Original Article

DRY BIOMASS AND GLYCOSIDES YIELD FROM S*tevia rebaudiana* LEAVES UNDER DIFFERENT HARVESTING TIMES

PRODUTIVIDADE DE BIOMASSA SECA E GLICOSÍDEOS EM *Stevia rebaudiana* CULTIVADA EM DIFERENTES PERÍODOS DA ESTAÇÃO DE CRESCIMENTO

Carlise PEREIRA[1]; Lindolfo STORCK[1]; Sidinei José LOPES[1]; Thomas Newton MARTIN[1]; Dilson Antonio BISOGNIN[1]

1. Engenheiro Agrônomo, Doutor, Programa de Pós-graduação em Agronomia, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil. carlise.pereira@gmail.com

ABSTRACT: The *Stevia rebaudiana* (Bert) Bertoni is a perennial plant native in the Amambay Hills in the South America. The leaves of this plant produce various natural sweeteners useful to replace the human needs of saccharine. The aims of this study were to evaluate the dry biomass and the glycoside concentration in the Stevia leaves along different growing periods of the growing season to determine the best time to harvest the crops. We selected and identified six groups of 20 plants for analyses. The highest plant yield was found in December, from the first harvest for the groups 3, 4 and 2 with 292.4; 285.2 and 206.7 g m^{-2}, respectively. The sweetener concentrations and the glycosides ranged within the harvests and the compounds analyzed. The highest concentrations of stevioside (12.16% - group 1 and 11.36% - group 5) and rebaudioside C (2.43% - group 5 and 1.95% - group 1) were found in January while rebaudioside A had the highest concentrations of 7.01% (group 6); 6.16% (group 4) and 6.15% (group 3) in December, February and March, respectively. The environmental conditions have influence in days to harvesting as well as in the concentration of glycosides.

KEYWORDS: Steviol glycosides. Environmental conditions. *Stevia rebaudiana.*

INTRODUCTION

Since the seventies, the international research community has investigating natural steviol-glycosides from the *Stevia rebaudiana* leaf extracts because they are sweetener than sucrose (BRANDLE; ROSA, 1992). The plant is native from the Paraguay, in South America where it has been sweeten drinks of indigenous people. The *Stevia rebaudiana* has widespread worldwide and currently is consumed and approved in many countries as Japan, Brazil, Colombia, China and Paraguay because of the human demand by natural products with low calories.

Different periods of luminosity affect many metabolic changes during the plant growth as the starch accumulation, respiration and photosynthesis (MICHELET; LISZKAY, 2012), and interfere with the movement of petals and leaves, stomata opening and metabolic processes (TAIZ; ZEIGER, 2009). This cyclic mechanism, called the circadian rhythm, governs the changes over the different climatic seasons of the year, allowing plants to adapt to new growth conditions according to the latitude and longitude (HIGUCHI et al., 2011). Plants can be classified in three different groups based on their photoperiod responses: short-, long- and neutral-day plants (TAIZ; ZEIGER, 2009). Short-day plants require less time of lighting than the critical day length and the reproductive stages are stimulated by the length of the dark period, when short nights are necessary to induce the plant flowering (TAIZ; ZEIGER, 2009). *Stevia rebaudiana* behaves as short-day plant, requiring about 13 hours of light (LIMA FILHO et al., 2004; CEUNEN et al., 2011). Plants adjust their photosynthetic apparatus based on the presence of light and their growth is the result of the light efficiency. Furthermore, photosynthetically active radiation ranges along region, growing season and it affects the plant phenology, biomass accumulation, and modify the planting dates (OROZCO et al., 2012).

The sweetener productivity is measured by the concentration of glycosides in the dry biomass of the leaves, which range within the environmental conditions (BRANDLE; ROSA, 1992; WOELWER-RIECK, 2012; SERFATY et al., 2013) and the stages of development. The greatest glycosides concentration is evaluated when plants are blooming and flowering (CEUNEN; GEUNS, 2013a), making this period optimal for harvesting the raw produce. Climatic factors such as solar radiation, temperature and day length (CEUNEN; GEUNS, 2013a) as well as the soil classification, soil fertility, population density and genetic factors also have influence on the synthesis of the glycosides (OROZCO et al., 2012). These factors are important in the photosynthetic processes and consequently have an effect on the crop yield and glycosides quality. However, no studies on the effects of the harvesting time on the glycosides productivity and quality are easily available, which

Received: 12/02/16
Accepted: 05/10/16

led us to develop this experiment under the environmental conditions of the central region of the Rio Grande do Sul State, in Southern Brazil. The aim of this study was to evaluate the growth and development of *Stevia rebaudiana* at different growing periods along the year to determine the best time for harvesting the leaves based on their dry biomass of leaves and glycosides concentration.

MATERIAL AND METHODS

Climatic parameters

We cultivated the Stevia plants under field conditions at 29° 41' SL and 53° 48' WL, and altitude of 95 m during the growing season 2011/2012 and 2012/2013. Based on Köppen's classification, this region has the Cfa weather conditions (humid subtropical), characterized by hot summers without dry season (HELDWEIN et al., 2009). The environmental temperature, solar radiation and rainfall data were obtained from the automatic meteorological station at 100 m from the experimental field. The mean solar radiation and the environmental temperature were estimated within a daily period of 24 hours. We used only positive values for solar radiation in these estimates. The day length of every growing season was the mean light hours for days from sprouting day (first day of plant evaluation) until the harvest. We recorded the rainfall using the water accumulation in millimeter within every period. In the Table 1, we printed the meteorological data.

Cropping and harvesting

In the soil fertilization, we applied 10 kg of N as urea, 90 kg of P_2O_5 (super-phosphate single), and 60 kg ha^{-1} of potassium chloride. The weed control was manual and when necessary non-selective herbicide was applied in a targeted manner.

Seeds harvested in the glasshouse of the Laboratory of Seed Science and Technology of the Departamento de Agronomia, Paraná State University at Maringá, Paraná State, in the facilities of the Iguatemi Research Farm were sown in October 19th 2011 in multicellular trays with 128 cells filled with commercial substrate for seeds and vegetables (H. Decker products). We raised the bedding plants under greenhouse conditions where we wetted them on a daily basis. Twenty-eight days after the sowing, we transplanted these bedding plants, into plastic black bags (8 x 10 x 13 cm) filled with the same growing media. The percentage of seed germination of 49% was counted within ten days after sowing, and sixty-seven percent of the bedding plants survived under field conditions. Some bedding plants were re-transplanted within 18 days. During the growing periods, from August 23th , 2012 to April 6th , 2013, 12 harvests was performed in plants from six groups. In December, 16th 2011, when the plants developed 10 pairs of expanded leaves, they were transplanted onto nursery beds under field conditions. Every field bed of 20.0 m in length and 5.0 m in width had the bedding plants established under spacing of 50 cm between the rows and 30 cm between the plants. We watered these plants every day. In March, 27th 2012, at the beginning of the flowering period, the plants were cut at 5.0 cm above the ground to grow up uniform their above ground parts. Thereafter, we applied nitrogen using urea as the N source based on recommendations (20 kg ha^{-1}) for crop growth (LIMA FILHO et al., 2004). The period between March and August, 2012 corresponded to the end of summer and beginning of winter time in the region, which is not favorable to the plant growing season. For this reason *Stevia* plants were not evaluated on this time. In August, 23th 2012, at the beginning of the new plant growing season, we selected, identified and standardized by cutting the first group of 20 sprouted plants. Thereafter, in August 23th, 2012, we selected and identified groups of 20 plants based on 21, 35, 46, 70 and 77 days after cutting the first group. We evaluated in this experiment the total of 120 plants. These six groups were established to afford different harvesting times. Uniform cutting was carried out when the plants with 5.0 cm above the ground had 50% of the plants in every group the floral bud breaking ($R_{1.5}$ stage) based on the reproductive stages of plant development (CARNEIRO, 2007). At the harvesting time the above ground parts were individually stored in paper bags and identified based on the individual plant number and group.

Biomass determination

The number of branches per plant was counted and the biomass was weighed, dried using forced air oven at 60°C until repetitive biomass. We estimate the dry biomass per plant using one digital scale with precision of 1.0 g and the dried material was stored in paper bags. The fresh and dry biomass were used to estimate the mean per point and per interval (1-p = 0.95) for the 20 plants from every group and respective harvest.

HPLC analysis

The steviol glycosides quantification in the *Stevia rebaudiana* leaves was performed at the Food Science and Technology Laboratory (Institut für

Ernährungs und Lebensmittelwissenschaften) at Bonn University, in Germany based on the governmental program for exchange young scientists between Brazil and Germany (Brazilian Science Program off the Borderlines) and sponsored by the Brazilian Agency of Research and Development (CAPES). *Stevia* leaves weighted and dried were ground (vibration grinding machine, MM 2000) to 0.12 mm in size. The extraction procedure started weighting 0.25 g of ground *Stevia* leaves in a 10 ml centrifuge tube. The samples were boiled with water in a heating block (Major Science) set at 102°C for 30 minutes. This process was repeated three times. Each extract was cooled in a bowl with ice and centrifuged (Hermle Z323K - 15 min, 8,500U). The aqueous phases were transferred to a 25 ml volume flask. This solution was used for the solid-phase extraction (SPE) in a Baker 10 SPE System. The solid-phased extraction consisted with the strata cartridges (Strata C18-E 100mg, 1ml, 55µm, 70Å) conditioned with methanol (3 ml) and water (3 ml). Aqueous sample (0.3 ml) were added to the cartridges and washed with water (3 ml) and acetonitrile/water (2:8 v/v) (5 ml). The samples were eluted with a mixture of acetonitrile/water (8:2 v/v) (2 ml). The mobile phase consisted of acetonitrile:water (85:15) (V/V) in a flow rate of 1ml/min at the temperature of 36 °C. The volume of injection was 20 µl. Liquid chromatography was performed on a Varian system consisting of a ProStar 210 pump, a Varian 510 autosampler, a 335 diode array detector set to a wavelength of 210 nm, a four-channel degasser, a Methaterm column thermostat and the Varian Galaxie chromatography data system. The device was equipped with a Luna HILIC, 5µm particle size, 250x4,6 mm Phenomenex with a fitting guardian-column. Steviol glycosides standards (stevioside, rebaudioside A and C) were obtained from WAKO Chemicals with minimum purity of 99%.

The evaluation method was determined by comparing the chromatograms obtained from the leaves samples with those of the standard solutions. The retention time of the peaks in the chromatograms of the samples were compared to those given by the standard solutions. To quantify the analytes an external calibration curve was made with steviol glycosides standard solutions with concentrations between 20 to 500 µg/ml in triplicate. The areas of the peaks in the chromatograms of the samples were measured. The relation between the retention times of the standard solution with those from the samples was used to the quantitative determination. Calibration plot equations calculated by the least-squares method were applied in order to quantify the steviol glycosides. The content of stevioside in the samples was calculated directly by the function of the calibration curve [$y = ax + b \leftrightarrow x = (y - b)/a$]. The rebaudioside A content could be calculated by using the factor 1,2 and the rebaudioside C content in a similar way using the factor 1,8. Considering the dilution and the dry weight the content of each steviol glycoside could be stated in g/100g.

Stevioside [g/100g]= (X[µg/ml] * F * 25 ml) / (E[mg] * 10)

Where:

F=3,33 if 0,33 ml of the extract and 1 ml of the mobile phase is used to eluate the analytes from SPE cartridge.

X=content of steviol calculated from the calibration curve (µg/ml).

E=weight of the sample (mg).

The principle of the quantitative analysis of steviol glycosides in stevia leaves used in this work followed the method described by WOELWER-RIECK (2010).

Statistical analysis

Mean and half-width of the confidence interval (1-p = 0.95), for every harvested group and character was performed using Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

Days to harvesting were different in every group and ranged from 61 to 89 days. This range was affected by the growing periods along the year and by their climatic factors (Table 1). These differences in the growing periods were also detected by Serfaty et al. (2013) where they reported the plant rate recovery (days to the beginning of sprouting, growing and flowering) ranged according to environmental conditions.

Table 1. Days to harvesting of *Stevia rebaudiana* plants in the different groups, days to the harvesting (DH) (growing period), day length (hours: min), rainfall (mm), mean temperature (MT, ^{o}C) and solar radiation (SRad, kJ m^{-2}).

Group	Cut	Beginning	Harvesting	DH	Day length	Rainfall	MT	SRad
N	Order	date	date	days	Hrs: min	mm	^{o}C	$kJ\ m^{-2}$
1	First	8/23/2012	10/23/2012	61	12:05	474.8	18.30	1021.6
1	Second	10/23/2012	1/16/2013	85	13:39	559.2	23.48	1670.4
1	Third	1/16/2013	4/06/2013	80	12:45	405.4	22.28	1552.0
2	First	9/13/2012	12/03/2012	81	13:01	367.86	20.77	1426.8
2	Second	12/03/2012	2/26/2013	85	13:48	547.4	24.04	1677.0
3	First	9/27/2012	12/17/2012	81	13:22	521.3	22.16	1504.2
3	Second	12/17/2012	2/27/2013	72	13:39	407.00	23.77	1689.6
4	First	10/08/2012	12/19/2012	72	13:32	420.6	22.67	1584.5
4	Second	12/19/2012	3/07/2013	78	13:33	459.00	23.42	1661.3
5	First	11/01/2012	1/17/2013	77	13:46	541.4	23.82	1727.8
5	Second	1/17/2013	4/06/2013	79	12:44	405.4	22.25	1545.7
6	First	11/08/2012	2/05/2013	89	13:49	504.4	23.91	1711.6

DH = Days to the harvesting (growing period).

The photoperiod also had influence on days to harvesting (bud sprouting). Although Stevia is sensitive to day length, the environmental temperature and the solar radiation are both equally important for the establishment and development of plants under field conditions. In the region where this study was established, the photoperiod was satisfactory for *Stevia* flowering in the six groups named (Table 1). Long days with long periods of luminosity allow the increased fresh biomass (SERFATY et al., 2013), despite it can also delay the flowering and the plant growth. In *Stevia rebaudiana* crops, day length has influence on the flowering and vegetative development (BRANDLE; ROSA, 1992) affecting the glycoside accumulation (CEUNEN et al., 2011). A decrease in the number of days of every growing period could be beneficial to provide a higher number of harvesting per growing season; however, this response also has effects on the dry biomass production. Planning the transplanting time to the field conditions will provide favorable environmental conditions essential for higher crop productivities. In this study, solar radiation began to increase in late September, reaching the maximal values from November to January; thereafter the levels decreased until the end of the experiment. The environmental temperature had similar behavior; however, this parameter had its highest values from December to February -- corresponding to the summer period. Rainfall was satisfactory and was not a limiting factor for the crop during the carried experiment. The availability of the soil water is important for plant development, because it has influence on root growth, absorption, and nutrient transport along the several plant components. Climatic factors, including temperature, solar radiation, humidity, and wind affect the plants during all the growing periods, affecting different processes as photosynthetic rate, evapotranspiration, water availability, plant growth and development, and their needs depend on the stage of development (SERFATY et al., 2013).

The first harvesting, carried out from plants in the groups 3, 4 and 2 whose standardized were in December, had the highest productivity (Table 2). However, this result did not recur for the following harvesting on the same groups. Evaluating the harvesting in all the groups, we can see that the second harvesting had lower productivity than the first one within the same group. This could indicate

that repeated harvesting negatively affects leaf yield because the photoperiod has an effect in plant development (sprouting, harvest time). Repeated cutting in the same group of plants interferes with the dry biomass weight, leading to a less productive second harvest, which may be linked to the date of first harvest, since it will define the recovery period for plants until the next harvest. These responses confirm the hypothesis from Serfaty et al. (2013), who concluded that *Stevia* plants submitted to many harvests at different growing seasons of the year had lower biomass productivity at the second harvest. In this case, the second harvest was done in October, period related to the beginning of autumn in the region. The main reason for this result, according to Lavini et al. (2008), could be related to temperature, solar radiation and photoperiod which can determine the optimum harvesting time of dry and fresh biomass. The yield parameters for *Stevia* (dry biomass and sweetener content) ranged according to the day length, which affects vegetative growth, as reviewed by Ramesh et al. (2006) who found these parameters higher in plants under long than short-day conditions.

The productivity of the above groups did not occur during the period in which solar radiation and temperature were too high. González et al. (2005) concluded that photosynthetic efficiency in the leaf is greater under low levels of solar radiation, because the leaves can easily be saturated with overload radiation when plants are directly exposed to the sun. Jarma et al. (2005) stated that the genotype "Morita 1" had a decrease in biomass accumulation when plants were exposed to higher radiation levels. Solar radiation compels plants to rearrange the leaf position in accordance to the radiation angle and quality of this radiation intercepted during the day (GONZÁLEZ et al., 2005). Based on temperature, the highest values can increase the respiration rate and decrease the photosynthetic efficiency (JARMA et al., 2005).

The temperature for *S. rebaudiana* development ranges from 15°C to 30°C (OROZCO et al., 2012). Lavini et al. (2008) demonstrated that high temperatures (above 36°C) can reduce productivity by affecting directly the quantity of dry biomass accumulated, with results varying from 4,6 t ha^{-1} at first harvest to 3,3 t ha^{-1} in the third one. Yields of 0,95 t ha^{-1} (dry biomass) were obtained in Israel during the spring/summer (SERFATY et al., 2013) and around 4,0 t ha^{-1} in China (YANG et al., 2013). In Colombia, the productivity can reach up to 5,5 t ha^{-1} but tends to decrease after the third harvest. In Brazil, *Stevia* crops are estimated to reach between 3,5 t and 4,5 t ha^{-1} (LIMA FILHO et al., 2004). In this study the dry biomass ranged among the groups because of the harvesting period when the estimates ranged from 0.6 to 2.9 t ha^{-1}. The yield range in the current experiment may be associated to the propagation method because of the genetic variability of the seeds that could be responsible for these values in conjunction with the needs of plant adaptation to many stressful situations. Based on results from Skirycz and Inzé (2010), plants initially respond to stress by an acute inhibitory response followed by recovery, and later there is an adaptation to the new conditions. These authors argue that this response depends on genetic variability of the species, and can range from days to hours when the plants need to adapt to newly situations. Plant growth and development are related to the ability of adapting to changing climates, which can be seen by the photosynthetic efficiency (ALMEIDA et al., 2004), because the leaf efficiency to convert light into chemical energy will affect the final dry biomass. Solar radiation, favorable temperatures and suitable rainfall are important conditions for cultivating *Stevia rebaudiana* in the central region of Rio Grande do Sul State.

Table 2. Mean and half-width of the confidence interval for number of branches per plant, dry biomass of leaves (DMLf, g plant^{-1}) and branches (DMB g plant^{-1}), leaf humidity (LfHu, %) and branches humidity (BrHu, %), dry biomass of leaves per branch (Lf/Br. g branch^{-1}) and dry biomass leaves productivity (DMLfP g m^{-2}) of Stevia rebaudiana among the different groups.

		Branches		DMLf		DMB		LfHu		BrHu			DMLfP	
Group	Harvesting	Mean	±HW	Mean	±HW	Mean	±HW	Mean	±HW	Mean	±HW	Lf/Br	Mean	±HW
1	10/23/2012	33	10	9.2	2.4	5.3	1.4	75.9	1.9	79.2	1.2	0.30	61.1	15.9
1	1/16/2013	57	15	24.5	7.3	42.2	12.6	65.0	2.2	64.7	1.4	0.46	163.5	48.5
1	4/06/2013	29	11	8.7	3.5	13.9	6.3	71.8	1.3	67.1	1.4	0.33	57.8	23.6
2	12/03/2012	57	12	31.0	8.3	23.6	8.1	71.2	1.1	71.5	7.6	0.62	206.7	55.6
2	2/26/2013	50	15	20.2	6.0	27.7	12.8	70.8	1.0	72.3	2.1	0.45	134.6	40.3
3	12/17/2012	56	13	43.9	12.6	35.6	11.3	71.6	1.4	74.7	1.8	0.82	292.4	84.3
3	2/27/2013	54	15	16.4	6.0	20.7	11.5	71.2	1.8	71.4	2.5	0.30	109.3	40.2
4	12/19/2012	64	12	42.8	12.3	30.1	9.4	71.5	1.3	74.4	1.2	0.72	285.2	81.8
4	3/07/2013	62	17	17.4	7.3	23.5	11.0	73.1	2.2	70.5	2.0	0.26	115.8	49.0
5	1/17/2013	55	13	26.4	9.0	35.5	11.8	68.1	1.4	67.4	1.1	0.51	176.2	59.8
5	4/06/2013	42	13	9.2	3.0	15.0	6.6	75.3	1.8	70.7	2.4	0.24	61.0	20.2
6	2/05/2013	48	12	21.9	10.0	29.8	15.4	67.7	1.3	67.6	1.1	0.46	146.3	66.4

(±HW, 1-p = 0.95) = Confidence intervals;

As for the geographic position, the current region has well-defined weather seasons with cold winters and very hot summers. Thus, the plant growing season have to start preferentially in October because September still has lower temperatures entirely unfavorable to plant development. Sweetener concentration and yield from the different harvesting times can be seen in Table 3, where we can see the difference among the periods. The harvesting time clearly defined the sweetener content in the *Stevia rebaudiana* leaves and the bud differentiation was presumed as the stage when high sweetener contents can be found (CEUNEN; GEUNS, 2013b)

Table 3. Contents of stevioside, rebaudioside A (Reb A) and rebaudioside C (Reb C)(%) on the dry biomass of *S. rebaudiana* leaves and sweetener productivity. The calculation followed the method of Woelwer-Rieck et al., 2010.

Group	Cutting	Stevioside	RebA	Reb C	Productivity (g m^{-2})		
					Stevioside	Reb A	Reb C
1	10/23/2012	7.90	2.94	1.54	4.83	1.80	0.94
1	1/16/2013	12.16	5.02	1.95	19.88	8.21	3.19
1	4/06/2013	6.89	3.37	1.82	3.98	1.95	1.05
2	12/03/2012	10.00	5.06	2.10	20.67	10.46	4.34
2	2/26/2013	10.07	5.61	1.55	13.56	7.55	2.09
3	12/17/2012	11.11	4.28	1.70	32.48	12.51	4.97
3	2/27/2013	8.41	6.15	1.78	9.19	6.72	1.95
4	12/19/2012	8.34	6.16	1.74	23.79	17.57	4.96
4	3/07/2013	9.90	4.95	1.58	11.47	5.73	1.83
5	1/17/2013	11.36	5.77	2.43	20.02	10.17	4.28
5	4/06/2013	7.35	3.53	0.89	4.48	2.15	0.54
6	2/05/2013	9.36	7.01	1.71	13.69	10.25	2.50
Mean		9.40	4.99	1.73	14.84	7.92	2.72

Stevioside, rebaudioside A and C were the three steviol glycosides identified and quantified in every one of the 12 harvests. The glycosides contents were related to higher temperature and solar radiation as well as day length (Table 1). As a short-day plant, *Stevia rebaudiana* requires from 12 to 13 hrs of light for the maximum accumulation of glycosides (MANDAN et al., 2010; CEUNEN; GEUNS, 2013b). The day length we found for all groups during the growth period was consistent with those found in the literature cited earlier. The harvesting in December, January and February provided greater glycosides content because of the sun light energy. Rebaudioside A had the highest content from December to February while rebaudioside C had the highest content in December and January. The results found in our study were also reported by Orozco et al. (2012) in their study from different growing periods. The SVglys tend to range in accordance with the solar radiation and temperature. According results found by Ceunen; Geuns (2013a), the accumulation of SVglys is influenced by the photoperiod, and the concentration in the leaves is higher in plants under short-day conditions comparing to long-day. In our study, short-day conditions influenced the steviol glycosides concentrations. The SVglys were higher during summer time (December, January and February) and when the photoperiod was around 13 hours. High temperatures may cause stress and alterations in biochemical reactions (ZOBAYED et al., 2005; LIANOPOULOU et al., 2014), triggering energy deviation from the primary to the secondary metabolism. (MÜLLER et al., 2013). Isopentenyl diphosphate (IPP) is the basic unit of terpene establishment (terpene chain) (TAIZ; ZIEGER, 2009) and can be synthesized via methylerythritol phosphate (TOTTÉ et al., 2000). Thereafter, it is involved in the synthesis of diterpene glycosides including stevioside, rebaudiosides A and C. IPP synthesis is influenced by light, because of the carbon fixation (CO_2), the main compound required for its biosynthesis depends on light energy (GUEVARA-GARCIA et al., 2005). Once the

glycoside molecules are synthesized into the terpene chain, it is possible to figure out that luminosity has a positive effect on its synthesis (GUEVARA-GARCIA et al., 2005). Zobayed et al. (2005), investigating temperature stress in secondary metabolite concentrations in *Hypericum perforatum* stated that temperature affects the leaf transpiration rate and modifies concentrations of active compounds of these species. Under field conditions, the climatic factors related to high steviol glycosides concentrations do not act individually; otherwise, the solar radiation is associated to high temperatures that directly are related to the transpiration rate and evapotranspiration.

The results of this study indicate that the optimum growing periods for *Stevia rebaudiana* are distinct and this factor is important to maximize the crop productivity and the quality of glycosides, because of specific conditions of temperature, solar radiation and photoperiod found in the Central Region of Rio Grande do Sul. The period should provide good conditions for yield (dry biomass volume and sweetener contents). Considering the greatest dry biomass yield obtained in December and days to harvesting ranged from 71 to 81, the transplanting should be in the second-half of September. This growing period is at the beginning of spring allowing plant growth and development under good temperature and solar radiation conditions, as well as day length above 12 hrs.

CONCLUSIONS

Temperature and solar radiation affected the steviol glycosides concentration as well as the yield of *Stevia rebaudiana* leaves.

Stevia crops should be established when the solar radiation and photoperiod are increasing in the region (from October to December), in order to maximize the dry biomass productivity and steviol glycosides content.

In the region where the study was carried out, the best time for harvesting considering the biomass was found in December whereas for the steviol glycosides the highest concentration was in December and January.

AKNOWLWEDGMENTS

The authors are thank to CAPES and CNPq for supplying the scholarships; Dr. Woewer-Rieck for the HPLC analysis, and INRES (Gartenbauwissenschaft Institute - Dr. Lankes and Dr. Hunsche) for supporting during the SwB program and Professor José Walter Pedroza Carneiro for supplying the seeds.

RESUMO: Originária na serra de Amanbaí, Paraguai, América do Sul, a *Stevia rebaudiana* é uma planta perene que possui em suas folhas edulcorantes naturais com alto poder adoçante e que podem substituir a sacarose. Objetivou-se com esse experimento avaliar a biomassa seca e a concentração de glicosídeos em folhas de *S. rebaudiana* em diferentes períodos do ano para determinar a melhor época para a colheita. Seis grupos com 20 plantas foram selecionados e identificados perfazendo um total de 120 plantas. As maiores produtividades de biomassa seca foram alcançadas no mês de dezembro quando ocorreu o primeiro corte nos grupos 3 com 292,4, 4 com 285,2 e 2 com 206,7 g m^{-2} . A concentração dos glicosídeos variou entre os períodos de crescimento de cada corte e entre os compostos analisados. As concentrações mais altas de esteviosídeo (12,16% - grupo 1 e 11,36% - grupo 5) e rebaudiosídeo C (2,43% - grupo 5 e 1,95% - grupo 1) foram observadas nas colheitas realizadas em janeiro, enquanto que para o rebaudiosídeo A (7,01% - grupo 6; 6,16% - grupo 4 e 6,15% - grupo 3), as maiores porcentagens foram alcançadas nos meses de dezembro, fevereiro e março.

PALAVRAS-CHAVE: *Stevia rebaudiana.* Fatores climáticos. Glicosídeos de esteviol.

REFERENCES

ALMEIDA, L. P. de. Crescimento inicial de plantas de *Cryptocaria aschersoniana* Mez. submetidas a níveis de radiação solar. **Ciência Rural,** v. 34, n. 1, p. 83-88, 2004. http://dx.doi.org/10.1590/S0103-84782004000100013

BRANDLE. J. E.; ROSA. N. Heritability for yield, leaf: stem ratio and stevioside content estimated from a landrace cultivar of *Stevia rebaudiana.* **Canadian Journal of Plant Science,** v. 72, p. 1263-1266, 1992. http://dx.doi.org/10.4141/cjps92-159

CARNEIRO, J. W. P. *Stevia rebaudiana* (Bert.) Bertoni: Stages of plant development. **Canadian Journal Plant Science**, v. 87, p. 861-865, 2007. http://pubs.aic.ca/doi/pdf/10.4141/P06-040

CEUNEN, S.; WERBROUCK, S.; GEUNS, J. M. C. Accumulation pattern of steviol glicosides in *Stevia rebaudiana* Bertoni and its stimulation by red light. Proceedings of the 5th *Stevia* symposium. organized by EUSTAS 2011 – ***Stevia*: Break-through in Europe**. 209p, 2011.

CEUNEN, S.; GEUNS, J. M. C. Influence of photoperiodism on the spatio-temporal accumulation of steviol glycosides in *Stevia rebaudiana* (Bertoni). **Plant Science**, v. 198, p. 72-82, 2013a. http://dx.doi.org/10.1016/j.plantsci.2012.10.003

CEUNEN, S.; GEUNS, J. M. C. Spatio-temporal variation of the diterpene steviol in *Stevia rebaudiana* grown under different photoperiods. **Phytochemistry**, v. 89, p. 32-38, 2013b. http://dx.doi.org/10.1016/j.phytochem.2013.01.007

GONZÁLEZ, A. Z.; TREJO, J. A. M.; LOPEZ, J. M.; JIMENEZ, L. I. Reflectividad y absortibidad de la radiación en tres densidades de planta y su relación con el rendimiento de maíz (variedade Cafime). **Agrociência**, v. 39, p. 285-292, 2005. http://www.redalyc.org/articulo.oa?id=30239305&iCveNum=1634

GUEVARA-GARCIA, A.; SAN ROMAN, C.; ARROYO, A.; CORTES, M. E.; GUTIERREZ-NAVA, M. L.; LEON, P. Characterization of the Arabidopsis clb6 mutant illustrates the importance of posttranscriptional regulation of the methyl-D-erythritol 4-phosphate pathway. **The Plant Cell**, v. 17, n. 2, p. 628-643, 2005. www.plantcell.org/cgi/doi/10.1105/tpc.104.028860

Heldwein, A. B.; Buriol, G. A.; Streck, N. A. O clima de Santa Maria. RS. **Ciência and Ambiente**, v. 38, p. 43-58, 2009.

HIGUCHI, Y. et al. Constitutive expression of the GIGANTEA ortholog affects circadian rhythms and suppresses one-shot induction of flowering in *Pharbitis nil,* a typical short-day plant. **Plant Cell Physiology,** v. 52, n. 4, p. 638-650. 2011. http://dx.doi.org/10.1093/pcp/pcr023

JARMA, A.; RENJIFO, T.; ARAMENDIZ-TATIS, H. Aspectos fisiológicos de estevia (*Stevia rebaudiana* Bertoni) en el Caribe Colombiano: I. Efecto de la radiación incidente sobre el área foliar y la distribuición de biomassa. **Agronomia Colombiana,** v. 23, n. 2, p. 207-216, 2005. http://www.redalyc.org/articulo.oa?id=180316955003

LAVINI, A.; RICCARDI, M.; PULVENTO, C.; LUCA, S de; SCAMOSCI, M.; D'ANDRIA, R. Yield, quality and water consumption of *Stevia rebaudiana* Bertoni growth under different irrigation regimes in Southern Italy. **Italian Journal of Agronomy,** v. 2, p. 135-143, 2008. http://dx.doi.org/10.4081/ija.2008.135

LIMA FILHO, O. F.; VALOIS, A. C. C.; LUCAS, Z. M. Estévia. Sistemas de Produção. Embrapa Agropecuária Oeste. Dourados. MS, 2004.

LIANOPOULOU, V.; BOSABALIDIS, A. M.; PATAKAS, A.; LAZARI, D.; PANTERIS, E. Effects of chilling stress on leaf morphology. Anatomy, ultrastructure, gas exchange, and essencial oil in the seasonally dimorphic plant *Teucrium polium* (Lamiaceae). **Acta Physiologiae Plantarum,** v. 36, p. 2271-2281, 2014. http://dx.doi.org/10.1007/s11738-014-1605-x

MANDAN, S.; AHMAD, S.; SINGH, G. N.; KOHLI, K.; KUMAR, Y.; SINGH, R.; Garg, M. *Stevia rebaudiana* (Bert.) Bertoni – a review. **Indian Journal of Natural Products and Resources,** v. 1, n. 3, p. 267-286, 2010. http://nopr.niscair.res.in/bitstream/123456789/10287/1/IJNPR%201%283%29%20267-286.pdf

MICHELET, L.; LISZKAY, A. K. Reactive oxygen intermediates produced by photosynthetic electron transport are enhanced in short-day grown plants. **Biochimica et Biophysica Acta,** 1817, p. 1306-1313, 2012. http://dx.doi.org/10.1016/j.bbabio.2011.11.014

MÜLLER, V.; LANKES, C.; ZIMMERMANN, B. F.; NOGA. G.; HUNSCHE, M. Centelloside accumulation in leaves of *Centella asiatica* is determined by resource portioning between primary and secondary metabolism while influenced by supply levels of nitrogen, phosphorus or potassium. **Journal of Plant Physiology,** v. 170, p. 1165-1175, 2013. http://dx.doi.org/10.1016/j.jplph.2013.03.010

OROZCO, J. A.; AYALA, C. C.; HERRERA, C. F. Efecto de la temperatura y radiación en la producción de glucósidos de esteviol en *Stevia rebaudiana* en el Caribe húmedo Colombiano. **Revista U.D.C.A Actualidad and Divulgación Científica,** v. 15, n. 2, p. 339-347, 2012.

RAMESH, K.; SINGH, V.; MEGEJI, N. W. Cultivation of stevia (*Stevia rebaudiana* (Bert.) Bertoni): A comprehensive review. **Advances in Agronomy**, v. 89, p. 137-177, 2006. doi:10.1016/S0065-2113(05)89003-0

SERFATY, M.; IBDAH, M.; FISCHER, R.; CHAIMOVITSH, D.; SARANGA, Y.; DUDAI, N. Dynamics of yield components and stevioside production in *Stevia rebaudiana* grown under different planting times, plant stands and harvest regime. **Industrial Crops and Products,** v. 50, p. 731-736, 2013. http://dx.doi.org/10.1016/j.indcrop.2013.08.063

SKIRYCZ, A.; INZÉ, D. More from less: plant growth under limited water. **Current Opinion on Technology,** v. 21, p. 197-203, 2010. http://www.sciencedirect.com/science/article/pii/S0958166910000418

TAIZ, L.; ZEIGER, E. Fisiologia Vegetal. Porto Alegre: Artmed, 2009.

TOTTÉ, N.; CHARON, L.; ROHMER, M.; COMPERNOLLE, F.; BABOEUF, I.; GEUNS, J. M. C. Biosynthesis of diterpenoid steviol, an *et*-kaurene derivative from *Stevia rebaudiana* Bertoni. via methylerythritol phosphate pathway. **Tethraedron Letters,** v. 41, p. 6407-6410, 2000. http://dx.doi.org/10.1016/s0040-4039(00)01094-7

WOELWER-RIECK, U. Improved HPLC method for the evaluation of the major steviol glycosides in leaves of *Stevia rebaudiana.* **European Food Research Technology**, v. 231, p. 581–588, 2010. http://dx.doi.org/10.1007/s00217-010-1309-4

YANG, J.; LIU, X.; SHI, Y. Effect on different mixer fertilizer on yield, quality and economic benefits in *Stevia rebaudiana* Bertoni. **Advanced Journal of Food Science and Technology,** v. 5, p. 588-591, 2013. http://www.academia.edu/5065966

ZOBAYED, S. M. A.; AFREEN, F.; KOZAI, T. Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. John's wort. **Plant Physiology and Biochemistry,** v. 43, p. 977-984, 2005. http://dx.doi.org/10.1016/j.plaphy.2005.07.013