthetic pigments: All the effects of the studied factors on the content of photosynthetic pigments were highly significant, but the effect of K was significant on the LC content (Table 3). The

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Table 3. Effects of drought stress (D) and K foliar spray (K) on chlorophyll a, chlorophyll *b*, carotenoids, electrolyte leakage (EL), relative water content (RWC), and proline of stevia

S.O.V.	df	Sum of squares					
		Chl *a*	Chl *b*	Car	EL	RWC	Proline
Replication	2	0.00002^{ns}	0.000003^{ns}	0.01^{ns}	191.04^{**}	31.86^{**}	0.00004^{ns}
Drought stress (D)	2	0.007^{**}	0.0003^{**}	0.35^{**}	460.40^{**}	1004.11^{**}	0.002^{**}
Potassium foliar spray (K)	2	0.0009^{**}	0.0001^{**}	0.10^{*}	2.32^{ns}	1.67^{ns}	0.0001^{*}
D×K	4	0.001^{**}	0.0001^{**}	0.13^{**}	1.97^{ns}	0.54^{ns}	0.0007^{**}
Error	16	0.00002	0.00002	0.02	16.54	2.06	0.00002
CV (%)	-	26.8	25.6	17.4	10.3	1.89	18.04

* and **: (*P ≤0.05 and **P ≤0.01) respectively and ^{ns}: Nonsignificant

Table 4. Mean comparison of drought stress (D) and K foliar spray (K) interaction on chlorophyll a, chlorophyll *b*, carotenoids, and proline of stevia

Drought stress (D)	K foliar spray (K)	chl *a*	chl *b*	Car	Proline
		(mg.g^{-1} FW)			
40	0	0.122^{abc}	0.029^{abc}	1.49^{ab}	0.065^{e}
	1	0.127^{a}	0.035^{ab}	1.58^{ab}	0.061^{e}
	1.5	0.128^{a}	0.042^{a}	1.65^{a}	0.073^{de}
70	0	0.08^{e}	0.018^{de}	1.22^{cd}	0.094^{b}
	1	0.97^{d}	0.025^{bcd}	1.32^{bc}	0.088^{bc}
	1.5	0.10^{cd}	0.024^{bcd}	1.37^{bc}	0.081^{cd}
100	0	0.035^{g}	0.015^{e}	0.99^{d}	0.115^{a}
	1	0.062^{f}	0.02^{cd}	$1.4a^{bc}$	0.110^{a}
	1.5	0.79^{e}	0.023^{bcd}	1.17^{cd}	0.092^{b}

In each column, means with the same letter(s) have no significant difference (LSD, P ≤0.05)

treatments of zero-stress × 1.5% K with an average of 0.128 mg.g^{-1}, zero-stress × 1% K with an average of 0.127 mg.g^{-1}, and zero-stress × non-foliar spray showed the highest amount of chlorophyll *a*. The minimum amount of chlorophyll a was related to severe stress treatment and no foliar spraying of K, with an average of 0.035 mg.g^{-1}. The variations of chlorophyll b and carotenoid had a similar pattern to that of chlorophyll *a*. The maximum amount of chlorophyll b and carotenoids was obtained in the zero-stress treatment × foliar spray of 1.5% K, which was not significantly different from the two zero-stress × foliar sprays of 1% and non-foliar spraying of potassium. The amount of chlorophyll b and carotenoids in the treatment of severe stress × non-foliar spraying of potassium were at the lowest level, with an average of 0.015 and 0.99 mg.g^{-1}, respectively (Table 4).

Relative water content, electrolyte leakage of the leaf, and proline: The effect of drought stress on the relative moisture of the leaf and the electrolyte leakage from the leaf was significant (at a probability level of 1%). However, the application of potassium and the interaction of the two factors had no significant effect on changes in these two traits. The impact of both experimental treatments and their interaction on proline content was significant (Table 3). Mean comparison between different levels of stress showed that with increasing drought stress, the amount of electrolyte leakage increased so that in severe stress treatment, the amount of electrolyte leakage was 5.53 and 14.19% higher than mild stress and zero-stress treatments, respectively (Figure 1).

The maximum percentage of RWC was obtained in the zero-stress treatment, 12.65% higher than the mild stress treatment and 20.98% higher than the severe stress treatment (Figure 2).

The maximum amount of proline was obtained in the treatment of severe stress × non-foliar spraying of potassium (0.115 mg.g^{-1} FW), which was not significantly different from the treatment of severe stress × foliar spray of 1% K (0.110 mg.g^{-1} fresh weight of the sample). The results also showed that zero-stress treatments at different levels of potassium foliar spray produced the lowest amount of proline (Table 4).

Stem height, dry matter, leaf yield, and harvest index: Considering the results obtained from the analysis of variance (Table 5), the effect of drought stress, K foliar spraying, and the interaction between the two studied factors on stem height (SH), dry matter yield (DMY), and leaf yield (LY) were significant (P ≤ 0.01). However, in the harvest index, only the effect of drought stress and the interaction of the two factors under study were significant (P ≤ 0.01) (Table 5). The mean comparison results indicated that zero-stress × foliar spray of 1.5% K led to the highest average SH of 58.90 cm, and it was not significantly different from zero-stress × foliar spray of 1% K with an average of 58.33 cm. The lowest SH (an average of 41.36 cm) was related to the treatment of severe stress × no foliar spraying of K (Table 6). Treatment of zero-stress × foliar spray of 1.5% K produced the highest number of leaves with an average of 846.47 kg.ha^{-1}, which showed a 55.48% increase compared to the treatment of severe stress × non-foliar spraying of K (544.41 kg.ha^{-1}). Also, the highest yield of the DMY of the whole plant was an average of 1280.72 kg.ha^{-1} in the zero-stress treatment ×

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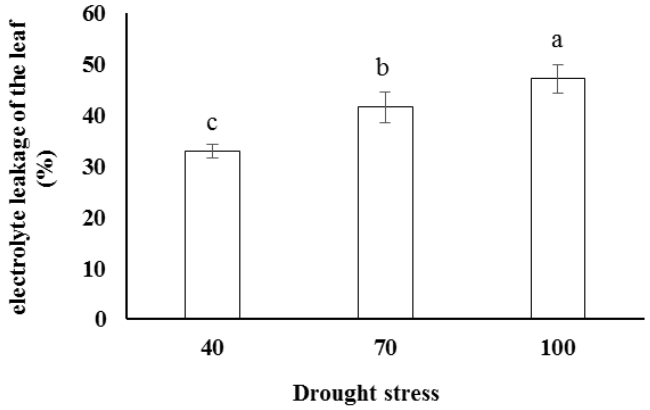


Figure 1. Effect of drought (D) on electrolyte leakage from leaf tissue of stevia

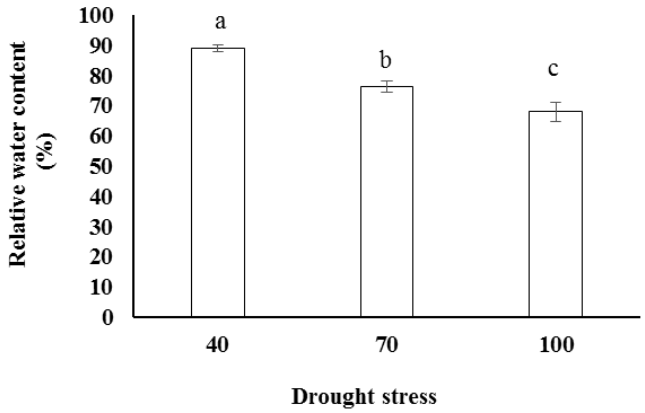


Figure 2. Effect of drought (D) on relative water content of stevia

Table 5. Effects of drought stress (D) and K foliar spray (K) on height, dry leaf yield, dry matter yield, and harvest index of stevia

S.O.V.	df	Sum of squares			
		Height	Dry leaf yield	Dry matter yield	Harvest index
Replication	2	1.44^{ns}	1985.5^{**}	321^{ns}	0.0005^{ns}
Drought stress (D)	2	437.00^{**}	138993.7^{**}	94418^{**}	0.06^{**}
Potassium foliar spray (K)	2	41.70^{**}	7391.2^{**}	52890^{**}	0.001^{ns}
D×K	4	119.03^{**}	36625.4^{**}	38910^{**}	0.01^{**}
Error	16	1.78	43.4	833	0.0007
CV (%)	-	12.40	16.00	9.73	12.80

* and **: (*P ≤0.05 and **P ≤0.01) respectively and ^{ns}: Nonsignificant

1.5% K foliar spraying, which was not significantly different from the lowest DMY being obtained in the treatment of severe stress × no foliar spraying of K (918.88 kg.ha^{-1}). The highest leaf harvest index (66.09%) was obtained in the treatment of zero-stress × 1.5% K, which, in this regard, did not show a significant difference with the treatment of zero-stress × no foliar spraying of K. However, the lowest HI was obtained in the treatment of severe stress × foliar spray of 1% K (50.40%), which showed a decrease of 15.69% compared to the treatment of zero-stress × foliar spray of 1.5% K (Table 6).

Amount of nitrogen, phosphorus, potassium, stevioside, and riboside A: The results of ANOVA (Table 7) indicated that the effect of drought stress and the interaction of the two factors under study ($P \leq 0.01$) were significant on the following traits: Percentage of nitrogen (N), potassium (K), phosphorus (P), stevioside, and riboside A of the leaf. The effect of K spray ($P \leq 0.01$) was significant only in terms of the percentage of nitrogen and leaf K (Table 7).

The highest percentages of N and P in the leaves were observed in the zero-stress treatment without considering the amount of K foliar spray. However, with increasing the intensity of drought stress, the level of these two traits decreased so that the lowest of N was

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Table 6. Mean comparison of drought stress (D) and K foliar spray (K) interaction on height, dry leaf yield, dry matter yield, and harvest index of stevia

Drought stress (D)	Potassium foliar spray (K)	Harvest index (%)	Dry matter yield ($kg.ha^{-1}$)	Dry leaf yield ($kg.ha^{-1}$)	Height (cm)
40	0	64.79^{ab}	1204.68^{b}	780.6^{c}	55^{b}
	1	63.71b	1279.88^{a}	815.42^{b}	58.33^{a}
	1.5	66.09^{a}	1280.71^{a}	846.47^{a}	58.9^{a}
70	0	59.76^{c}	1058.43^{e}	632.56^{f}	45.33^{e}
	1	55.48^{d}	1193.27^{b}	662.04^{e}	47.66^{cd}
	1.5	58.75^{c}	1173.65^{bc}	689.55^{d}	49.33^{c}
100	0	59.25^{c}	918.88^{f}	544.41^{i}	41.36^{f}
	1	50.40^{f}	1103.33^{de}	556.07^{h}	44.53^{e}
	1.5	52.54^{e}	1129.72^{cd}	593.50^{g}	46.05^{de}

In each column, means with the same letter(s) have no significant difference (LSD, P ≤0.05)

Table 7. Effects of drought stress (D) and K foliar spray (K) on N, P, K, stevioside, riboside A, and of stevia

S.O.V.	df	Sum of squares				
		Riboside A	Stevioside	Potassium	Phosphorus	Nitrogen
Replication	2	0.000004*	0.0001^{ns}	23.1**	0.12^{ns}	0.000002^{ns}
Drought stress (D)	2	0.005**	0.008**	0.002**	38.1**	0.0003**
Potassium foliar spray (K)	2	0.0000009^{ns}	0.00001^{ns}	1246**	1.11^{ns}	0.00005**
D×K	4	0.001**	0.002**	699**	9.91**	0.0001**
Error	16	0.00003	0.00006	2.70	0.49	0.000003
CV (%)	-	29.60	15.90	5.88	9.73	7.85

* and **: (*P ≤0.05 and **P ≤0.01) respectively and ^{ns}: Nonsignificant

Table 8. Mean comparison of drought stress (D) and K foliar spray (K) interaction on N, P, and K of stevia

Drought stress (D)	Potassium foliar spray (K)	Phosphorus (ppm)	Potassium (ppm)	Nitrogen (%)
40	0	17.04^{a}	248.6^{de}	2.39^{a}
	1	17.25^{a}	266.1^{b}	2.41^{a}
	1.5	17.36^{a}	282^{a}	2.47^{a}
70	0	14.22^{bc}	237.36^{g}	2.14^{cd}
	1	15.36^{b}	246.33^{e}	2.23^{bc}
	1.5	15.15^{b}	256.63^{c}	2.30^{b}
100	0	12.75^{d}	232.53^{h}	1.92^{e}
	1	13.06^{cd}	241.46^{f}	2.09^{d}
	1.5	13.50^{cd}	250.46^{d}	2.15^{cd}

In each column, means with the same letter(s) have no significant difference (LSD, P ≤0.05)

observed in the treatment of severe stress × no foliar spraying of K (1.92%). In severe stress × no foliar spraying of K, the lowest P level was observed in the leaf (12.75 ppm), which was not significantly different from the treatments of severe stress × foliar spray of 1 and 1.5% K. The highest and lowest K levels in the leaf were observed in the treatments of zero stress × foliar spray of 1.5% K and severe stress × no foliar spraying of K (an average of 282 ppm and 232.53 ppm, respectively) (Table 8).

Drought stress increased the percentage of stevioside and riboside A in stevia leaves. Severe drought stress at each level of foliar spraying of potassium showed the highest rate of stevioside. Therefore, under severe stress, no significant difference was observed between the different foliar spray levels. The highest percentage of riboside A, with an average of 3.49%, was obtained in severe stress evaporation × no foliar spray of potassium (Figure 3).

This treatment showed a 2.18% increase in the amount of this compound compared to the treatment of zero-stress × no foliar spray of K, which led to the lowest amount of riboside A. The lowest percentage of stevioside was observed in the treatments of zero-stress × no foliar spray and foliar spray of 1% K (Figure 4).

Discussion

Drought stress leads to a decrease in dry matter (DM), harvest index (HI), and photosynthetic pigments in stevia plants. This stress can negatively impact plant growth and yield through various physiological, morphological, and phenological changes (Havrlentova *et al*., 2021). Drought stress affects the membrane structure, transpiration rate, and nutrient movement in the soil, which in turn reduces the absorption and transfer of nutrients within the plant (Hu and Schmidhalter, 2005). Potassium (K) can help mitigate the effects of drought stress by maintaining cellular osmotic pressure, regulating osmotic balance, controlling the opening and closing of stomata,

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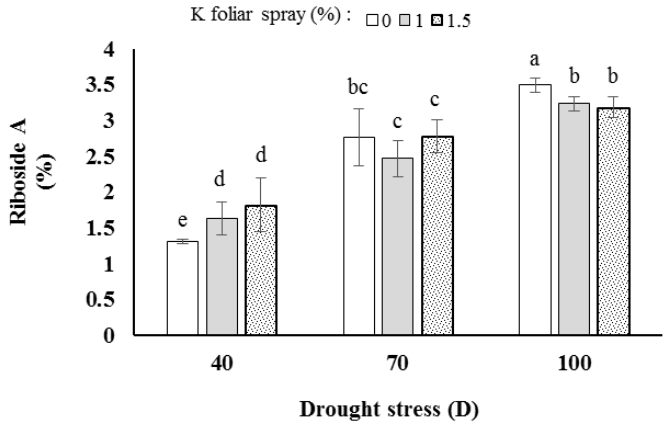


Figure 3. Effect of drought stress (D) and K foliar spray (K) (%) on leaf riboside A percentage of stevia

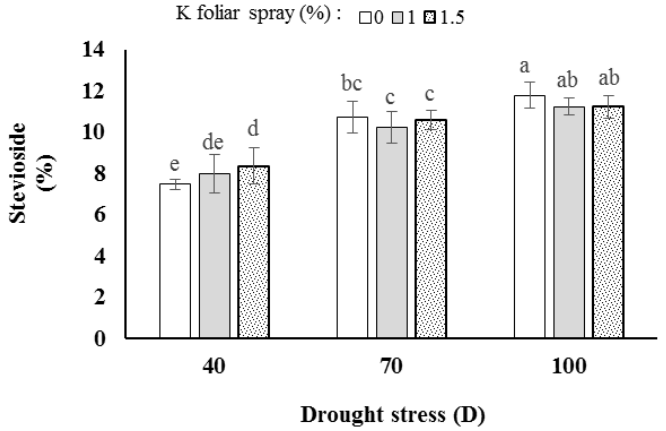


Figure 4. Effect of drought stress (D) and K foliar spray (K) (%) on leaf stevioside percentage of stevia

facilitating carbon dioxide fixation, and preventing oxidative damage in plants (Cakmak, 2005).

Leaf photosynthetic pigments: In this experiment, due to the decomposition of pigments in intense drought stress conditions and their non-regeneration, the amount of chlorophyll *a*, *b*, and carotenoids showed a significant decrease (Yang *et al.,* 2021). However, K had the potential to reduce photosynthetic pigments significantly in response to stress. So, the amount of chlorophyll *b* in the treatments of severe and mild stress × foliar spray did not show a significant difference from the treatment of zero. In a study on the interaction of foliar spray of potassium and moisture stress on sugar beet yield, Farazi *et al.* (2018) showed that foliar spray of potassium under mild stress (75% water requirement) increased the chlorophyll index by 21%.

Relative leaf moisture and electrolyte leakage of the leaf: The amount of leaf water in different plants depends on the amount of water in the soil and the opening and closure of the stomata. Under drought stress conditions, the RWC will decrease due to reduced available water in the soil (Pantin *et al.,* 2012; Prado and Maurel, 2013). Brown (1995) reported in his studies that the relative water content in leaves is both directly and indirectly related to a plant's response to drought stress. His research found that plants subjected to 40 mm of evaporation had a higher percentage of relative humidity in their leaves compared to those experiencing mild and severe stress. It appears that, in plants under drought stress, the intercellular spaces and water content are reduced due to an increase in osmotic materials within the plant tissues. As a result, water is drawn into the soil more forcefully. Which reduces the amount of relative water under drought stress (Havrlentova *et al.,* 2021).

Relative leaf moisture, electrolyte leakage of the leaf, and proline: The electrolyte leakage from leaf tissue increased with increasing stress intensity, in a way that it was 14.19% higher under severe stress compared to the zero-stress conditions. Considering that under drought stress conditions, some parts of the phospholipid layers of the membrane become hexagonal, and pores emerge in the membrane structure, there will be an increase in lipid peroxidation and ultimately a decrease in the cell membrane stability index (Mirjalili, 2005). Ultimately, the cell membrane structure is damaged. Therefore, as drought stress intensity increases, the membrane stability decreases, and there will be an increase in the electrical conductivity of the solution leaking from the leaf tissue (Porahmadi *et al.,* 2015). Quan *et al.* (2004) reported that electrolyte leakage increases in maize under drought stress.

Proline is an amino acid that serves as a major

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component of many proteins involved in the osmotic regulation of cell walls and membranes (Szabados and Savoure, 2009). Under drought stress, proline maintains the osmotic potential, removes free radicals and ROS, protects macromolecules from denaturation, and regulates cellular pH. In addition, proline acts as a nitrogen and carbon source for plants under severe stress and increases plant tolerance to stress (Amini *et al.,* 2015). Therefore, plants under drought stress spend a large amount of carbon and nitrogen resources on the synthesis of osmotic regulators such as proline to maintain the turgor pressure of their cells (Aranjuelo *et al.,* 2011). Under stress conditions, the rapid increase in proline coincides with the decrease in leaf water potential. Any factor that reduces the water potential leads to proline accumulation (Fatemeh and Arian, 2015). This experiment indicated that the proline level increased with increasing stress intensity. However, with the increase in the potassium level used, the proline concentration decreased slightly at any stress level, considering the neutralization of the adverse effects of stress through potassium consumption.

Stem height, dry matter, and leaf yield and harvest index: The results showed that, in general, with the increased drought stress and decreased plant access to water, there will be evidence of damage to cell membranes, increased electrolyte leakage, and decreased photosynthetic pigments (Tables 4 and 5). As a result, the height of the plant, leaf yield, leaf dry matter, and the harvest index also decreased. In the meantime, due to its role in the plant's water regulation, potassium reduced the effects of drought stress. This experiment found that under severe stress conditions, foliar spray of K could lead to a dry matter yield equivalent to mild stress conditions. On the one hand, potassium increases water absorption due to the increased osmotic pressure of root cells. On the other hand, considering its participation in the opening and closing of stomata, potassium could prevent water from leaving the plant, and the combination of these factors makes the plant resistant to drought stress in the presence of potassium (Marschner, 1995). However, the application of 1.5% K under severe stress could not increase leaf production and leaf harvest index, such as dry matter yield. The plant allocates most of its photosynthetic resources to shoot growth under severe stress, and it prefers to reduce the number of its leaves generated due to a lack of water to prevent severe evaporation from the leaves (Yang *et al.,* 2021).

Amount of nitrogen, phosphorus, and potassium: This experiment showed that the amount of NPK decreased with increasing stress intensity. The decline was particularly pronounced in relation to phosphorus (P). Lack of moisture in the soil reduces the rate of diffusion and mass flow of elements in the soil, which reduces the transfer of elements to the root surface and consequently reduces the absorption of elements (Azizabadi *et al.,* 2014; Malakouti and Homaee, 1994). Under drought stress, the phosphorus release rate from the soil to the root surface decreases more than that of other nutrients because phosphate ions adhere to the clay particles and are less available to the plant roots (Marschner, 1995).

Amount of stevioside and riboside A: The levels of stevioside and riboside A increased under stress. This increase in secondary metabolites is probably an adaptation to drought stress in Stevia. Ceunen and Geuns (2013) stated that to maintain cellular integrity in plant tissues and keep water in leaf cells to increase the intracellular osmotic potential under stress, we could observe an increase in the content of steviol glycosides. Benhmimou *et al.* (2018) reported that the highest levels of acetyl clickoside were obtained to treat severe drought stress.

Conclusions

Drought stress could reduce the amount of chlorophyll and carotenoids, leaf and dry matter yield, leaf harvest index, and various elements in the leaf. On the other hand, drought stress increases secondary metabolites, including proline, stevioside, and riboside A levels. Management of essential nutrients such as K can reduce the effects of drought stress. Foliar spray of 1.5% K could reduce these traits by improving plant growth conditions. In general, it could be concluded that under mild and severe stress conditions, the foliar spray of 1 and 1.5% K, respectively, could reduce the negative effects of DS on plant growth and yield in stevia.

Conflict of interest

The authors have no conflicts of interest to declare.

Author's Contribution

All authors contributed equally to this work.

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