

The Effect of Different Plant Population and Plant Age on Some Yield and Quality Parameters of Sugar Grass (*Stevia Rebaudiana* Bertoni L.)

erkan boydak

erkanboydak@gmail.com

Bingöl Üniversitesi <https://orcid.org/0000-0002-3466-5356>

irfan omay

Bingöl Üniversitesi <https://orcid.org/0009-0000-8816-4736>

Research Article

Keywords: Sugar Grass, Stevioside, Rebaudioside, Plant Density, Harvest Times

Posted Date: August 2nd, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4835071/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: The authors declare no competing interests.

Abstract

This study was established in 2017 and 2018 to determine the yield and quality parameters of sugar grass (*Stevia rebaudiana* Bertoni L.) at different plant densities (25x50, 30x50, 35x50, 40x50 cm) and number of harvesting (1st harvest and 2nd harvest). The experiment was carried out according to 'split plots experimental design' with 4 replications. Fresh herb yield, dry herb yield, stevioside (steviol glycoside) ratio, different sugar components in leaves, rebaudioside A, B and C (Reb A, Reb C, Reb D) were analysed. As a result, the highest fresh herb yield was obtained at 25x50 cm plant density with 502.33 kg/da and 417.90 kg/da in the first and second year, respectively in the 2nd form, while the lowest fresh herb yield was obtained at 40x50 cm plant density in the 1st form with 129.99 kg/da and 223.76 kg/da, respectively. The highest dry herb yield was obtained in the first and second year with 150,32 kg/da and 164,50 kg/da at 25x50 cm plant density in the 2nd form, and the lowest dry herb yield was obtained with 37,12 kg/da and 69,93 kg/da at 40x50 cm plant density in the 1st form. Stevioside ratios were between 7.47 and 5.71 in the first year and between 7.69 and 6.58 in the second year, the amounts of different sugar components in the leaves were between 13.27 mg/ml and 10.37 mg/ml in the first year and between 13.47 mg/ml and 10.85 mg/ml in the second year, RebaudiosideA ratios were between 13.27 mg/ml and 10.37 mg/ml in the first year and between 13.47 mg/ml and 10.85 mg/ml in the second year, 09 to 2.39 in the first year and 3.20 to 2.58 in the second year, RebaudiosideC rates varied between 1.00 and 0.76 in the first year and between 1.15 and 0.76 in the second year, and RebaudiosideD rates varied between 1.56 and 1.07 in the first year and between 1.56 and 0.00 in the second year. According to the results of the two-year study, it was determined that frequent planting, especially 25x50 cm planting frequency, gave better results in Hayrat district conditions.

GİRİŞ

Stevia leaves contain bioactive compounds such as steviol glycosides, phenolics, flavonoids and vitamins which give sweetness and can be used in food and pharmaceutical industries. Compared to the first planting year, the second year values (leaf dry yield, polyphenol accumulation and antioxidant activities) are higher. Growth, crop yield and quality are strongly dependent on genotype, harvest time and crop age. The status of these traits may be different in different cultivars. The amounts of Rubusoside, Dulcoside A, Stevioside, Rebaudioside A, C, D, D, E and M may vary over the years. For these reasons, it is necessary to develop stevia breeding programmes (Clemente et al., 2021).

It is reported that stevioside in stevia has 300–350 times more sweetening properties than normal sugar. Due to its low glycaemic index, it can be safely used by both diabetics and glycaemic patients. It may also contain a bitter taste due to some essential oils and tannins. As the amount of nitrogen fertiliser per unit area increases, plant height and leaf area increase, while leaf area index and total soluble solids decrease. In other words, when sown in dense plant populations, the quality is at high levels when the amount of nitrogen is increased (Shivani et al., 2021).

Steviz provides more dry leaf yield and steviol at high plant density regardless of plant age, and the results increase in direct proportion when fertilised with nitrogen fertiliser up to a certain point (Mitali et al., 2021).

It is reported that different environmental conditions have the potential to improve the vegetative and quality traits of stevia through hybrid and mutant populations, and different genotypes can be developed in suitable environments (Amien et al., 2021).

Plant height, number of branches, total leaf area, plant fresh weight, plant dry weight, sativoside and leaf dry yield can increase 2 to 3 times when treated with organic and non-organic nitrogen fertilisers and some micro-organisms together and separately (youssef et al. 2021).

It has been reported that environment, environmental interactions (GEI) (5.20%) and genotype effects (2.43%) are important in the total variation on stevia yield, thus increasing stevia leaf yield is highly influenced by genotypes and environment and their interactions, environmental effects contribute 92.38% of the total variation (Amien et al., 2022).

Stevia (*Stevia rebaudiana* Bertoni L.), known as sugar grass, is a perennial, herbaceous, shrub-like plant from the Asteraceae family, is not found naturally in the flora of Turkey (Gedik and Tansı 2017), and has a stevioside content (total glycoside) ranging from 4–20% on a dry weight basis, depending on the variety and growing conditions (Kennelly 2001), It is known that stevioside ratios obtained in dry leaf analyses vary between 4–20%, that this variation depends on the variety of the sugar grass plant and the growing conditions in the regions where it grows, that dulcoside A, one of the sweeteners, is 50–120 times more equivalent to sucrose, and that the rate of rebaudioside F in leaves is 2. 7% (Starrat et al, 2002). The original plant *Eupatorium rebaudianum* has more than 240 natural species. It grows naturally in semi-tropical and humid regions of South America where annual rainfall is 1400–1800 mm and humidity is around 75–86%. The ideal soil pH for its growth should be 6.5–7.7, soil salinity should be zero or very low, the soil should be sandy and rich in organic matter, well drained and highly permeable (Landazuri and Tigrero 2009).

The leaves are dried and traditionally chewed and consumed by the Paraguayan Indian Guaranis, and these dried leaves are also used in various teas and medicines. 3.8% Rebaudioside A, 0.6% Rebaudioside C, 0.3% Dalcoside and 9.1% Stevioside can be obtained from the leaves of the wild Sugar Grass plant. In addition, 100 g of dry leaves of *Stevia rebaudiana* B. contain 4.65 g moisture, 11.2 g protein, 1.9 g fat, 6.3 g ash and 15.2 g crude fibrin (Goyal et al., 2010). The leaves of the sweetgrass plant contain more than one steviol glycoside; Rebaudioside A (2–4%), Rebaudioside C (1–2%), Dulcoside A (0.5-1%), Rebaudioside B (< 1%), Rebaudioside D (< 1%), Rebaudioside E (< 1%), Rebaudioside F (< 1%). Among the steviol glycosides found in the sugar grass plant, the most valuable is Rebaudioside A (Reb A) compound. Rebaudioside A compound is 400–500 times sweeter than normal sugar. *Stevia* leaves contain 0.6% rebaudioside C, 3.8% rebaudioside A and 9.1% stevioside (Lemus- Mondaca et al., 2012). The fresh herb yields and dry herb yields of rebaudioside, which is largely responsible for the sugar taste in the sugar grass plant used in the treatment of obesity and diabetes, show similar trends (Moraes et al.,

2013). Some scientific researches related to the study on *Stevia* are given below. Kohda et al. (1976), in a research study on diterpene glycosides of sugar grass, found that the rate of Stevioside in the leaves was 2.18%. They also reported that the sweetness of Rebaudioside A was 300–450 times sweeter than sucrose and the rate of Rebaudioside A in leaves was 1.43%, the sweetness of Rebaudioside B was 300–350 times sweeter than sucrose and the rate of Rebaudioside B in leaves was 0.44%, Steviolbioside was 100–125 times sweeter than sucrose and its rate of presence in leaves was 0.04%.

Bian (1981) stated that harvesting should be done before flowering or at the beginning of flowering because flowering causes the stevioside rate in the leaves to decrease. Shyu (1994) reported that 3 different harvests in May, July and September significantly affected the plant height of sugarcane, dry leaf yield, leaf size, Rebaudioside and Stevioside ratio were dependent on the thickness of the leaf formed in the plant, total herb dry yield was 1150 kg/ha, dry leaf yield was 720 kg/ha and Stevioside ratio was 7.19%. Huang et al. (1995) reported that they obtained 19.57% Stevioside from dry leaves. Weng et al. (1996) showed that the amount of Rebaudioside depends on the high photosynthesis value, the surface shape of the leaf, the high protein values of the leaves and the chlorophyll content of the leaves. They reported that the total stevioside content of the leaves of sweetgrass plants is associated with high rebaudioside A content, large leaf area, high photosynthesis, high chlorophyll and protein content at the seedling stage and when the plant is mature. Fronza and Folegatti (2003) reported that they obtained 4.3 tonnes/ha of dry leaves and 6.5% Stevioside from a total of two harvests at a plant density of 6.6 plants/m². Andolfi et al. (2006) reported that they obtained 301 kg/ha in the first and 630 kg/ha in the second harvest from a plant density of 4.5 plants/m² and that the ratio of stevioside and rebaudioside A in dry leaves varied between 4–13% and 2–4%, respectively. Kovylyayeva et al. (2007) determined the amounts of glycoside components in 100 g of dry leaves as Stevioside, Rebaudioside A and Rebaudioside C % in Russia (5.8; 1.2; 0.5), Ukraine (4.8; 1.3; 0.3) and the % Stevioside, % Rebaudioside A and % Rebaudioside C found by studies conducted in different countries were found in South Korea (5.5; 2.5; 1.4), China (6.6; 3.7; 2.1), Paraguay (4.6; 1.9; 0.9), Japan (7.7; 1.9; 0.9), Canada (5.0; 0.3; 0.1), Vietnam (15.5; 3.8; 1.4).

Kumar et al. (2012) reported that plants growing in the sun grew faster, plants growing in the shade had longer plant height values than those growing in the sun, plants in the sun and in decreasing amounts of light in the order reached a higher number of leaves, with the decrease in light, the leaves were larger in terms of length and width, that is, plants growing in the shade produced larger leaves than plants growing in the sun. Again in this experiment, leaf stem ratio increased with decreasing light. Plants growing in full sun produced 3.83% stevioside and 0.99% rebaudioside, those in 25% shade produced 3.73% stevioside and 0.93% rebaudioside, those in 50% shade produced 3.78% stevioside and 1.07% rebaudioside, and plants in 75% shade produced 3.60% stevioside and 0.84% rebaudioside.

Taleie et al. (2012) investigated the effect of plant density and sowing date on stevioside, flavonide and phenol yield of sugarcane plant under the conditions of Gilan region of Iran, 4 different sowing densities were determined as 50 cm between rows and 20, 25, 35 and 50 cm above rows (50x20 cm, 50x25 cm, 50x35 cm, 50x50 cm) and 3 different sowing times (15 March, 30 March and 15 April) on the yield and

quality of sugarcane plant. They stated that the highest plant height was 80 cm in plants sown with a frequency of 50 × 20 cm on 15 March, fresh branch yield increased with the increase in the number of plants per unit area at all 3 sowing times, the highest fresh branch weights, the highest fresh herb yield, the highest dry branch weights, the highest dry herb yield and the highest fresh branch yield were obtained at 50 × 20 cm sowing frequency on all 3 dates. They reported that 15 March was the most suitable date for transplanting sugar grass seedlings to the field under the conditions of Gilan region of Iran, and that plant height, fresh and dry herb weight, Stevioside, phenol and flavonide yields of seedlings transplanted on 15 March were significantly positively affected. Steviol, Stevioside, Rebaudioside A, B, D, E, Dalkoside A, B, D, E, DalkosideA, B were determined as the main glycosides and Ascorbic acid, β Carotene, K, P, Fe, Zn, Mg, Cr, Co, Sn, Riboflavine, Thiamine etc. as other components in the leaves of sugar grass plant. Samadpourrigani (2014) reported that fresh leaf yield (429.7 kg/ha), dry leaf yield (110.9 kg/ha), dry herb weight per plant (228.7 g/plant), fresh twig yield (335.6 kg/ha), dry twig yield (95.9 kg/ha) were obtained at a plant density of 30 × 60 cm.

Sözmen (2015) reported that the highest fresh herb yield was obtained at 15 kg/da nitrogen dose and the highest dry herb yield was 750 kg/da at 15 kg/da N dose. Tadesse et al (2016) used five row spacing (20cm, 25cm, 30cm, 35cm and 40cm) and three row spacing (40cm, 50cm and 60cm) in their study. They reported that in 2014 and 2015, the highest fresh herb yield was 27.547 kg/ha and 23.619,8 kg/ha and the highest dry leaf yield was 4773,7 kg/ha and 4314 kg/ha at 40x20 cm plant density. Although the study showed that the highest sugar grass yield per unit area was recorded at 40x20 cm spacing, they stated that 40x25 cm plant density was the best spacing for sugar grass to obtain maximum yield considering the difficulties in weeding and irrigation. Gedik and Tansı (2017) reported that in 30x60, 45x60, 60x60 cm plant density applications, the highest fresh herb yield (1265.8 kg da) was recorded at 45x60 cm planting frequency, the highest leaf yield (268.9 kg da) and the highest flower yield (184.5 kg da) were recorded in 2015 in 3-year-old plants and 45x60 cm planting frequency, and the yields decreased in the 4th harvest year.

MATERIAL AND METHOD

The experiment was established in a farmer's field in Hayrat district of Trabzon province. The stevia plant materials used in the study were obtained from a trader who cultivates stevia (*Stevia rebaudiana* Bertoni L.) from the same region. The altitude of the experimental area is approximately 180 metres. The climate is warm and rainy in summer and cool and rainy in winter. The district, which is very hilly in terms of land structure, has a slope of about 30–35 degrees and receives a lot of rainfall, is very rich in terms of vegetation (Anonymous 2016).

Table 1
Climatic Data of Trabzon Province in 2017–2018

	Average Temp. (°C)		Relative Humidity (%)		Total Precipitation (mm)	
Months	2017	2018	2017	2018	2017	2018
May	14,8	19,6	81,3	84,4	116,9	143,1
June	18,8	23,8	83,4	80,4	69,5	58,1
July	21,5	25,2	85,5	81,3	63,2	116,6
August	22,6	25,2	90,2	80,1	107,1	119,3
September	19,8	22,4	83,1	82,1	67,7	175,4
October	14,2	18,8	83,2	85,5	133,9	167,0
Averages	18,6	22,5	84,5	82,3	-	-
Total	-	-	-	-	558,3	779,5

Source: Republic of Turkey Ministry of Agriculture and Forestry General Directorate of Meteorology

In 2017, the average temperature in the 6-month (May-October) growing season was 18.6 0C, the average relative humidity was 84.5%, and the total rainfall was 558.3 mm. In 2018, the average temperature was 22.5 0C, the average relative humidity was 82.3% and the total rainfall was 779.5 mm. Since there was sufficient rainfall during the vegetation period, there was no need for irrigation. The parameters analysed and the methods of obtaining them are given below.

Soils previously taken from 0–30 cm soil depth from certain points to represent the area where the research will be established were mixed and the samples taken were analysed by Trabzon Special Provincial Administration Agricultural Services Directorate in the Soil Analysis Laboratory. The results are given in Table 2.

Table 2
Soil Analysis Results of The Research Area

Sample Depth (cm)	Soil Composition	pH	Salt Content (%)	Organic Matter (%)	P ₂ O ₅ (kg/da)	K ₂ O (kg/da)	Lime (%)
0–30 cm	clayey	6,43	0,12	3,95	24,82	310,3	-----

According to the results of soil analyses; pH was found to be slightly acidic, salt-free in terms of salt content, good organic matter content, very high level of P2O5, no lime content, high K2O content (Anonymous 2013).

The experiment was established and conducted according to the 'Split Plots Experimental Design' with four replications. Each plot consisted of 4 rows. The length of the plots was 3 m and the width was 2 m and the size of the plots was 6 m². The space between blocks and plots was set as 1 m. On 19 May 2017, the plants were planted in the pits.

The analyses of 100 g of steviol glycoside (Stevioside, RebaudiosideA, RebaudiosideB, Rebaudioside C, RebaudiosideD and Dulcoside etc.) values in the leaves of sugar grass plants were performed in the Chromatography Laboratory of the Food Engineering Department of Adana Alparslan Türkeş Science and Technology University.

The dried and ground dry leaves of sugar grass dried at 38 °C for 72 hours in ETUV, AS 220 RS device, magnetic stirrer (IKA C-MAG HS 10) device, Universal 320 R centrifuge device, HPLC device and isocritical standard system method were used in the laboratory. 0 ml/min flow rate of the mobile phase at constant concentration and the separation of steviol glycoside substances from each other depending on the retention time in the columns; 50 mg of the dried and ground powder of the sugar grass leaf was taken with purified water and Acetonitrile (ACN). 50 mg was weighed in beaker in AS 220 RS device, 50 ml of pure water/ACN (7/3) ratio was added to the weighed sugar grass powder. The mixture was diluted, stirred evenly and the steviol glycosides in the dry leaves were released into the mixture and stirred for 5 minutes in a magnetic stirrer (IKA C-MAG HS 10).

It was placed in a centrifuge (Universal 320 R). The mixture was stirred at 5000 rpm for 10 minutes at room temperature (22 °C) in Universal 320 R device. A 2 ml portion of the mixture was taken and completed to 10 ml with pure water/ACN mixture and the mixture was taken into the vials with a 0.45 ml syringe. The mixture was placed in the HPLC (Agilent Teknologies 1260 Infinity) device with the vials. In the HPLC device, C18 column (Capcell Pak C18) and solvent Na₂H₂PO₄ (68/32 ACN) ACN mobile phase was set as buffer and the flow rate and time 1 ml/min isocritical flow method was set as single mobile phase. The steviol glycoside values were calculated by running the C18 column in the HPLC device at the ideal temperature (40 °C) for 30 minutes. Stevioside, RebaudiosideA, RebaudiosideC and RebaudiosideD, to which the sugar components (Glucose, Fructose, Sucrose, Rhamnose and Sorbitol) bound to the obtained steviol glycosides were bound, were calculated as % of the glycoside values in mg/ml.

Row spacing was 50 cm in each plot. The places on the row where the plants were to be planted were dug 5–6 cm deep and made ready for planting sugar grass seedlings and the seedlings were planted by hand.

Plant densities were as follows;

In 25x50 cm plot; 52 plants (13 x 4) (80000 plants/ha) were planted.

In 30x50 cm plot; 44 plants (11 x 4) (66666 plants/ha) were planted.

In 35x50 cm plot; 40 plants (10 x 4) (60000 plants/ha) were planted.

In 40x50 cm plot; 36 plants (9 x 4) (53333 plants/ha) were planted.

Pure 6 kg/da nitrogen, 6 kg/da phosphorus and 6 kg/da potassium (20-20-20) NPK compound fertiliser (10 kg / 320 m²) was mixed into the soil by sprinkling. After planting, the plants were given life water. Top fertiliser urea (46% N) was applied to the plots at a rate of 4 kg nitrogen per decare.

When the plants reached approximately 5–10% flowering level, after removing the edge effects of the plot, 1 plant each at the beginning and end of the plants in the middle 2 rows were discarded and all the plants remaining in the middle 2 rows were harvested. The second harvest was done when the plants reached approximately 5–10% flowering level. Sugar grass leaves were dried in an ETUV machine at 38 °C for 72 hours and ground into powder.

Analysed Properties and Methods

Fresh Herb Yield (kg/ha): Fresh herb yield (kg/da) was determined by removing the edge effects of the middle 2 rows of each plot, discarding 1 plant each at the beginning and end of the plants in the middle 2 rows and harvesting all the remaining plants. **Dry Herb Yield (kg/ha):** The plants used for fresh herb yield were dried in an ETUV machine at 38 °C for 72 hours and dry herb yield (kg/ha) was calculated.

Stevioside Rate (%): Dried leaves were determined by HPLC (High Performance Liquid Chromatography) device as specified in the method and calculated as (%). **Different Sugar Constituents in Leaves (mg/ml):** The dried leaves were determined by HPLC (High Performance Liquid Chromatography) as specified in the method and calculated as (%). **Rebaudioside A (RebA) Rate (%):** Rebaudioside A (RebA) ratio was determined and calculated as (%) in dried leaves by HPLC (High Performance Liquid Chromatography) device as specified in the method. **Rebaudioside C (RebC) Ratio (%):** In dried leaves, RebaudiosideC (RebC) ratio was determined and calculated as (%) by HPLC (High Performance Liquid Chromatography) as specified in the method. **Rebaudioside D (RebD) Ratio (%):** RebaudiosideD (RebD) ratio was determined and calculated as (%) in dried leaves by HPLC device as specified in the method.

The findings obtained from the study were analysed and evaluated using JUMP statistical package programme. All statistically significant values were subjected to L.S.D. (0,05) test.

RESEARCH FINDINGS AND DISCUSSION

1.Fresh Herb Yield (kg/ha)

The mean values and groupings of fresh herb yield per plant for different harvest times and different plant density obtained from the experiment are given in Table 3.

Table 3

Mean Values of Fresh Herb Yield of Sugar Grass at Different Plant Densities and Different Harvesting Times Under Rize Conditions and The Resulting Groups

Fresh Herb Yield (kg/da)						
2017				2018		
Plant Density	1. Harvest	2. Harvest	Averages	1. Harvest	2. Harvest	Averages
25x50 cm	318,65 c	502,33 a	410,49 A	406,70 a	417,90 a	412,30 A
30x50 cm	247,69 d	418,30 b	332,99 B	317,43 c	335,43 b	326,43 B
35x50 cm	193,00 e	331,25 c	262,12 C	257,63 f	288,50 d	273,06 C
40x50 cm	129,99 f	322,65 c	226,32 D	223,76 g	275,50 e	249,63 D
Averages	222,33 B	393,63 A		301,38 B	329,33 A	
L.S.D. (0,05)	Harvest Time** (26,3353) Plant Density** (15,4829) Interaksiyon*(21,8962)			Harvest Time (6,32) Plant Density (8,69) Interaction(12,29)		

As can be understood from the examination of Table 3, the highest average fresh herb yield per plant was 393.63 kg/ha at the 2nd harvest time and the lowest average fresh herb yield was 222.33 kg/ha at the 1st harvest time in 2017. The highest fresh herb yield average was 410,49 kg/da at 25x50 cm plant density and the lowest fresh herb yield average was 226,32 kg/da at 40x50 cm plant density. According to the harvest time x plant density interaction, the highest fresh herb yield was obtained at 25x50 cm plant density (502.33 kg/da) in the 2nd harvest period and the lowest fresh herb yield was obtained at 40x50 cm plant density (129.99 kg/da) in the 1st harvest period. The data of 2018 were in parallel with the results of 2017 and although the numbers were different, the best results were obtained from 25 x 50 cm plant density and the lowest results were obtained from 40 x 50 cm plant density, the second harvest average gave better results than the first harvest average and the fresh herb yield decreased in direct proportion as the row spacing increased (Table 3).

As a result of the research, fresh herb yield values were found to be statistically significant and it was observed that plant height, number of branches and leaf yield tended to increase as the plant density increased and harvest time was delayed. The fact that the increase in 2nd harvest fresh herb yield was higher than the 1st harvest was due to the fact that the longer the vegetation period of the plant, the more fresh herb yield was obtained. It is thought that this is due to the fact that as the harvest time is delayed, the leaves of the plant form more nutrients in the plant. This situation is thought to increase the fresh herb yield depending on the irregularities in air temperatures during the growth and development periods of sugar grass plants and plant densities depending on the length of the vegetation period. The findings obtained as a result of our study are lower than the findings of Aladakatti (2011), Taleie et al. (2012), Tadesse et al (2016) and parallel to the findings of Samadpourrigani (2014).

2. Dry Herba Yield (kg/ha)

The mean values and groupings of dry herb yield at different harvest times and different plant density obtained from the experiment are given in Table 4.

Table 4

Mean Values of Dry Herb Yield of Sugar Grass at Different Plant Densities and Different Harvesting Times Under Rize Conditions and The Resulting Groups

Dry Herb Yield (kg/da)						
	2017			2018		
Plant Density	1. Harvest	2. Harvest	Averages	1. Harvest	2. Harvest	Averages
25x50 cm	85,66 d	150,32 a	117,99 A	135,45 b	164,50 a	149,98 A
30x50 cm	68,31 e	123,06 b	95,68 B	101,20 e	139,20 b	120,20 B
35x50 cm	53,76 f	100,74 c	77,25 C	80,25 f	129,20 c	104,73 C
40x50 cm	37,12 g	95,34 c	66,23 D	69,93 g	114,00 d	91,97 D
Averages	61,21 B	117,36 A		96,71 B	136,73 A	
L.S.D. (0,05)	Harvest Time** (5,293974) Plant Density** (4,294743) Interaksiyon* (6,073697)			Harvest Time (4,84) Plant Density (3,55) Interaction(5,03)		

As it can be understood from the examination of Table 4, the highest average dry herb yield per plant was determined at the 2nd harvest time with 117,36 kg/da and the lowest average dry herb yield was determined at the 1st harvest time with 61,21 kg/da. The highest average dry herb yield was 117,99 kg/da at 25x50 cm plant density and the lowest average dry herb yield was 66,23 kg/da at 40x50 cm plant density. According to the harvest time x plant density interaction, the highest dry herb yield was obtained at 25x50 cm plant density (150.32 kg/da) in the 2nd harvest period and the lowest dry herb yield was obtained at 40x50 cm plant density (37.12 kg/da) in the 1st harvest period. The data of 2018 were in parallel with the results of 2017 and although the numbers were different, the best results were obtained from 25 x 50 cm plant density and the lowest results were obtained from 40 x 50 cm plant density, the second harvest average gave better results than the first harvest average and the dry herb yield decreased in direct proportion as the inter-row distances increased (Table 4).

As a result of the research, dry herb yield values were found to be statistically significant and it was observed that dry herb yield values increased as the plant density increased. In the 2nd harvest, the increase in dry herb yield was higher than the 1st harvest. It is thought that the reason why the increase in dry herb yield was higher than the 1st harvest is that the longer the vegetation period of the plant, the more fresh leaf yield was obtained in the plant, which led to an increase in dry herb yield, and more dry herb yield was obtained in the 2nd harvest due to carding in the leaves. With the increase in plant density,

vegetative parts increased, yield increased and as the plant density increased, dry herb yield also increased depending on fresh herb yield. The reason for this is thought to be the similarity between fresh herb yield and dry herb yield in terms of yield (Moraes et al., 2013) and the increase in dry herb yield due to irregularities in air temperatures and the length of vegetation period.

The findings obtained as a result of our study are lower than the findings of Shyu (1994), Tadesse et al (2016) and parallel to the findings of Samadpourrigani (2014).

3.Stevioside Rate (%)

The mean values and groupings of Stevioside ratio in sugar grass plant for different harvest times and different plant density obtained from the experiment are given in Table 5. As can be understood from the examination of Table 5, the highest Stevioside ratio was determined at the 2nd harvest time with 7,01 mg/ml % and the lowest Stevioside ratio average was determined at the 1st harvest time with 6,30 mg/ml %. The highest mean Stevioside ratio was 6,88 mg/ml % at 30x50 cm plant density and the lowest mean Stevioside ratio was 6,38 mg/ml % at 35x50 cm plant density. According to harvest time x plant density interactions, the highest Stevioside ratio was obtained at 30x50 cm plant density (7,47 mg/ml %) in the 1st harvest period and the lowest Stevioside ratio was obtained at 40x50 cm plant density (5,71 mg/ml %) in the 2nd harvest period.

Table 5
Mean Values of Stevioside Content of Sugar Grass at Different Plant Densities and Different Harvesting Times Under Rize Conditions and The Resulting Groups

Stevioside Content (%)						
	2017			2018		
Plant Density	1. Harvest	2. Harvest	Averages	1. Harvest	2. Harvest	Averages
25x50 cm	6,29 ab	7,43 a	6,86	7,27 ab	7,35 ab	7,310 A
30x50 cm	7,47 a	6,29 ab	6,88	7,69 a	6,58 c	7,133 AB
35x50 cm	5,73 b	7,03 a	6,38	6,85 bc	6,85 bc	6,846 B
40x50 cm	5,71 b	7,28 a	6,50	6,96 bc	7,22 abc	7,090 AB
Averages	6,30	7,01		7,193 A	6,997 B	
L.S.D. (0,05)	Harvest Time (NS) Plant Density (NS) Interaksiyon* (1,1846)			Harvest Time (0,051) Plant Density (NS) Interaction(0,653)		

In the 1st harvest period, the highest Stevioside ratio was found in 30x50 cm plant density (7,47 mg/ml %), while the lowest Stevioside ratio was found in 40x50 cm plant density (5,71 mg/ml %). In the 1st harvest period, it was determined that as the plant density increased, Stevioside ratio increased. In the 2nd harvest period, the highest Stevioside ratio was realised at 25x50 cm plant density (7,43 mg/ml %),

while the lowest Stevioside ratio (6,29 mg/ml %) was realised at 40x50 cm plant density. In 2018, the highest value (7.69%) was obtained at the first harvest date and 30 x 50 cm plant density, while the lowest value (6.58%) was obtained at the second harvest date and 30 x 50 cm plant density. In the second year values, both plant density averages, harvest date averages and interaction data formed different groups. In general, it can be said that stevioside ratio increased in dense plantings (Table 5).

As a result of the research, no statistically significant difference was found between plant density and harvest times. Stevioside was positively correlated with RebaudiosideA / Stevioside ratio in leaf thickness (Shyu 1994), Stevioside values were higher at 30x50 cm plant density at the 1st harvest, this is estimated to be due to Bian (1981) that Stevioside ratio increases in the beginning period of flowering in sugar grass leaves and Weng et al. (1996) that it is affected by many parameters.

The findings obtained in our study were higher than the findings of Kumar et al. (2012), in parallel with the findings of Shyu (1994), Fronza and Folegatti (2003), Samadpourrigani (2014), and lower than the findings of Huang et al. (1995), Andolfi et al. (2006), Sözmen (2015).

4. Different sugar components (glycosides) in leaves (mg/ml)

The mean values and groupings of different sugar components (mg/ml) in leaves for different harvest times and different plant density obtained from the experiment are given in Table 6. As can be understood from the examination of Table 6, the highest mean of Total Steviol Glycoside (TSG) of different sugar components in leaves was determined at the 2nd harvest time with 11,99 mg/ml, and the lowest mean of Total Steviol Glycoside (TSG) of different sugar components in leaves was determined at the 1st harvest time with 11,14 mg/ml. Different sugar components of Total Steviol Glycoside (TSG) in leaves between plant densities were found to be statistically insignificant and no group was formed. The highest average of Total Steviol Glycoside (TSG) of different sugar components in leaves was obtained at 40x50 cm plant density with 11,82 mg/ml and the lowest average of Total Steviol Glycoside (TSG) of different sugar components in leaves was obtained at 35x50 cm plant density with 10,87 mg/ml.

Table 6
Mean Values of Different Sugar Constituents of Sugar Grass at Different Plant Densities and Different Harvesting Times Under Rize Conditions and The Resulting Groups

Different Sugar Constituents (mg/ml)						
	2017			2018		
Plant Density	1. Harvest	2. Harvest	Averages	1. Harvest	2. Harvest	Averages
25x50 cm	11,06 c	12,52 ab	11,79	11,96 c	12,52 b	12,24 A
30x50 cm	12,69 a	10,85 c	11,77	13,47 a	10,85 e	12,16 AB
35x50 cm	10,43 c	11,32 bc	10,87	10,92 de	11,32 d	11,12 B
40x50 cm	10,37 c	13,27 a	11,82	11,80 c	13,27 a	12,54 C
Averages	11,14 B	11,99 A		12,04	11,99	
L.S.D. (0,05)	Harvest Time** (0,66207) Plant Density (NS) Interaksiyon* (1,3497)			Harvest Time (NS) Plant Density (0,304) Interaction(0,431)		

According to the interactions of harvest time x plant density, the highest Total Steviol Glycoside (TSG) of different sugar components in leaves was obtained in the 2nd harvest period at 40x50 cm plant density (13,27 mg/ml), while the lowest Total Steviol Glycoside (TSG) of different sugar components in leaves was obtained in the 1st harvest period at 40x50 cm plant density (10,37 mg/ml). When the 1st harvest period was examined, the highest level of different sugar components Total Steviol Glycoside (TSG) in the leaf was obtained at 30x50 cm plant density (12,69 mg/ml), while the lowest level of different sugar components in the leaf was obtained at 40x50 cm plant density (10,37 mg/ml). In the 2nd harvest period, Total Steviol Glycoside (TSG) was the highest at 40x50 cm plant density (13,27 mg/ml), while the lowest TSG was the lowest at 30x50 cm plant density (10,85 mg/ml). As the plant density increased, it was determined that Total Steviol Glycoside (TSG) of different sugar components in leaves did not increase. When the second year values were analysed, the highest value was obtained from the 2nd harvest period and the most sparse planting 40 x 50 cm plant density, while the lowest value was obtained from the 2nd harvest period 30 x 50 cm plant density with 10.85, but the other results were very similar to the first year results (Table 6). As a result of the research, the values of different sugar components (Total Steviol Glycoside) (mg/ml) in the leaves were found to be statistically significant. In the 2nd harvest, the values of different sugar components (mg/ml) in the leaves were higher than the 1st harvest. According to the sugar to which different sugar components were bound in the leaf; Stevioside (Steviol + Glucose sugar bound), RebaudiosideA (Steviol + Glucose sugar bound), RebaudiosideC (Steviol + Rhamnose sugar + Glucose sugar bound) and RebaudiosideD (Steviol + Glucose sugar bound) were determined. Since the sum of Stevioside + RebaudiosideA + RebaudiosideC + RebaudiosideD gives the sum of different sugar components in the leaf (mg/ml), the different sugar components in the leaf were determined as 11,99 mg/ml in the 2nd harvest. It is estimated that the rate of Steviol Glycoside increases in the period when

the flowering is 5–10% in the sugar grass leaves, therefore, the steviol glycoside values bound in the leaf are determined as high in the 2nd harvest in the HPLC device. Kovylyaeva et al. (2007) found that the % of glycoside constituents Stevioside, RebaudiosideA and RebaudiosideC in 100 g dry leaves of sugar grass plants were found in Russia (5.8; 1.2; 0.5), Ukraine (4.8; 1.3; 0.3), South Korea (5.5; 2.5; 1.4), China (6.6; 3.7; 2.1), Paraguay (4.6; 1.9; 0.9), Japan (7.7; 1.9; 0.9), Canada (5.0; 0.3; 0.1), Vietnam (15.5; 3.8; 1.4), respectively. The findings of our study were in parallel with the findings of Samadpourrigani (2014) on different sugar components in leaves.

5. RebaudiosideA (RebA) Rate (%)

The mean values and groupings of Rebaudioside A ratio in sugar grass plants for different harvest times and different plant density obtained from the experiment are given in Table 7.

As can be understood from the examination of Table 7, the highest Rebaudioside A ratio was determined at the 2nd harvest time with 2,85 mg/ml % and the lowest Rebaudioside A ratio average was determined at the 1st harvest time with 2,62 mg/ml %. The highest average of Rebaudioside A ratio was 2,80 mg/ml % at 30x50 cm plant density and the lowest average of Rebaudioside A ratio was 2,58 mg/ml % at 35x50 cm plant density. According to the harvest time x plant density interaction, the highest Rebaudioside A ratio was obtained in the 2nd harvest period at 40x50 cm plant density (3,09 mg/ml %) and the lowest Rebaudioside A ratio was obtained in the 1st harvest period at 35x50 cm plant density (2,39 mg/ml %).

Table 7
Mean Values of Rebaudioside A of Sugar Grass at Different Plant Densities and Different Harvesting Times Under Rize Conditions and The Resulting Groups

Rebaudioside A (%)						
	2017			2018		
Plant Density	1. Harvest	2. Harvest	Averages	1. Harvest	2. Harvest	Averages
25x50 cm	2,65 abc	2,94 ab	2,79	3,01 abc	2,94 abcd	2,98
30x50 cm	3,01 ab	2,58 bc	2,80	3,20 a	2,58 d	2,89
35x50 cm	2,39 c	2,78 abc	2,58	2,70 cd	2,78 bcd	2,74
40x50 cm	2,42 c	3,09 a	2,75	2,90 abcd	3,09 ab	2,99
Averages	2,62	2,85		2,95	2,85	
L.S.D. (0,05)	Harvest Time (NS) Plant Density (NS) Interaksiyon* (0,4444)			Harvest Time (NS) Plant Density (NS) Interaction(0,387)		

As a result of the research, the highest Rebaudioside A ratio was realised at 30x50 cm plant density (3,01 mg/ml %) and the lowest Rebaudioside A ratio was realised at 35x50 cm plant density (2,39 mg/ml %).

%) in the 1st harvest period. In the 2nd harvest period, the highest Rebaudioside A ratio was realised at 40x50 cm plant density (3,09 mg/ml %), while the lowest Rebaudioside A ratio (2,58 mg/ml %) was realised at 30x50 cm plant density. In the 1st and 2nd harvest periods, it was determined that the increase in plant density did not cause an increase in Rebaudioside A ratio. The values of RebaudiosideA (Steviol + Glucose sugar) sugar component in the leaf were found to be statistically significant. In the 2nd harvest, RebaudiosideA values in the leaves were found to be 3.09% at 40x50 cm plant density by HPLC device. When we examined the 2018 values, the highest value was obtained from the first harvest period and 30 x 50 cm plant density with 3.20%, followed by the 2nd harvest period and 40 x 50 cm plant density with 3.09%, and the lowest value was obtained from the second harvest period and 30 x 50 cm plant density with 2.58%. However, when the obtained data are evaluated in general, it is seen that they are in great similarity with the first year data (Table 7). As a result of the research, it was found that there was no statistically significant difference in RebA ratio in terms of averages. The interaction was statistically significant and it was observed that RebA ratio tended to increase in the 1st form of dense sowing and 2nd form of sparse sowing. It is thought that this situation can be explained by the opinion of Shyu (1994) who reported that there is a positive relationship between leaf thickness and RebA/Stevioside ratio.

The findings of our study were in parallel with the findings of Andolfi et al., (2006), Goyal et al., (2010) on Rebaudioside A ratio and lower than the findings of Sözmen (2015).

6. Rebaudioside C (Reb C) Rate (%)

The mean values and groupings of Rebaudioside C ratio in sugar grass plants for different harvest times and different plant density obtained from the experiment are given in Table 8.

As can be understood from the examination of Table 8, the highest Rebaudioside C ratio was determined at the 2nd harvest time with 0.91 mg/ml % and the lowest Rebaudioside C ratio average was determined at the 1st harvest time with 0.88 mg/ml %. The highest mean Rebaudioside C ratio was 0,97 mg/ml % at 40x50 cm plant density and the lowest mean Rebaudioside C ratio was 0,78 mg/ml % at 35x50 cm plant density. According to the harvest time x plant density interaction, the highest Rebaudioside C ratio was obtained in the 1st harvest period at 40x50 cm plant density (1,00 mg/ml %) and the lowest Rebaudioside C ratio was obtained in the 2nd harvest period at 35x50 cm plant density (0,76 mg/ml %). In the 1st harvest period, the highest Rebaudioside C ratio was obtained at 40x50 cm plant density (1,00 mg/ml %) and the lowest Rebaudioside C ratio was obtained at 35x50 cm plant density (0,80 mg/ml %). In the 2nd harvest period, the highest Rebaudioside C ratio was realised at 25x50 cm plant density (0,96 mg/ml %), while the lowest Rebaudioside C ratio (0,76 mg/ml %) was realised at 35x50 cm plant density. In the 1st and 2nd harvest periods, it was determined that the increase in plant density did not cause an increase or decrease in Rebaudioside C ratio.

Table 8
Mean Values of Rebaudioside C of Sugar Grass at Different Plant Densities and Different Harvesting Times Under Rize Conditions and The Resulting Groups

Rebaudioside C (%)						
	2017			2018		
Plant Density	1. Harvest	2. Harvest	Averages	1. Harvest	2. Harvest	Averages
25x50 cm	0,87 bc	0,96 a	0,92 A	1,06 b	0,96 c	1,010 A
30x50 cm	0,99 a	0,86 bc	0,92 A	1,15 a	0,86 d	1,005 A
35x50 cm	0,80 cd	0,76 d	0,78 B	0,93 cd	0,76 e	0,845 B
40x50 cm	1,00 a	0,94 ab	0,97 A	1,11 ab	0,94 cd	1,025 A
Averages	0,91	0,88		1,063 A	0,880 B	
L.S.D. (0,05)	Harvest Time (NS) Plant Density (0,6105) Interaksiyon* (0,0863)			Harvest Time (0,089) Plant Density (0,058) Interaction(0,082)		

When the values obtained in the second year are examined, the highest value was obtained from the first harvest period and 30 x 50 cm plant density with 1.15%, while the lowest result was obtained from the second harvest period and 35 x 50 cm plant density with 0.76%. When both these results and the averages of harvest time and plant density are analysed, it will be seen that they are very similar to the first year values. As a result of the research; it is seen that the averages of the RebC ratio in terms of the number of harvesting periods are not statistically significant, and in terms of plant densities, although it is statistically significant, it is not statistically significant except for 30x50 cm plant density. It is thought that the difference in the mean of 30x50 cm plant density and the values in the 1st and 2nd form interactions is due to the fact that the plots were affected differently due to the fact that the land where the experiment was established has a slope of more than 30 degrees.

The findings obtained from our study were found to be higher than the RebC ratio of Kohda et al. (1976).

7. Rebaudioside D (Reb D) Rate (%)

The mean values and groupings of Rebaudioside D ratio of sugar grass plants at different harvesting times and plant density are given in Table 9.

As it can be understood from the examination of Table 9, the highest average Rebaudioside D rate was determined at the 2nd harvest time with 1,32 mg/ml % and the lowest average Rebaudioside D rate was determined at the 1st harvest time with 1,19 mg/ml %. The highest mean Rebaudioside D ratio was 1,40 mg/ml % at 40x50 cm plant density and the lowest mean Rebaudioside D ratio was 1,16 mg/ml % at 30x50 cm plant density. According to the interactions of harvest time x plant density, the highest

Rebaudioside D ratio was obtained in the 2nd harvest period at 40x50 cm plant density (1,56 mg/ml %) and the lowest Rebaudioside D ratio was obtained in the 1st harvest period at 35x50 cm plant density (1,07 mg/ml %).

Table 9

Mean Values of Rebaudioside D of Sugar Grass at Different Plant Densities and Different Harvesting Times Under Rize Conditions and The Resulting Groups

Rebaudioside D (%)						
	2017			2018		
Plant Density	1. Harvest	2. Harvest	Averages	1. Harvest	2. Harvest	Averages
25x50 cm	1,25 bc	1,20 c	1,22 B	0,00 f	1,20 c	0,600 D
30x50 cm	1,22 c	1,11 c	1,16 B	0,67 e	1,11 d	0,890 A
35x50 cm	1,07 c	1,43 ab	1,25 B	0,00 f	1,43 b	0,715 C
40x50 cm	1,24 bc	1,56 a	1,40 A	0,00 f	1,56 a	0,780 B
Averages	1,19	1,32		0,168 B	1,325 A	
L.S.D. (0,05)	Harvest Time* (NS) Plant Density (1,3368) İnteraksiyon* (0,1890)			Harvest Time (0,090) Plant Density (0,063) İnteraksiyon(0,089)		

In the 1st harvest period, the highest Rebaudioside D ratio was realised at 25x50 cm plant density (1,25 mg/ml %), while the lowest Rebaudioside D ratio was realised at 35x50 cm plant density (1,07 mg/ml %). In the 2nd harvest period, the highest Rebaudioside D ratio (1,56% mg/ml) occurred at 40x50 cm plant density (1,56% mg/ml), while the lowest Rebaudioside D ratio (1,11% mg/ml) occurred at 30x50 cm plant density. In the 1st and 2nd harvest periods, it was determined that the increase in plant density did not cause an increase or decrease in Rebaudioside D ratio. When 2018 data were analysed, no significant results were observed in the first harvest period, but the second harvest period was similar to the first year data. Again, plant density averages and harvest period averages are in parallel with the first year results. As a result of the research; RebD ratio was higher both in the first and second harvest periods and in the average harvest period at 40x50 cm plant density. When evaluated in terms of plant densities, it is seen that RebD decreases as the plant density increases, although it is not directly proportional. In terms of the number of cuttings, although high data was obtained in the 2nd cuttings, it was not found to be statistically significant.

Our findings are in harmony with the findings of Kohda et al. (1976) who stated that RebD in the leaf increased during the flowering period of 5–10% and as a result, RebD increased and RebD in the leaf was around 1.43%.

NOTIFICATION

The first year figures of this study were taken from the master's thesis of a student named İrfan OMAV.

References

1. Aladakatti, Y.R. 2011. Response of Stevia (stevia rebaudiana Bertoni.) to Irrigation Schedule, Planting Geometry and Nutrient Levels. Department of Agronomy College of Agriculture, Dharwad University of Agricultural Sciences, Dharwad. Ph.d. thesis. 214 P.
2. Amien S, Maulana H, Ruswandi D, Nurjanah S (2022). Stevia (*Stevia rebaudiana* B.) Genotypes assessment for leaf yield stability through genotype by environment interactions, AMMI, and GGE biplot analyses. *SABRAO J. Breed. Genet.* 54 (4) 767-779.
<http://doi.org/10.54910/sabrao2022.54.4.8>
3. Amien S, Maulana H, Ruswandi D, Nurjanah S. 2021. Genetic gain and relationship of yield and yield attributes of mutant and cross-bred stevia (*Stevia rebaudiana*) genotypes. *Biodiversitas* 22: 3119-3126. <https://doi.org/10.13057/biodiv/d220805>
4. Andolfi, L. Macchia, M. and Ceccarini L. 2006. Agronomic-productive Characteristics of Two Genotype of Stevia Rebaudiana in Central Italy. *Italian Journal of Agronomy*, 1: 257-263.
5. Anonymous, 2007. Medicinal and Aromatic Plant Cultivation Strategy. Uttaranchal Decentralized Watershed Development Project. 34 P. AZE,
6. Anonymous 2013, T.C. Trabzon İl Özel İdaresi Tarımsal Hizmetler Müdürlüğü Toprak Analiz Raporu ve Gübre Tavsiyeleri
7. Anonymous 2016, <http://www.hayrat.gov.tr/ilcemiz> (Hayrat ilçe Kaymakamlık sayfası erişim tarihi 13.02.2018)
8. Anonymous 2018c, <http://www.hayrat.gov.tr/ilcemiz> (erişim tarihi 13.02.2018)
9. Bian Y M. 1981. Studies on Stevia rebaudiana a new sweet-tasting plant: refining stevioside and determination of its concentration. *Plant Physiol. Commun* 3: 15-17 [in Chinese, English abstract.].
10. Clemente, C.; Angelini, L.G.; Ascrizzi, R.; Tavarini, S., 2021. Stevia rebaudiana (Bertoni) as a
11. Fronza, D., and Folegatti, M.V., 2003. Water Consumption of the Stevia rebaudiana Bert. Crop Estimated through Microlysimeter. *Scientia Agricola*, 60 (3): 595-599.
12. Gedik ve Tansı, 2017. Çukurova Koşullarında Bitki Yoğunluğu ve Bitki Yaşının Şeker Otu (*Stevia rebaudiana* Bertoni)'nın Verimine Etkisi Araştırma Makalesi / Research Article Iğdır Üni. Fen Bilimleri Enst. Der. / Iğdır Univ. J. Inst. Sci. & Tech. 7(3): 285-292.
13. Goyal, S.K., Samsher, A., and Goyal, R.K., 2010. Stevia (*Stevia rebaudiana*) a Bio-Sweetener: a Review. *International Journal of Food Sciences and Nutrition*, 61 (1): 1–10.
14. Huang Y S, Guo A G, Qian Y, Chen L Y, Gu H F. 1995. Studies on the variation of steviosides content and selection of type R-A in Stevia rebaudiana. *J. Plant Res. Environ* 4: 28-32 [In Chinese, English summary.]
15. Kennelly, E.J. 2001. Sweet and non-sweet constituents of *Stevia rebaudiana*. *Stevia: The Genus Stevia*, pp. 68-85, Ed. Douglas Kinghorn.

16. Kohda, H., Kasai, R., Yamsaki, K., Murakami, K., and Tanaka, O., 1976. New Sweet Diterpene Glucosides from *Stevia rebaudiana*. *Phytochemistry*, 15 (6): 981-983.
17. Kovvilyaeva, G.I., Bakaleinik, G.A., Strobrykina, I.Yu., Gubskaya, V.I., Sharipova, R.R., Alfonsov, V.A., Kataev, V.E., and Tolstikov, A.G., 2007. Glycosides From *Stevia rebaudiana*. *Chemistry of Natural Compounds*, 43 (1): 81-85.
18. Kumar, R. Sharma, S. Ramesh, K. Prasad, R. Pathania, V.L. Singh, B. and Singh, R.D. 2012. Effect of agro-techniques on the performance of natural sweetener plant–stevia (*Stevia rebaudiana*) under western Himalayan conditions. *Indian Journal of Agronomy*, 57 (1): 74-81.
19. Landazuri, A.P., and Tigrero, S.J., 2009. *Stevia rebaudiana* Bert. *Planta Medicinal*. Escuela Politecnica del Ejercito. Departamento de Ciencias de la Vida, Carrera de Ingeniería en Ciencias Agropecuarias, 33 P.
20. Lankes, C., and Pude R., 2008. Possibilities for Growth of *Stevia* in European Temperate Zones. In *Steviol Glycosides: Technical and Pharmacological Aspects*. 2 *Stevia Symposium* Organized by Eustas. Kuleuven, 7: 103- 115.
21. Lemus-Mondaca, R., Vega-Gálvez, A., Zura-Bravo, L., Ah-Hen, K. 2012. *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: a comprehensive review on the biochemical, nutritional and functional aspects. *Food Chem.*, 132: 1121–1132.
22. Mitali Mahajan, Anuradha, Probir Kumar Pal, 2021. Attaining higher biomass and steviol glycosides yields of *Stevia rebaudiana* through adjustment of plant population and nitrogen rate. *Industrial Crops and Products*, Volume 165, <https://doi.org/10.1016/j.indcrop.2021.113426>.
23. Moraes RM, Donega MA, Cantrell CL, Mello SC, McChesney JD, 2013. Effect of harvest timing on leaf production and yield of diterpene glycosides in *Stevia rebaudiana* Bert: A speciality perennial crop for Mississippi. *Industrial Crops and Products*. 51. 385– 389.
24. Multifunctional and Sustainable Crop for the Mediterranean Climate. *Agriculture*, 11, 123. <https://doi.org/10.3390/agriculture11020123>
25. Samadpourrigani E. (2014) Çukurova koşullarında şeker otu (*Stevia rebaudiana* B.)’nda farklı ekim sıklıkları, biçim zamanları ve biçim sayılarının verim ve kaliteye etkisi tez çalışması
26. Shivani Kumari, Gautam Ghosh, M.R. Meshram, 2021. TSS, Yield and Energetics of *Stevia* as Influenced by Nitrogen Levels and Spacing under Eastern U.P. Conditions. ***Agricultural Science Digest, Volume 41 Issue 2: 319-323***
27. Shyu Y T. 1994. Effects of harvesting dates on the characteristics, yield, and sweet. *J. Agric. Res. China* 43: 29-39.
28. Sözmen, U. E. (2015). Şeker Otu (*Stevia rebaudiana bertonii*) Bitkisinin Bazı Verim ve Kalite Özellikleri Üzerine Farklı Azot Dozlarının Etkisi. Akdeniz Üniversitesi, Fen Bilimleri Enstitüsü, Tarla Bitkileri Anabilim Dalı, Doktora Tezi, Antalya.
29. Starrat A N, Kirby C W, Pocs R, Brandle J E. 2002. Rebaudioside F a diterpene glycoside from *Stevia rebaudiana*. *Phytochemistry* 59: 367-370 Crossref, Medline.
30. Strauss S. 1995. The perfect sweetener. *Technol. Rev.* 98: 18-20.

31. Tadesse N, Gebere A, Lulie B, Hordofa M, 2016. Influence of plant population density on growth and yield of *Stevia* (*Stevia rebaudiana* Bertoni L.) at Wondo Genet South Ethiopia. Acad. Res. J. Agri. Sci. Res. 4(6): 321-329.
32. Taleie, N., Hamidoghli, Y., Rabiei, B., and Hamidoghli, S., 2012. Effects of Plant Density and Transplanting Date on Herbage, Stevioside, Phenol and Flavonoid Yield of *Stevia rebaudiana* Bertoni. International Journal of Agriculture and Crop Sciences., 4 (6): 298-302.
33. Weng X Y, Sun J Y, Zang R C. 1996. Study on the growth and physiological characteristics of *Stevia rebaudiana* SM4. J. Zhejiang Agric. Univ 22: 538-540 [in Chinese, English abstract.]
34. Youssef, M.A., Yousef, A.F., Ali, M.M. 2021. Exogenously applied nitrogenous fertilizers and effective microorganisms improve plant growth of stevia (*Stevia rebaudiana* Bertoni) and soil fertility. *AMB Expr* 11, 133 (2021). <https://doi.org/10.1186/s13568-021-01292-8>