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The Effects Of Planting Density, Cutting Stage and Cutting Number On Yield and Quality Of *Stevia rebaudiana* B.

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Abstract: To avoid harmful health effects of synthetic sugar and also due to human attention to supply sugar from natural sources, the interests of the sugar plants cultivation, especially *Stevia rebaudiana* have been increased. A field experiment was conducted to study the effect of planting density, cutting stage and cutting number on *Stevia*. In Çukurova region, which is classified as Mediterranean climate. The experiment conducted in a split plot design consisted of three planting density, 30 × 60, 45 × 60 and 60 × 60 cm in the main plot and two cutting time in various growth stages in subplots with three replications. Since there were two cuttings in the season, the differences between cuttings, statistical analysis was valued by using split-split plot design in time. Planting density significantly affected the dry leaf yield and the higher yield (110.9 kg da-1) was obtained from the plants, spaced 30 × 60 cm. Plants cutting at flowering stage had high dry leaf weight (20.55 g plant-1) and yield (84.70 kg da-1). Also, more dry leaf weight and yield were obtained from first cuttings. Leaves of closer plant densities produced high content of stevioside. Pre-flowering stage had significant effect on stevioside in the affirmative way.

Keywords: *Stevia*, cutting, density, stevioside, yield

Stevia rebaudiana B.'nin Verim ve Kalitesi Üzerine Bitki Sıklığı ve Biçim Zamanlarının Etkisi

Öz: Sentetik şekerin sağlığa zararlı etkilerinde kaçınmak, ayrıca doğal kaynaklardan şeker tedarik etmeye insanoğlunun ilgisi nedeniyle, şeker bitkilerine özellikle şeker otunun tarımına olan ilgi artmaktadır. Ekim sıklığı, biçim dönemi ve biçim sayısının şeker otu bitkisine etkisini incelemek amacıyla bir tarla denemesi yürütülmüştür. Akdeniz iklimine sahip olduğu bilinen Çukurova bölgesinde, Bölünmüş parseller deneme deseninde ekim sıklığı, 30 × 60, 45 × 60 ve 60 × 60 cm, ana parselleri ve değişik zamanlardaki biçimler alt parselleri oluşturmak üzere 3 tekrarlamalı bir deneme yürütülmüştür. Bir yetiştirme sezonunda 2 biçim olduğu için biçimler arası fark zamanda katılarak Bölünen bölünmüş deneme deseni kullanılarak istatistiksel olarak değerlendirilmiştir. Ekim sıklığı kuru yaprak verimine önemli etkide bulunmuş ve 30x60 cm mesafesindeki bitkilerden daha yüksek verim (110.9 kg da-1) elde edilmiştir. Çiçeklenme dönemi biçilen bitkiler yüksek kuru yaprak ağırlığı (20.55 g plant-1) ve kuru yaprak verimine (84.70 kg da-1) sahip olmuşlardır. Ayrıca birinci biçimden daha yüksek kuru yaprak ağırlığı ve verimi elde edilmiştir. Artan bitki sıklıklarından elde edilen yapraklar daha yüksek stevioside içeriğine sahip olmuştur. Çiçeklenme öncesi dönem stevioside üzerine önemli derecede olumlu etki yapmıştır.

Anahtar Kelimeler: Şeker otu,biçim, yoğunluk, stevioside,verim

1. Introduction

World population growth increased demand for food and human food security is threatened, particularly in the developing countries. Many indigenous medicinal plants are also widely used as foods (Hill 1952). On the other hand, with changing consumer relish the worldwide demand for high-potency sweeteners is expected to increase, especially with the new practice of blending various sweeteners (Brandle et al. 1998). The sweet herb, Stevia rebaudiana Bertoni plants have been used for centuries by

the indigenous Guarani Indians of South America, as a traditional sweetener. The plant belongs to the Asteraceae family (Brandle et al. 1998; Geuns 2003), which comprises of approximately 50.000 species of perennial herbs and shrubs. Dry leaves are the economic part in *Stevia* plant. Stevia dry leaves have taste 20-30 times sweeter than sugar cane but without any calories, importantly. Hence, Stevia is a potential natural source of no calorie sweetener, alternative to the synthetic sweetening agents like saccharine, aspartame, asulfam-K that are available in the market to the diet conscious consumers and diabetics (Aladakatti et al. 2012). The leaves of *Stevia* content steviol glycosides, stevioside and rebaudioside, which estimated to be 300 times sweeter than sugar but also have no effect on blood sugar, so it is beneficial for hypoglycaemia and type 2 diabetes (Soejarto 2002; Ramesh et al. 2006). Stevioside is described as a glycoside comprising three glucose molecules attached to an aglycone, the steviol moiety (Barriocanal et al. 2008). As stevioside is the largest part of the steviol glycosides in the plant, *Stevia* leaf extracts are sometimes mistakenly called stevioside (Anonym 2010). Stevioside has a sweetening power comparable to that of artificial sweeteners presently. The leaves, as well as the pure stevioside extract, can be used in its natural state or cooked, and are thermostable at temperature up to 200°C (Lemus-Mondaca et al. 2012). Toxicological studies have shown that stevioside does not have mutagenic, teratogenic or carcinogenic effects. Likewise, allergic reactions have not been observed when it is used as a sweetener (Pól et al. 2007). Also, literature survey reveals *Stevia*'s versatile applications in treatment of obesity, weight loss (Gregersen et al. 2004). The glycosides found mainly in the leaves of the plant, make up to 15% of the content, depending on variety (Giraldo et al. 2005). The amount sweet glycosides in the leaves of *Stevia* is influenced by growing conditions (Pól et al. 2007), as well as on the adoption of modern agronomical techniques (Geuns 2003). Among the different factors affected on plant growth and development, climatic conditions have an important role in the efficiency of crops. For prosperous cultivation of any production, crop should be exposed to optimum climatic conditions during the growing cycle, so as to get maximum growth and yield of *Stevia* plant (Maheshwar 2005). Agricultural factors such as plant density, transplanting date, harvest time and quality can be obtained is another important requirement for profitable cultivation of this crop. It is also important to harvest the crop when both leaf biomass and steviol glycoside content are at maximum level. Taleie et al. (2012) reported that transplanting date and spacing significantly affected plant height, herbage (leaf and stem) fresh and dry weights and also stevioside yield.

This study was conducted to determine effects of planting density, ontogenetic stage and cutting on yields and stevioside content of Stevia rebaudiana under the Çukurova conditions in Turkey.

Table 1. Some meteorological data of experiment region during crop growth (2013)
Çizelge1.*Ürünün yetişme süresince deneme alanına ilişkin bazı iklim değerleri (2013*)

Month	Temperature (˚C)			Rain (mm)	Average of Relative Humidity (%)	Average of Day Length (hour)
	Minumum	Maximum	Average			
April	13.20	25.40	19.30	100.20	63.25	13.08
May	17.90	29.90	23.90	61.50	64.38	14.11
June	20.80	31.50	26.20	0.90	59.63	14.47
July	24.00	34.00	29.00	0.00	63.97	14.32
August	23.90	35.40	29.70	19.80	67.31	13.47
September	20.60	32.30	26.50	31.90	59.09	12.34
October	13.80	27.86	20.80	40.10	49.86	11.13
November	13.20	24.40	18.80	6.10	61.35	10.17
December	6.40	15.90	11.20	21.50	48.96	9.48

Source: Turkish State Meteorological Service

2. Methodology

This study was conducted in the research field of field crops department, Çukurova University (37°00'54.06" N and 35°21'26.17" E, 14 m below sea level) that is a part of Çukurova region, Turkey.

Most of the Çukurova region is a large stretch of flat, fertile land which is among the most agriculturally productive areas of the world that region is located in the mediterranean climate of Turkey with relatively mild and wet winters and warm and dry summers (Table 1) *Stevia rebaudiana* Bertoni seeds as the experiment material previously were supplied from Paraguay. Seeds germination was done under controlled conditions in the germination room with 24 ˚C constant temperature and continuous fluorescent light in 21 March 2013. After 5-days, the first germination was determined in plastic cups (sized 20x20 cm). Also, seed bed was formed from equal proportions of soil, barnyard manure and turf combination.

When the seeds germinated and reached to 2-3 cm height, they were transferred to the greenhouse. After that when seedlings grew to 6-8 leaf stage were transferred to field on 29 April 2013 and transplanted. A split plot field experiment was conducted in a randomized complete block design with three replications. Main plot consisted of three planting density, 30 × 60, 45 × 60 ve 60 × 60 cm, providing approximately 55556.5, 37037 and 27777.8 plants ha-1 respectively. The subplot consisted of two cutting time in various growth stages, pre-flowering and beginning of flowering. Two thirds of Nitrogen and whole of the P and K was applied at the time of transplanting and the remaining of N was applied after 1st and 2nd cuttings equally. Irrigation and other field practices was done during the seasons. Also, date of cuttings, is shown in the Table 2.

Stevia plants were flowered in short days normally, but in our experiment, plants flower first time in July (14 h long day) after transplanted in April because of temperature as meteorological factors and from seed or desirable wide range photoperid (Table 2).

Table 2. Cutting dates and duration of vegetation of Stevia rebaudiana
Çizelge 2. *Stevia rebaudiana'nın hasat tarihleri ve vegetasyon süresi*

Stage	Cutting dates		Duration of vegetation (Day)	
	1th Cutting	2nd Cutting		
Pre-flowering	15.07.2013	20.09.2013	77	144
Flowering	30.07.2013	08.10.2013	92	162

Before cutting, plant height was measured from the ground to the top of plant, then eight plants randomly were cut from about 10 cm from each plot and the other measurements were implemented. After separating the leaves, stems and inflorescences, these parts were dried at 38 °C for 72 hours.

For Stevioside approximately 10 g of each dried sample with three replication was powdered with grinder, then 1 g of powdered sample was weighted and extracted with 20 ml of aqueous ethanol (80 %, v v-1). Reaction mixture was placed in boiling water bath for 80 min at 80 °C. Extracted samples were centrifuged at 5000 prm for 15 min and supernatant be waited until evaporating ethanol. The residue was dissolved with 5 ml of distilled water then filtered before HPLC analysis (Miron and Schaffer, 1991).

As for the sugars, the liquid chromatographic apparatus (Shimadzu LC-10Avp) consisted of an in-line degasser, pump and controller coupled to a refractive index detector equipped with an automatic injector (1 µL injection volume) interfaced to a PC running Class VP chromatography manager software (Shimadzu, Japan). Separations were performed on a 250 · 4.6 mm i.d., 5 µ, reverse-phase Intersil NH_2 analytical column (Shimadzu, Japan) operating at 40 °C temperature with a flow rate of 1 mL min-1. Elution was effected using an isocratic elution of Acetonitrile:Water by ratio of 60:40 as a solvent. Components were identified by comparison of their retention times with those of

authentic standards under analysis conditions and quantified by external standard method. A 15 min equilibrium time was allowed between injections. The reproducibility of the chromatographic separation of the components was determined by making five injections of the standard solutions and *Stevia* sample.

The data were analyzed by software MSTAT-C. Since there were two cuttings in the season, for to determine the differences between cuttings, analysis was performed by using split-split plot design in time. The treatment means were compared by using the LSD test as described by Steel and Torrie (1980).

3. Results and Discussion

3.1. Plant height

Plant height was influenced significantly by densities and stages. Interactions were not significant (Table 3).

Table 3. Effect of planting density, cutting stage and cutting number on *Stevia* plant height, fresh leaf weight and fresh leaf yield

Çizelge 3. *Bitki yoğunluğu, biçim zamanı ve biçim sayısının Stevia'nın bitki boyu, taze yaprak ağırlığı ve taze yaprak verimi üzerine etkisi*

Treatments	Plant height (cm)	fresh leaf weight (g plant^{-1})	fresh leaf yield (kg da^{-1})
Densities			
30x60	58.74 a	66.03	429.7 a
45x60	53.91 b	71.41	251.5 b
60x60	52.29 b	68.03	165.9 c
LSD (5 %)	2.14	ns*	70.42
Stages			
Pre-flowering	51.27 b	65.65	265.7
Flowering	58.70 a	71.33	299.1
LSD (5 %)	3.64	ns*	ns*
Cuttings			
1st cutting	55.78	94.37 a	381.9 a
2nd cutting	54.18	42.61b	182.9 b
LSD (5 %)	ns*	7.74	32.06

ns: non-significant

The highest plant height (58.74 cm) was obtained from the lowest densities (30*60 cm) (Table 3). Similarly Aladakatti (2012) reported that as distance of plants decreases plant height considerably increased. Plant height was increased at closer spacing. This finding was consistent with the data reported by Taleie et al. (2012) who observed that taller *Stevia* plants were achieved by the closer spacing (50×20 cm). In contrast, Lee et al. (1980) had reported that plant height was unaffected by plant density of 50- 70 cm inter-row and 10-30 cm intra-row spacing. In terms of ontogenetic stage plant height were varied significantly. The highest plant height was determined in flowering stage (Table 3). As similar to findings (Shyu et al. 1994; Kumar et al. 2012b; Maheshwar 2005) reported that plant height increased as duration of growing increased.

3.2. Fresh leaf weight and yield

Fresh leaf weight per plant not affected significantly by different densities but fresh leaf yields were affected significantly (Table 3). The highest fresh leaf yield (429.7 kg da-1) was obtained from the lowest plant spacing (30x60 cm) (Table 3). Fresh leaf weight and fresh leaf yield were affected by different cutting numbers x cutting stages interaction. The highest fresh leaf weight and yield were obtained from first cutting and flowering stage (Table 4). These results showed great similarity to Talei (2012), reporting that this increasing density not only raises plants performance per m^2 but also reduces the weed growth. Also, fresh leaf yield was affected by different cutting times x cutting stages x plant densities interaction. The highest fresh leaf yield (680.6 kg da-1) was obtained from the plots with highest plant density (30x60 cm), in the first cutting during the pre flowering stage (Table 5)

Table 4. The values of fresh leaf weight and fresh leaf yield regarding cutting stage x cutting number interaction

Çizelge 4. *Biçim zamanı xbiçim sayısı interaksiyonuna ilişkin taze yaprak ağırlığı ve taze yaprak verimi değerleri*

Cutting Stages	Fresh leaf weight (g plant $^{-1}$)		Fresh leaf yield (kg da $^{-1}$)	
	Cutting number		Cutting number	
	1th	2nd	1th	2nd
Pre flowering	79.52 b	51.78 c	313.5 b	218.2 c
Flowering	109.22 a	33.44 d	450.6 a	147.6 d
LSD (5 %)	10.94		45.34	

Leaf yield increased with increasing density from 8300 to 11100 plant/da in *Stevia* for the first year of production Madan et al. (2010). The decrease in fresh leaf yield per unit area until area with increasing plant spacing was reported by Madan et al. (2010) in *Stevia.* Also, Taleie et al. (2012) reported that decreased in fresh leaf yield with increased plant spacing in *Stevia*. Fresh leaf weight and yield increased as stages develops from pre flowering to flowering because of length of time of life but these increases were not statistically significant (Table 3)..

Table 5. The values of fresh leaf yield regarding planting density x cutting stage x cutting number interaction (kg/da)

Çizelge 5. *Biçim zamanı x biçim sayısı x bitki yoğunluğu interaksiyonuna ilişkin taze yaprak verimi değerleri (kg/da)*

Plant Densities	Pre-flowering stage		Flowering stage	
	Cutting number		Cutting number	
	1th	2nd	1th	2nd
30x60	407.9 b	348.6 bc	680.6 a	281.8 ce
45x60	312.7 cd	195.3 f	404.9 b	93.0 g
60x60	218.9 ef	110.7 g	266.2 df	68.0 g
LSD (5 %)	78.53			

The fresh leaf per plant and fresh yield obtained from first cutting were the higher than those of second cutting (Table 3). First cuttings were made in long days (Table 1). Fresh leaf weight and fresh leaf yield were affected by day lenght in different cutting times. Similarly Metivier and Viana (1979), reported that the biomass accumulation of *Stevia* plants are markedly affected by environmental conditions. Ramesh et al. (2006) reported that growing plants under long day conditions provides a good opportunity to enhance leaf mass. Allam et al. (2001) reported that research conducted in Egypt revealed that climatic conditions, such as temperature, and length and intensity of photoperiod, greatly affect *Stevia* production and quality.

3.3. Dry leaf weight and yield

Despite the fact that the effect of plant density was not significant, the density of 30×60 cm had the maximum dry leaf weight per plant (Table 6). Plants which were planted with a close distance on the rows (30×60 cm) produced significantly higher dry leaf yield (110.9 kg da-1) than 45×60 cm and 60×60cm spacing levels, but there wasn't any significant difference between 45×60 cm and 60×60 cm, statistically (Table 6). Ramesh et al. (2006) reported that *Stevia* plug plants are then planted in to the field on either 53 cm or 61 cm row spacing with a total plant density on the order of 100.000 plants per hectare. However, different climatic conditions would influence *Stevia* cultivation, so it is advisable to carry out trials in each planting zone to establish adequate plant population density for that particular area.

Plants harvested at flowering stage had high dry leaf weight and yields than pre-flowering stage, respecively 20.55 g plant-1, 84.7 kg da-1 (Table 6.) Dry leaf weight per plant at flowering stage was higher than pre-flowering stage. Increasing dry leaf weight per plant may be associated with increasing number of branches and leaves per plant.

Naturally, the plants harvested at flowering stage had more time for development and completion their maturity than the plants

harvested at pre-flowering stage. Hence, dry leaf weight per plant was higher at flowering stage. Kumar et al. (2012a) reported that along the increasing development stage of *Stevia*, dry leaf weight per plant increased.

Table 6. Effect of plant density, cutting stage and cutting number on dry leaf weight, dry leaf yield and stevioside content of *Stevia*

Çizelge 6. *Stevia'nın kuru yaprak ağırlığı, kuru yaprak verimi ve stevioside oranı üzerine bitki yoğunluğu, biçim zamanı ve biçim sayısının etkisi*

Treatments	Dry leaf weight (g plant^{-1})	Dry leaf yield (kg da^{-1})	Stevioside (%)
Densities			
30x60	19.96	110.9 a	5.67 a
45x60	18.89	70.0 b	4.68 ab
60x60	16.45	45.7 b	4.62 b
LSD (5 %)	ns*	27.99	0.82
Stages			
Pre-flowering	16.32 b	66.3 b	5.44 a
Flowering	20.55 a	84.7 a	4.53 b
LSD (5 %)	2.953	12.23	0.54
Cuttings			
1th cutting	25.20 a	101.2 a	4.48 b
2nd cutting	11.67 b	49.9 b	5.49 a
LSD (5 %)	2.887	11.98	0.52

ns: non-significant

Stevia plants start flowering from July to September in the northern hemisphere. Subsequent flowering occur in rapid succession as regrowth from the plant crown grows shorter each time until winter in July (Shock 1982). Cutting stage and cutting number significantly affected dry leaf weight per plant (Table 6). The highest leaf weight and yields (25.20 g plant-1, 101.2 kg da-1) were obtained at first cuttings, in mid July (Table 6). Mishra et al. (2010) reported that, the quantity of dry leaves can be harvested ranging from 15 to 35 g plant-1. Also Serio (2010) reported, the quantity of dry leaves varied from 100 to120 kg da -1.The temperature is avaliable for growing of *Stevia* plants from transplanting (April) to first cutting (mid July). Dry leaf yields were affected negatively by high temperature from first cutting (mid July) to second cutting (October) during summer (Table 6) First cuttings were performed under long day conditions (Table 3). Long-day conditions, as compared with short days, increase internode length, leaf area, and dry weight (Yadav et al. 2011). The *Stevia* plant actively grew in the spring-summer period (from April to September), when the mean temperatures were above 20 ◦C. In autumn, the vegetative growth decreased and the aboveground portion of plant gradually became dry (Angelini and Tavarini (2014) Day length draw in from first cutting to second cutting (Table 1). In this period, relatively long day in July and August affected constructively on growing plants. High temperature and short day, together with flowering, caused by fall leaves in the lowest part of plant in second cutting (Table 7).

Table 7. The value of dry leaf weight and dry leaf yield regarding cutting stage x cutting number interaction

Çizelge 7*.Biçim zamanı ve biçim sayısı interaksiyonundan elde edilen kuru yaprak ağırlığı ve kuru yaprak verimi değerleri*

Stages	Dry leaf weight (g plant $^{-1}$)		Dry leaf yield (kg da $^{-1}$)	
	cutting number		cutting number	
	1th	2nd	1th	2nd
Pre flowering	19.90 b	12.73 c	79.1 b	53.5 c
Flowering	30.50 a	10.61 c	123.2 a	46.2 c
LSD (5 %)	4.08		16.95	

Also, dry leaf weight and dry leaf yield were affected by different cutting stages. The highest dry leaf weight and yields were obtained from the plots in the first cutting during the flowering stage (Table 7). Also, Ramesh et al. (2006) and Sh et al. (2006) noted that growing plant under long day conditions provides a good opportunity to enhance leaf mass.Similar results were reported by Metivier and Viana (1979), Ceunen et al. (2012) and Tavarini and Angelini (2013). et al (2006) reported the growth and flowering of *Stevia* are affected by radiation, day length, temperature, soil moisture, and wind. The stevioside content in *Stevia rebaudiana* leaves varies according to genotype, phenological stage, and growth conditions (Brandle and Rosa 1992; Yadav et al. 2011). It is generally accepted that stevioside content increases gradually up to the budding phase and the onset of flowering (Bondarev 2003; Ceunen and Geuns 2013). Stevioside is influenced by environmental and soil conditions. During ontogeny, a gradual increase in the stevioside concentration was observed in both mature leaves, and this process lasted to the budding phase at the onset of flowering (Bondarev et al. 2003). Kang and Lee (1981) demonstrated that the maximal content of stevioside in leaves is achieved during the formation of flower buds and it then gradually declines. All this information may indicate that the steviol glycosides are transported to generative organs. Once flowering is initiated, glycoside concentrations in the leaves start declining (Singh and Rao 2005). Since glycoside synthesis is reduced at or just before flowering, delaying flowering with long days allows more time for glycoside accumulation (Metivier and Viana 1979; Singh and Rao 2005). Second cutting had significantly high content of stevioside in leaves than 1st cutting (Table 6). The number of long day in second cutting were higher than first cutting. Thus *Stevia* production is best suited to a long day environment, where vegetative growth is longer and steviol glycoside yields will be higher. This was confirmed further by Metivier and Viana (1979). Additionaly, Angelini and Tavarini (2014) were stated that the steviol glycoside yield was significantly influenced by the timing of cutting. Also, Angelini and Tavarini (2014) and Megeji et al. (2005), reported that dry leaf yield and stevioside production varied depending on the

3.4. Stevioside content

Stevioside content significantly varied with densities, ontogenetic stages and cuttings (Table 6). Stevioside in the dry leaves was changed between 4.48 and 5.67 % (Table 6). Similiarly, Stevioside was reported to be the most abundant Stevia glycoside (4–13 %) found in the plant leaves (Makapugay et al. 1984). Also, Andolfi et al. (2006) reported that Stevioside ranged from 3 to 10 %. Yadav (2011) reported, stevioside content in dry leaves was 5-10 %. There are reports of stevioside content (total glycosides) ranging between 4 and 20% on a dry weight basis, depending on the cultivar and growing conditions (Kennely 2002; Starrat et al. 2002). Stevioside content (%) in leaves was significant affected by different planting densities (Table 6). By increasing the distance between the plants on the rows, stevioside content decreased and ranging from 4.62 % to 5.67 %. Tateo et al. (1998) concluded that environmental and agronomic factors have more influence on stevioside production. Plants harvested at pre-flowering stage significantly produced high stevioside content than flowering stage, (5.44 and 4.53 % respectively) (Table 6). Similarly were reported harvesting should be before flowering at flower bud appearance as the stevioside content of the leaves falls when flowering commences (Bian 1981 and Hoyle 1992). *Stevia* plants were flowered twice in July and October in 2013 (Table 2). Dwivedi (1999) reported, the sweetness in the leaves is two times higher than that in inflorescence. Plants starts florescence during relatively long days in first cutting and short day in second cutting under our conditions (14.32,11.13 h) (Table 1). *Stevia* is short days plant. *Stevia* is highly sensitive to the day length and it requires 12-16 h of sun light (Ramesh et al. 2006). However, Valio and Rocha (1966) reported that a photoperiod of 13-14 h might be necessary. Sh

harvest time and they concluded that the best results in terms of leaf dry yield and stevioside production were achieved in the 1year-old crop, harvested in September. Although dry leaf yield was found to be low our conditions, we, similarly, found that stevioside production were higher in the second harvest in September and early October in the 1year-old crop. Because day length decreased from first cutting to second cutting transition time from vegetative to generative stage was shorter (Table 6), adversly affecting dry leaf yield.The period between first cutting and second cuttings coincided with hot mounts in summer seasons (Table 1). Also, according to the Table 1, it seems that the long growing duration and suitable environmental conditions have led to high dry leaf weight per plant at first cutting, but same tendency was not shown in stevioside value in first cutting. Metivier and Viana (1979) and Kang and Lee (1981) were reported that the long day conditions significantly increased leaf biomass and stevioside content in *Stevia* leaves. Second cutting was occurred in short days in our conditions the fact remains that vegetation duration (162 day) is higher than first cutting (144 day) (Table 2). Although, this results look like incompatible with Metivier and Viana (1979) and Kang and Lee (1981). Our results compatible with results reported by other authors that, a stevioside is not only influenced by photoperiod but also by the cultivar variations (Blumental 1996), temperature growing conditions and genotype (Bondarev et al. 2003) and also reported that Nakamura and Tamura, (1985) and Tamura et al. (1984), propagation by seeds does not allow the production of homogeneous populations, resulting in great variability in important features like sweetening levels and composition.

4. Conclusions

Stevia rebaudiana was cultivated for the first time under the Çukurova conditions in Turkey with this study. *Stevia* originates from the highland areas of tropical Paraguay, but has successfully adapted to the cooler, temperate climate of Mediterranean. The results show that such as plant density and cutting stage and cutting number could significantly affect leaf yields and stevioside content. The optimal cutting time for high stevioside content is at the pre-flowering. Further work is necessary focusing on high densities less row spacing applied from ours.

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