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Effects on Water Stress on Daily Stomatal Conductivity of *Stevia rebaudiana*

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Authors' contributions

This work was carried out in collaboration between all authors. Author AK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HK, GEA and CK managed the analyses of the study. Authors HK, GEA and CK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Stevia rebaudiana (Bertoni), an herbaceous perennial plant belong to Asteraceae family, is one of the important source of natural sweetening agents with non-calorie that can be used as an alternative to artificial sweeteners. Plant originating in Paraguay and the south-west of Brazil, have some usage possibilities in many sector and can accumulate glycosides which tastes about 300 times sweeter than sugar cane. The previous studies have shown that stevia plant is affected by water stress. In case of water stress, the plants close their stomata and reduced the rate of transpiration. In this study which was carried out at the Akdeniz University in 2016 under a rainout shelter, it was aimed to determine changes in daily stomatal conductance before and after irrigation (T) for consecutive 15 days. In this scope, stevia plants were grown under 6 different irrigation regimes (I) including a control (I100), plants irrigated with 100% restitution of water consumption and additionally 120% (I120), 80% (I80), 60% (I60), 40% (I40) and 20% (I20) of the control treatment and 2 nitrogen (N) levels including zero N (control, N0) and recommended nitrogen level

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of 10 kg N/da (N10) in a split plot experimental design with three replicates. Irrigation schedule was based on A-Class evaporation pan and soil moisture level. As a result of study, it was determined that stomatal conductivities were decreased with increasing water stress at each N levels.

Keywords: Irrigation regime; nitrogen level; water consumption; plant stress; stomatal conductance

1. INTRODUCTION

Stevia rebaudiana (Bertoni), officially discovered by Dr. M. S. Bertoni in 1887, belonging to the family Compositae is a recent high demanding secondary metabolite in herbal world. Health causing diseases by natural caloric sweetener as well as by synthetic sweeteners (aspartame, saccharine, sucralose) make the life risk. So the focus came to on stevia which is completely natural and non-caloric plant [1]. Stevia leaves were used either to sweeten mate or as a general sweetening agent by Guarani Indians of the Paraguayan highlands. Seeds were sent to England in 1942 for cultivation but it is not successful. The first commercial cultivation was carried out in Paraguay in 1964 [2,3,4]. In the following years, cultivation started in many countries such as Japan, China, Brazil, Korea, Mexico, America, Indonesia, Canada etc. [4-11]. Since 2004, stevia listed as a positive herb plant by the Ministry of Food Agriculture and Livestock began to be grown first time in the province of Antalya in Turkey and the first harvest was realized in 2012.

Plant extracts have been used in Japan since 1968 as a low calorie sweetener in the food sector and in alternative diabetics for the treatment of diabetes, but studies that have examined the effect of sugar on health have supported the finding that steviol glycosides are safe for long term and regular consumption by humans [12-14]. In addition, no carcinogenic or mutagenic effects have been reported [15-17]. It can be consumed by both healthy people and diabetes patients [18]. The use of extracts increases the amount of antioxidants taken with the diet, and it also to take stevioside instead of sugar makes it easier for obese patients to lose weight. In addition, regular consumption of these compounds reduces blood sugar and cholesterol levels [19], enhances cell renewal and blood clotting, strengthens blood vessels and reduces blood pressure [20-22]. Nowadays, stevia extract is widely used in products such as carbonated beverages, dried seafood, confectionery, ice cream, chewing gum, yoghurt as well as toothpaste and mouthwash. It has become popular all over the world in recent years. In addition, powder products or refined extracts obtained from leaves which have antioxidant properties are also consumed as food supplements [23,24].

It is known that the concentration of stevioside in stevia leaves varies greatly depending on plant breeding conditions and agricultural practices. Especially water scarcity is one of the important limiting factor to plant production. Plants express a variety of symptoms when under stress. Some symptoms are visible (wilting, color changes, reduced growth, and death) while measurements to indicate disease severity using leaf transpiration, stomatal resistance, stomatal conductance [25]. Stomatal behavior is a complex phenomenon involving feedback controls which interact with a wide range of environmental factors [26], such as light, temperature and water status of the leaves [27, 28]. Plants have developed certain acclimation strategies to react rapidly in a changing, restrictive environment. Amongst the strategies, stomatal regulation plays a key role in plant response to water stress, because the stomatal regulation causes rapid variation in water use efficiency [27,29]. Thus, there has been increasing interest in studying the stomatal behaviors of various plant species [27]. In this article, the changes in the stomata conductance of different irrigation regimes and nitrogen applications in stevia, which are an important raw material in the food sector and have a wide variety of uses, have been examined.

2. MATERIALS AND METHODS

This study was carried out in the Research and Application Fields of the Faculty of Agriculture of Akdeniz University in 2016 under a rainout shelter. The geographic coordinates of the experimental area are 36°54'15" N and 30°38'30" E [30]. In the research area where Mediterranean climate is dominant, summers are warm and dry, winters are cool and rainy. The average annual temperature is 18.0 °C, the coldest with January 9.2°C and the warmest at 28.2°C in July. Annual mean relative humidity, total precipitation and evaporation were 63%, 1063.5 and 1886.3 mm respectively [31].

Stevia plant as one of the most important sources of non-calorie natural sweeteners is used as a material in this study. The best development is in areas with an annual average temperature of 31 °C and a precipitation of 1400 mm. During the development stages the plant is highly sensitive to cold and produce more leaves in regions with minimum frost events, high light intensity and high temperatures. If the sun's rays are too dense, they need a shadow in the summer months. The most suitable soil for stevia cultivation is areas with high yield and no drainage problems. The plant's nutritious roots are very close to the soil surface. For this reason, shallow soils are not a problem for plant growth.

The soil of the experimental area is from Gölbası territory series. These soils, developed on massive travertines, are included in the Entisol order because they are young soils and do not show much profile development. They have AC horizon with clay-loam texture in all profiles and almost flat topography. Their permeability is good and there is no drainage problem [32]. The physical and chemical properties of the experimental soil are given in Table 1.

Treatments consisted of six irrigation regimes and two N levels in a split plot experimental design with three replicates. Six water regimes were tested: the control (I100), in which plants received 100% of the soil water consumption, and 120% (I120), 80% (I80), 60% (I60), 40% (I40) and 20% (I20) of the control treatment and 2 nitrogen (N) levels including zero N (control, N0) and recommended nitrogen level of 10 kg N/da (N10). Irrigation applications were realized by drip irrigation method. The first fertilization was applied on 15 June (10%) and the other applications were in split application of 15%, 20%, 25% and 30% on 1 July, 16 July, 2 August and 16 August, respectively. All treatments of irrigation regime in N10 main parcels were equally fertilized irrespective of the irrigation regime. Stevia planting was performed with 30 cm spacing above the row and 60 cm spacing between the rows.

The amount of irrigation water was calculated based on measured amount of evaporation from Class A Pan. In addition, gravimetric sampling was performed every 10 days for control purposes.

Stomatal conductance measurements were performed based on Doubledee et al. [25] during 15 days from July 27th to August 12th by using an SC-1 Leaf Porometer (Decagon Devices Inc., Pullman, WA) with a range of 0 to 1000 mmol m^{-2} s^{-1} and a sample chamber aperture of 6.35 mm [33]. Measurements were realized by clipping the sensor of the leaf porometer onto a leaf at the top of the canopy just below the newest green leaf. For accurate readings, stomatal conductance was measured when the sun is highest in the sky between the hours of 11:00 A.M. and 2:00 P.M. and the sky was clear to prevent interference from clouds.

The experimental data were analyzed by the General Linear Model (GLM) using SAS statistical analysis software package. Duncan's Multiple Range test was used, if necessary, to separate the means of the data at 0.05 level of significance.

3. RESULTS AND DISCUSSION

In order to evaluate the effect of irrigation regimes (I) and nitrogen applications (N) on stomatal conductance values averaged over all measurements including before and after irrigations, variance analysis results are given in Table 2. According to the statistical analyses, no statistically significant difference was detected between N levels and the interaction between I×N. However, a high significant difference ($P<.001$) was obtained among stomatal conductance values of irrigation regimes. In general, a significant decrease in stomatal conductance was determined as the water stress increased at each N levels (Table 2 and Fig. 1). Under nitrogen conditions, the highest stomatal conductance was obtained from I120 treatment whereas I120 and I100 treatments were the highest under N0. For both (N0 and N10), the lowest stomatal conductance values measured from the I20 treatments which were not significantly different from that of I40 treatment. The highest stomatal conductance averaged over N levels was determined for I120 (124.0 mmol m^{-2} s^{-1}) and lowest for I40 and I20 (66.9 and 55.4 mmol m^{-2} s^{-1}, respectively). Although there was a decrease trend in stomatal conductance with increased water stress, I100 treatment was not significant different from I80 and I60 treatments. However, in these treatments, the plant was found to have higher stomatal conductance compared to I40 and I20 treatments.

Table 1. Some physical and chemical properties of the experimental soil

Depth (cm)	Texture	Field capacity %	Wilting point %	Bulk density gr/cm^3	EC_e	pH
0-5	CL	25.63	17.66	1.28	0.65	8.06
5 15	CL	26.90	18.88	1.34	0.68	8.08
15-25	CL	26.15	17.37	1.41	0.57	8.13
25-35	CL	26.58	17.61	1.39	0.55	8.13
35-50	CL	25.35	16.36	1.37	0.62	8.15

Table 2. Effect of N level and irrigation regimes on stomatal conductance values averaged over before and after irrigations

	I120	I100	I80	I60	I40	I20	Mean of N
N10	132.5a	106.0b	96.1bc	79.9cd	66.6de	54.2e	**89.2** ns
N0	115.4a	93.2ab	85.3bc	89.1bc	67.1cd	56.6d	**84.4** ns
Mean of I	**124.0 a**	**99.6 b**	**90.7 b**	**84.5 b**	**66.9 c**	**55.4 c**	

Significance
N: ns
Irrigation regime: **
N x Irrigation regime : ns
Means followed by the different letters in each row are significantly different at 5% level by Duncan test.
ns: non-significant; *: P<.01 (significant); ** P<.001 (highly significant).

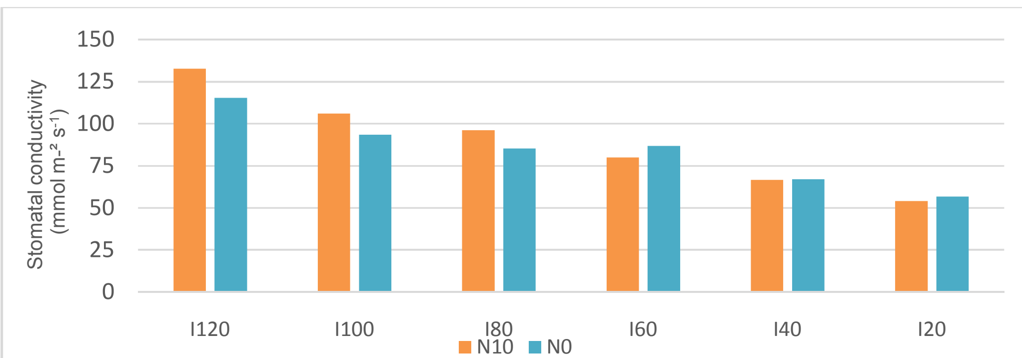

Fig. 1. Stomatal conductivity for different N levels and irrigation regimes between 27 July and 12 August

In order to evaluate the effect of irrigation regimes (I) on stomatal conductance values measured before and after irrigations (T), variance analysis results are given in Table 3 and 4 for N0 and N10. In addition, the differences in stomatal conductance values measured for 15 days before and after irrigation for each N level are shown in Figs. 2 and 3 respectively. According to Table 3 and 4, it was concluded that stomatal conductance values showed significant differences between measurement time (before and after irrigation, T) and also among irrigation regimes (I) at 0.001, in addition to interaction between T×I at 0.01 level of significance for both N levels. If the stomatal conductance values averaged over all irrigation regimes values were compared for before and after irrigation, it was determined that the highest stomatal conductance occurred after irrigation in both N0 and N10 applications (135.2 mmol m^{-2} s^{-1}, 134.4mmol m^{-2} s^{-1}, respectively) (Table 3, 4). Furthermore, if N0 application is considered, the highest value measured from I120 (127.5 mmol m^{-2} s^{-1}) and the lowest value from I40 and I20 applications (70.2 and 57.7 mmol m^{-2} s^{-1}) (Table 3). Similarly, when the effects of irrigation regimes on stomatal conductance values averaged over before and after irrigation were examined, it was determined that the highest values measured from I120 and I100 (136.6 and 114.9 mmol m^{-2} s^{-1}) and the lowest values from I40 and I20 (67.1 and 54.6 mmol m^{-2} s^{-1}) treatments at N10 (Table 4).

According to interaction between measurement time and irrigation regime at N0, the highest stomatal conductance was determined from after irrigation measurements of I120 and I100 (193.8 and 163.8 mmol m^{-2} s^{-1}, respectively) (Table 3 and Fig. 2) whereas at N10, from after irrigation measurements of I120, I100 and I80 (190.9, 171.3, 155.2 mmol m^{-2} s^{-1}, respectively) (Table 4 and Fig. 3). Similarly, interaction between measurement time and irrigation regime both at N0 and N10, the highest stomatal conductance was determined from before irrigation measurements of I120 (61.1 and 82.3 mmol m^{-2} s^{-1}, respectively) treatment which was significantly different from other treatments (Table 3-4 and Figs. 2-3).

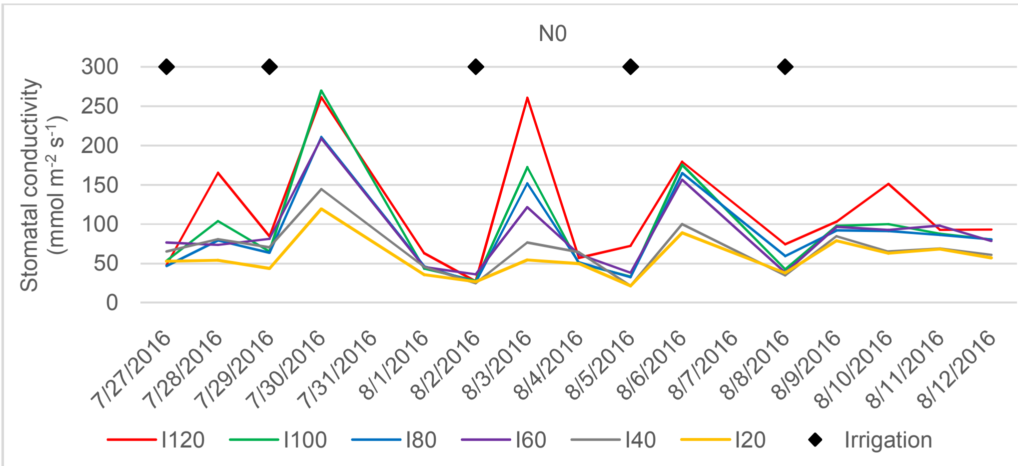

Fig. 2. Daily stomatal conductivity for different irrigation regime and before and after each irrigation at N0

Table 3. Effect of measurement time and irrigation regimes on stomatal conductance in N0 application

	I120	I100	I80	I60	I40	I20	Mean of T
Before Irrigation	61.1Ba	44.3Bbc	45.4Babc	53.9Bab	43.2Bbc	36.4Bc	**47.4**B
After Irrigation	193.8Aa	163.8Aab	139.5Abc	138.3bc	97.1Acd	78.9Ad	**135.2**A
Mean of I	**127.5a**	**104.0b**	**92.4bc**	**96.1b**	**70.2cd**	**57.7d**	

Significance
Time: **
Irrigation regime: **
Time x Irrigation regime: *
Means followed by the different small letters in each row or capital letters in each column are significantly different at 5% level by Duncan test.
*: P<0.01 (significant); ** P<0.001 (highly significant).

Table 4. Effect of measurement time and irrigation regimes on stomatal conductance in N10 application

	I120	I100	I80	I60	I40	I20	Mean of T
Before Irrigation	82.3Ba	58.5Bb	50.4Bbc	44.6Bbc	39.2Bc	38.6Bc	**52.3**B
After Irrigation	190.9Aa	171.3Aa	155.2Aab	123.6Abc	95.0Acd	70.6Ad	**134.4**A
Mean of I	**136.6 a**	**114.9ab**	**102.8bc**	**84.1dc**	**67.1de**	**54.6e**	

Significance
Time: **
Irrigation regime: **
Time x Irrigation regime: *
Means followed by the different small letters in each row or capital letters in each column are significantly different at 5% level by Duncan test.
*: P<0.01 (significant); ** P<0.001 (highly significant).

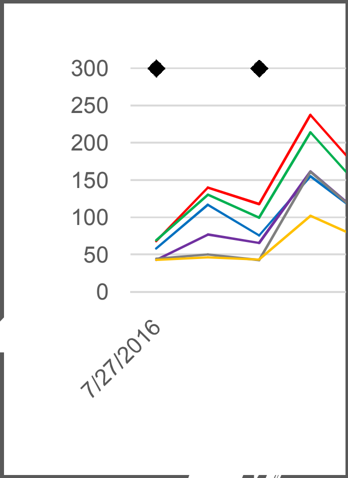

Fig. 3. Daily stomatal conducti

It was determined that, for both
stomatal conductivity measure
irrigation regimes at either bef
irrigation were highly different. In e
water stress conditions resulted l
conductance than the optimum wate

Ćosić et al. [34] reported that
regime has an impact on stomatal
the higher the level of irrigation
stomatal conductance. Chouzouri
[35] showed that there was a goo
between drought tolerance and the
stomatal conductance, as soil wat
became restricted. Cifre et al. [36]
stomatal closure is among the fi
occurring in the leaves in respons
Similarly, Liu et al. [37], Sabir an
have found evidence of reduc
conductance of plants under water
work on different plants. The res
from research are in parallel with th

4. CONCLUSION

This study was realized to inve
individual effects of water regime a
the interaction of them on t
conductance values in stevia plan
show that there is no effect of N lev
conductance values averaged ove
regime and measurement time a
after irrigation. However, under N0 a
plots, stomatal conductance v
significant differences either for
time or for irrigation regime
Increased water stress causes a

sweetener in paraguay. Econ Bot. 1992; 46(3):336-337.

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