



Article

Irrigation Practice in Cultivation of *Stevia rebaudiana* Bertoni in Cooler Climates—Case Study in Poland

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Abstract: *Stevia rebaudiana* Bertoni originated in South America, but has gained popularity due to the concentration of sweet steviol glycosides present in its leaves. Stevia is also currently grown on other continents in many countries characterised with warm climate. This research aimed to determine whether different irrigation practices might have a significant influence on the yield and composition of the leaves of stevia plants grown in Poland, in cooler climate than native regions for stevia plants. Stevia plants were grown with four different irrigation regimes: irrigation twice a week, irrigation once a week, irrigation only during times of the drought and not irrigated at all. It was determined that different irrigation regimes have substantial effects on the yield, biometric characteristics and chemical composition of stevia leaves, but none of the irrigation regimes were clearly supreme to the others. Yield of stevia plants that were irrigated once a week or only during the drought times was the highest (2.42–2.58 kg per square meter). Stevia plants that were not irrigated were characterised with the lowest height and lateral width (47.61 and 25.35 cm). Leaves of un-irrigated stevia contained the greatest dry mass content (30.47%) and highest concentration of phenolic compounds (337.88 mg per 100 g of fresh stevia leaves). Stevia plants that were irrigated once or twice a week were characterised with a lower concentration of chlorophylls or carotenoids at the time of the harvest (1.20–1.24 and 2.54–2.58 mg per gram of fresh stevia leaves), but stevia plants that were not irrigated contained a greater amount of chlorophyll and carotenoids (1.58 and 3.17 mg per gram of fresh stevia leaves).

Keywords: water stress; yield; biometric measurements; elements



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1. Introduction

Stevia rebaudiana Bertoni is an herbaceous plant originating from Paraguay, grown primarily for its high concentration of glycosides, which are up to 300 times sweeter than the most commercially available sweetener—sugar [1]. The cultivation of this plant represents an increasing field of interest in agricultural research and sustainable food production. Remarkable sweetness of stevia is not its only advantage, as this plant is characterised with a low-calorie content and its consumption has a minimal influence on blood glucose levels [2]. Stevia is also a source of important antioxidants, such as polyphenolic metabolites including flavons, flavonols and chlorogenic acids [3].

Due to these properties, the stevia plant is widely used in the food, pharmaceutical and cosmetic industries [4]. As the consumer preferences are changing towards low-calorie options, the cultivation of this plant in diverse geographic regions, outside of South America, becomes more interesting to researchers and farmers [5]. Additionally, growing this plant in the country in which it would be used offers several advantages over shipping them from distant locations. It can result in decreased carbon emissions due to reduced transport distance [6]. Locally grown plants can reduce the costs of the final product due to

lower shipping costs. Additionally, plants grown locally can be sold fresh, without the need of extensive drying techniques, reducing costs and environmental impact and increasing quality of the final product [7].

The cultivation of plants in the country in which they will be consumed or used to produce various other products supports the local economy and decreases the reliance on the imported goods. Local production also allows for better traceability and transparency in the supply chain, which can increase food safety and consumer trust [7,8]. Furthermore, local cultivation can enable farmers to use cultivation practices that are typical for local environmental conditions and to produce plant material that would be acceptable for consumers from that particular region or country [7–9]. However, unfortunately, as stevia originated in South America, its physiology might not be suitable for cultivation in cooler climates.

Poland is a country with temperate seasonal climate, characterised with relatively cold winters and rather short summers. The cooler temperatures and varying precipitation patterns suggest a revaluation of traditional cultivation practices to optimise stevia's growth and yield in this region. One of the key factors in a successful cultivation of herbaceous plants is the irrigation regime, because various plants benefit greatly from the proper water management and lack of water during the cultivation of these plants can result in poor yield and unsatisfactory raw material quality [10–15]. Plants grown with an inadequate amount of water are also often characterised with worse organoleptic properties, such as taste and aroma, which is important for potential consumers of these products [11–13]. Water stress during growth of the plants can also influence the concentration of various elements or bioactive components, such as phenolic acids or vitamins; therefore, it is an important factor in the cultivation of plants, which could potentially be used in the production of food products [10]. During this study, four types of irrigation (or lack thereof) were investigated in *Stevia rebaudiana* plants grown in Polish climate: watering two times a week, watering one time a week, watering only during drought periods and no watering at all. The goal of this study was to determine whether *Stevia rebaudiana* can be grown in Poland using different irrigation regimes and assessment of the yield of the stevia plants gathered through this study. Additionally, biometric characteristics of stevia plants and the chemical composition of the raw material were analysed.

2. Materials and Methods

2.1. Study site, Experimental Design and Cultivation Conditions

Field experiments using *Stevia rebaudiana* plants were conducted in the Wrocław University of Environmental and Life Sciences: at the Research and Didactic Station in Psary (51°19'055913609418" N, 17°03'36781036313" E; 113 m over the sea level). Stevia plants used in the experiment were planted in black degraded soil characterised with humus content of 1.8% in the years 2014, 2015 and 2016. Table 1 shows the temperature and amount of rainfall during the time of the experiment at the stevia planting area.

Table 1. Rainfall and temperature on site of the experiment during the time of the stevia growth.

Month	Decade	2014		2015		2016	
		[°C]	[mm]	[°C]	[mm]	[°C]	[mm]
May	1st	11.4	30.3	13.3	12.3	15.4	14.0
	2nd	12.2	33.6	13.0	14.0	15.5	12.0
	3rd	17.3	42.8	14.0	0.5	20.9	0.0
	Monthly average	13.8	106.7	13.4	26.8	17.3	26.0
June	1st	18.4	8.2	18.1	11.0	21.1	9.4
	2nd	16.6	0.3	16.4	23.1	18.5	59.0
	3rd	15.7	15.4	15.8	32.2	23.8	0.8
	Monthly average	16.9	23.9	16.8	66.3	21.1	69.2

Table 1. Cont.

Month	Decade	2014		2015		2016	
		[°C]	[mm]	[°C]	[mm]	[°C]	[mm]
July	1st	20.5	20.0	20.5	11.0	20.5	39.5
	2nd	22.5	0.0	20.3	38.9	20.1	70.5
	3rd	22.6	26.1	19.9	3.3	23.8	24.5
	Monthly average	21.9	46.1	20.2	53.2	21.5	134.5
August	1st	21.7	17.5	27.2	0.0	20.4	5.8
	2nd	17.4	13.1	25.2	0.0	19.7	5.0
	3rd	15.9	35.0	23.1	2.1	21.3	13.3
	Monthly average	18.3	65.6	25.2	2.1	20.5	24.1
September	1st	17.2	24.1	17.1	16.4	21.2	11.5
	2nd	18.6	21.6	18.8	0.0	19.3	45.8
	3rd	14.0	14.3	14.1	0.0	15.0	0.0
	Monthly average	16.6	60.0	16.7	16.4	18.5	57.3

The experiment was performed in a one-factor randomised block design in quadruple repetitions, on the plots with the area of 1 m² with the total of sixteen plots. Total area of the experiment (experimental plots and uncultivated areas of the field used to divide the experimental plots) was equal to 33.25 m². The variable factor of the experiment was different types of irrigation practice. Four plots were not irrigated at all (0 W), four plots were irrigated once a week (1 W), four plots were irrigated two times a week (2 W) and four plots were irrigated only during the dry periods (no rainfall: NR) in the period from three weeks after planting the seedlings into the soil up to the harvest (phase of the intensive growth of stevia plants). The amount of water used during each watering (for the samples NR, 1 W and 2 W) was equal to 20 mm. The seedlings were obtained from our institution's own heated greenhouse, where seedlings were grown in plastic boxes filled with a peat substrate. Seeds of *Stevia rebaudiana* 'Sweet Herb' variety were sown in the first decade of April. Three weeks after sowing, stevia plants were transferred to 54-cell plastic trays filled with a peat substrate. In the second decade of May, plants with at least three pairs of foliage leaves were planted in 10 cm pots filled with a peat substrate. Stevia plants were planted during the first decade of June into the plots, which have been preplant-fertilised with ammonium nitrate at 100 kg N·ha⁻¹ and a mulched black agrotexile. In the third decade of July, plants were top-dressed-fertilised with ammonium nitrate (50 kg N·ha⁻¹). The concentration of phosphorus in the soil was equal to 45 mg·dm⁻³; concentration of potassium in the soil was equal to 310 mg·dm⁻³; concentration of calcium was equal to 2800 mg·dm⁻³; and concentration of magnesium was equal to 34 mg·dm⁻³.

2.2. Evaluated Variables

Plants were harvested in the second decade of September each year, before the flowering stage (average of 102 after transplanting to the field). After the harvest, plants were weighed to assess the total yield of the herbs and leaves (kg·m⁻²) and these data were then used to calculate the percentage of leaves in the yield. After weighing the single plants, leaves and shoots from single plants were also weighed to determine their percentage in the total yield. Additionally, once during June and once in September, after the harvest, healthy leaves gathered from the mid-plant height were used for chemical analyses. The amount of measurements per year was three in July and in September. Biometric measurements such as the plant height (cm), lateral width (cm), number of shoots (of the first order) and total number of leaves on the lowest lateral shoot were taken just before the harvest in September. Biometric measurements were performed for three plants from each field, to a total of twelve plants for one irrigation method. In leaves, dry mass content was measured by the gravimetric method (PN-90/A-75101/03) [16]. Subsequently, the concentration of ascorbic acid (vitamin C) was measured using Tillman's method (PN-90/A-75101/11) [17]. Content

of chlorophyll a, chlorophyll b and carotenoids was measured spectrophotometrically in the fresh plant material using a method by Bieniek (2012), while the total concentration of reducing sugars was measured using a method described previously by Godlewska et al. (2021) (PN-90/A-75101/07) [18–20]. The concentration of phenolic compounds in the stevia leaves was measured using the Folin–Ciocalteu method modified by Godlewska et al. (2021) and content of nitrate, phosphorus, potassium, magnesium and calcium was measured using a universal method by Nowosielski (1971) [20,21].

2.3. Statistical Analysis

Results were subjected to a statistical analysis in the Statistica program, using one-way ANOVA utilising the Duncan's test at confidence level $\alpha = 0.05$ to determine homogenous groups.

3. Results and Discussion

3.1. Yield of *Stevia*

Table 2 presents yield of *Stevia rebaudiana* grown in Poland, while using different irrigation practices throughout the growth period.

Table 2. Influence of irrigation on the yield of stevia plants.

Treatment	Year	Stevia Plant ¹	Stevia Leaves	Stevia Shoots	Stevia Plant Waste
		[kg·m ^{−2}]			
0 W	2014	1.21 ± 0.15 e	0.83 ± 0.06 e	0.33 ± 0.04 f	0.06 ± 0.01 e
	2015	2.56 ± 0.22 ab	1.64 ± 0.09 ab	0.78 ± 0.07 d	0.15 ± 0.04 c
	2016	2.46 ± 0.23 ab	1.50 ± 0.08 ab	0.87 ± 0.06 cd	0.09 ± 0.03 cde
Average for 0 W in the years 2014–2016		2.08	1.32	0.66	0.10
NR	2014	2.08 ± 0.18 c	1.14 ± 0.03 d	0.79 ± 0.06 d	0.12 ± 0.03 cde
	2015	2.82 ± 0.27 a	1.57 ± 0.10 ab	1.02 ± 0.05 bc	0.23 ± 0.06 b
	2016	2.83 ± 0.28 a	1.63 ± 0.18 ab	1.10 ± 0.05 ab	0.10 ± 0.02 cde
Average for NR in the years 2014–2016		2.58	1.45	0.97	0.15
1 W	2014	1.89 ± 0.18 d	1.04 ± 0.05 d	0.75 ± 0.05 d	0.10 ± 0.02 cde
	2015	2.33 ± 0.21 bc	1.15 ± 0.04 d	0.76 ± 0.04 d	0.42 ± 0.07 a
	2016	3.05 ± 0.19 a	1.75 ± 0.15 a	1.21 ± 0.09 a	0.09 ± 0.02 cde
Average for 1 W in the years 2014–2016		2.42	1.31	0.91	0.20
2 W	2014	1.70 ± 0.16 d	1.07 ± 0.05 d	0.55 ± 0.06 e	0.08 ± 0.01 de
	2015	2.24 ± 0.20 bc	1.12 ± 0.13 d	0.70 ± 0.03 de	0.42 ± 0.06 a
	2016	2.67 ± 0.22 ab	1.42 ± 0.12 bc	1.12 ± 0.08 ab	0.14 ± 0.02 cd
Average for 2 W in the years 2014–2016		2.20	1.30	0.79	0.21

¹ Values are expressed as the mean ($n = 30$) ± standard deviation. Mean values with different letters within the same column are significantly different ($\alpha = 0.05$) according to Duncan's test; 0 W—not irrigated, 1 W—irrigated once a week, 2 W—irrigated two times a week, NR—irrigated only during the dry periods (no rainfall).

Presented in Figure 1 are percentages of particular components of stevia (a leaf, shoots or so-called plant waste, i.e., dried leaves or stems) in the total yield of stevia grown using different irrigation practices.

Total average yield of stevia plants in the three-year period of the experiment was in the range of 2.08 kg to 2.58 kg·m^{−2}. Sample 0 W was characterised with the lowest total yield, while the result of 2.58 kg·m^{−2} was determined in the sample NR. Increasing the frequency of the irrigation of stevia plants had no significant impact on the total yield of the acquired biomass. Only 0 W was characterised with a different yield than other samples. These data suggest that the optimisation of the type of watering of stevia is not as important as just simple use of watering procedures to avoid drought periods during stevia cultivation. However, all of the analysed samples were characterised with a similar yield of stevia leaves, despite the differences in the total yield of the whole plants. The average yield of stevia shoots from the three-year period was greatest in the samples NR and 1 W (0.95 and 0.90 kg·m^{−2}) and smallest in 0 W (0.66 kg·m^{−2}). Yield of plant waste was similar for all analysed irrigation regimes and ranged from 0.10 to 0.22 kg·m^{−2}.

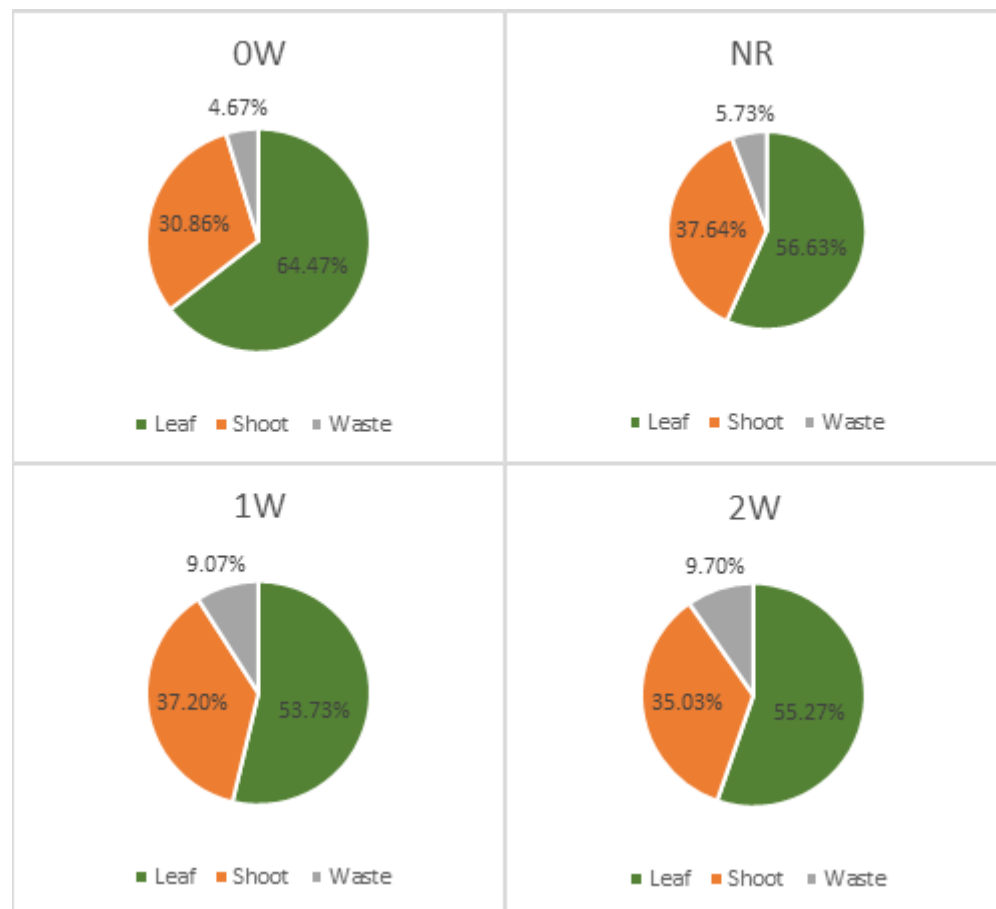


Figure 1. Percentage of yields of various components of stevia in total yield in stevia cultivated in different irrigation regimes. $n = 30$; 0 W—not irrigated, 1 W—irrigated once a week, 2 W—irrigated two times a week, NR—irrigated only during the dry periods (no rainfall).

The percentage of particular components in the total yield of stevia plants shows lesser homogeneity than yield of various stevia components. Samples 1 W and 2 W were characterised with a lower percentage of the leaf weight (53.73% and 55.27%, respectively) in the total yield than sample 0 W (64.47%). Typically, a farmer growing *Stevia rebaudiana* plants wants to acquire a large amount of leaves and, with the similar yield of all the analysed samples, a high percentage of the leaves from 0 W would mean that less total plant material from stevia plants that is not typically used by the industry (such as shoots and dried/damaged leaves) would be acquired. This result can be either an advantage or a disadvantage: the higher amount of redundant biomass could result in the increase in costs of utilisation, but, on the other hand, redundant biomass could be used for composting, cattle feed or other practices and prove valuable.

Studies by various other researchers, however, have shown variety of the influence of stevia watering on the yield of the plants. In the study of Aladakatti et al. [22], where stevia plants were grown using different irrigation regimes (0.8 IW/CPE—irrigation water per cumulative pan evaporation, 1.0 IW/CPE and 1.2 IW/CPE), stevia plants irrigated more frequently (1.0 and 1.2 IW/CPE) were characterised with greater total biomass yield than stevia plants irrigated less frequently, similarly as in our own study performed in Poland. However, difference in the total biomass yield also had a significant influence on the fresh leaf yield, contrary to the results acquired in our own study. On the other hand, the study by Lavini et al. [23] about growing stevia plants in Italy with adequate or deficit irrigation treatment (on the basis of soil water consumption) has shown that deficit irrigation (33 and 66% of soil water consumption) can decrease plant weight by even 20–25%. The study by Lavini et al. [23] has also shown that, similarly to this study

conducted in Poland, stevia plants grown with the lowest amount of water used were characterised with the greatest harvest index (i.e. ratio of leaf weight to plant weight) [23]. A study about stevia grown in Morocco, conducted by Benhmimou et al. [24], has shown that stevia plants grown with the highest irrigation practice (100% evapotranspiration) yielded a far greater amount of biomass than stevia plants grown with 80% or 50% evapotranspiration, but all the different watering regimes did not have an influence on the percentage of stevia leaves in the total plant weight. An analysis by Parris et al. [25] about stevia irrigation on the basis of soil water tension conducted in Ontario has shown, contrary to results acquired in stevia grown in Poland, that less frequent irrigation during stevia growth can reduce leaf yield of stevia by even 25%. Additionally, the different weather conditions in the years of the experiment also could have an influence on the stevia yield. However, the differences of the rainfall level in different years of the stevia cultivation during the experiment were effectively eliminated by the irrigation of the plants. Therefore, the most probable weather factor influencing the yield of stevia was the temperature. Lower stevia yields in 2014 could be caused by lower temperature than was noted in the following years. On the other hand, the average temperature in the years 2015 and 2016 was similar; however, in 2015, more temperature drops and increases could be noted, which could have also negatively affected the yield of stevia in 2015.

Results acquired in this study, as well as the results presented by the other authors, suggest that it is possible that quantity of the rainfall in Poland can be acceptable for the cultivation of stevia in cooler conditions present in the Polish region, but the various particular aspects of stevia cultivation (amount of water available for stevia connected with the amount of sunlight and average temperature) need to be analysed in the consequent studies.

3.2. Biometric Measurements of the Stevia Plants

Various biometric parameters such as the height of the plants, lateral width of the plant (measured at 2/3 of the plant height), number of shoots and number of leaves on the lowest lateral shoot were measured in this study. The results are shown in Table 3 and in Figure 2.

Table 3. Influence of irrigation on biometric characteristics of stevia plants measured in the years 2014–2016.

Treatment ¹	Number of Shoots of the First Order	Number of Leaves on the Lowest Lateral Shoot
0 W	7.75 ± 5.04 a	38.61 ± 12.24 a
NR	7.72 ± 4.88 a	42.64 ± 14.85 a
1 W	8.53 ± 5.78 a	43.25 ± 12.68 a
2 W	8.39 ± 4.37 a	43.44 ± 13.21 a

¹ Values are expressed as the mean ($n = 36$) ± standard deviation. Mean values with different letters within the same column are significantly different ($\alpha = 0.05$) according to Duncan's test; 0 W—not irrigated, 1 W—irrigated once a week, 2 W—irrigated two times a week, NR—irrigated only during the dry periods (no rainfall).

Height of the stevia plants ranged from 47.61 to 62.31 cm and was the lowest for sample 0 W. Lateral width of the plant was smallest in sample 0 W (25.35 cm) and sample 2 W (26.68 cm), while in sample 1 W, it was equal to 29.04 cm and in sample NR, to 33.92 cm. The total number of shoots on one plant was similar in stevia plants grown in all the watering regimes and ranged from 7.72 to 8.52. Similarly, different watering regimes did not have a significant influence on the number of leaves on the lowest lateral branches, which was in the range of 38.61–43.44. In the study of Aladakatti et al. [22], where stevia plants were grown using different irrigation regimes, stevia plants irrigated more frequently were characterised with a greater height and greater amount of branches per plant than stevia plants irrigated with the lowest amount of water. However, in the study of Aladakatti [22], differences between the amounts of stevia leaves were also significant, which is contrary to the results of this study in Poland. The fact that the study by Aladakatti [22] also consisted of different plant spacing connected to the different watering regimes could have

an influence on that fact and these results show that there is a possibility of existence of various correlating factors between the stevia plant spacing, stevia plant irrigation regime and amount of the leaves that the plant produces. These data can indicate an interesting research topic to be analysed in the future studies. In another study, by Lavini et al. [23], stevia plants grown with water deficiency in Italy were characterised with 5–10 cm lower height than plants grown with adequate water quantity. Similar results could be noted in the study by Benhmimou et al. [24], in which stevia plants grown with an inadequate amount of water were characterised with plant height lower by 10–15 cm from the plants grown with the addition of a greater amount of water. A study by Grad et al. [26] has shown that stevia plants grown in a greenhouse in Egypt, which were grown with an inadequate irrigation level (irrigation at 70 and 50% of the field capacity), were characterised with a lower number of branches per plant and plant height lower by 16–20 cm.

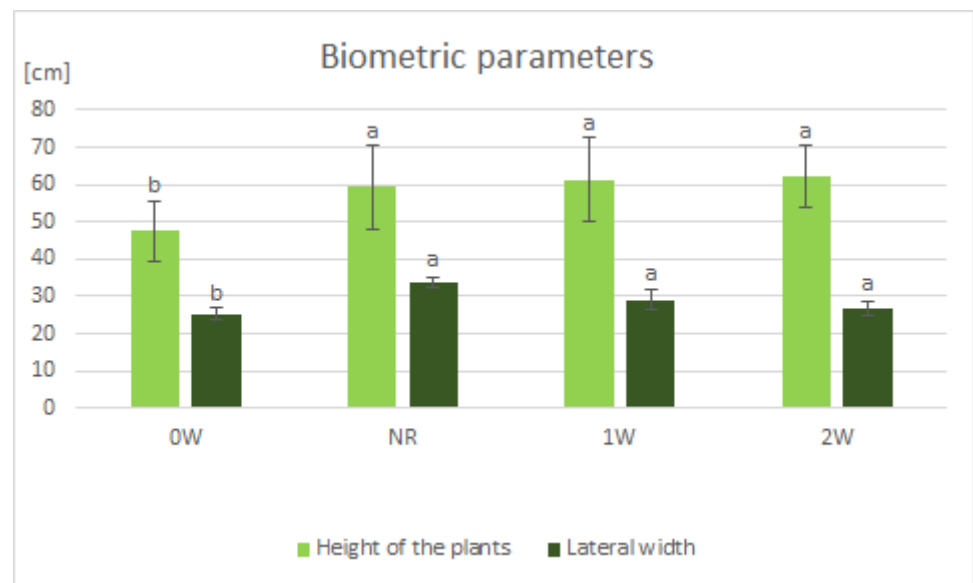


Figure 2. Influence of irrigation on biometric characteristics of stevia plants measured in the years 2014–2016. $n = 36$; 0 W—not irrigated, 1 W—irrigated once a week, 2 W—irrigated two times a week, NR—irrigated only during the dry periods (no rainfall). Different letters (a, b) within one parameter indicate significantly different values according to Duncan’s test.

3.3. Dry Mass Content and Concentration of Reducing Sugars, Phenolic Compounds and Ascorbic Acid in Stevia Leaves

Chemical analyses of the stevia leaves were performed at two different times: in July and just after the harvest, in September. Results of these analyses are shown in Table 4.

Table 4. Influence of irrigation on dry mass content and concentration of reducing sugars, phenolic compounds and ascorbic acid in leaves of stevia measured in the years 2014–2016.

Treatment	Dry Mass		Reducing Sugars		Phenolic Compounds		Ascorbic Acid	
	July ¹	September	July	September	July	September	July	September
0 W	23.14 ± 2.59 a	30.47 ± 2.99 a	0.76 ± 0.62 a	1.74 ± 0.60 a	270.26 ± 15.83 a	337.88 ± 31.88 a	139.99 ± 7.79 a	143.30 ± 7.21 a
NR	24.75 ± 2.28 a	27.87 ± 0.99 bc	0.77 ± 0.46 a	1.99 ± 0.80 a	229.73 ± 33.01 ab	241.03 ± 21.29 b	133.14 ± 24.24 b	124.66 ± 14.27 c
1 W	24.13 ± 2.51 a	28.82 ± 1.99 ab	0.82 ± 0.48 a	2.27 ± 0.25 a	185.62 ± 15.35 b	260.57 ± 24.18 b	121.02 ± 5.12 b	132.38 ± 10.07 bc
2 W	23.73 ± 2.54 a	26.52 ± 0.96 c	0.93 ± 0.71 a	1.95 ± 0.23 a	216.80 ± 21.94 b	246.46 ± 19.90 b	128.62 ± 7.22 ab	135.42 ± 6.15 ab

¹ Values are expressed as the mean ($n = 9$) ± standard deviation. Mean values with different letters within the same column are significantly different ($\alpha = 0.05$) according to Duncan’s test; 0 W—not irrigated, 1 W—irrigated once a week, 2 W—irrigated two times a week, NR—irrigated only during the dry periods (no rainfall).

The concentration of dry mass in the stevia leaves ranged from 23.14 (0 W) to 24.75% (NR) in July and from 26.52 to 30.47%. Differences in the dry mass content in July were not significant, while at the same time, the statistical analysis revealed three homogenous

groups in the average dry mass content in stevia leaves in September. Sample 0 W and 1 W were characterised with the highest concentration of dry mass in September and sample 2 W was characterised with the lowest, so it is not easy to determine whether a greater addition of water can always reduce dry mass content in the stevia leaves. A study by Badran et al. (2015) about drought stress on the *Stevia rebaudiana* properties has shown different results: stevia plants grown in worse drought conditions were characterised with lower dry weight content [27]. In the study by Aladakatti et al. [22], the impact of different irrigation methods was more clear-cut. Stevia plants that were irrigated more often were characterised with lower dry mass content in contrast to stevia plants that were irrigated less often. However, in the study of Benhmimou et al. [24] in which stevia plants were grown under water stress, leaves of plants that received a smaller quantity of water were characterised with lower dry mass content than leaves of the stevia plants that acquired a greater amount of water.

Different irrigation practices did not have an influence on the concentration of reducing sugars in stevia leaves, which were, in July, in the range from 0.76 for 0 W to 0.93% for 2 W and, in September, in the range from 1.74 for 0 W to 2.27% for 1 W. In the study of Karimi et al. [28], stevia plants grown with an inadequate water level (water deficiency) were characterised with a higher concentration of glucose and saccharose in stevia leaves. Studies conducted in Iran showed that the irrigation of stevia cultivated in fields as well as in greenhouses can result in an almost 20% increased concentration of sugars in the stevia plants [29]. These results suggest that it is possible that irrigation has a more substantial role in the formation of sugars in stevia in warmer climates.

The irrigation of stevia decreased the concentration of phenolic compounds in stevia leaves. In July, the lowest concentration of phenolic compounds was detected in the sample 1 W ($185.62 \text{ mg} \cdot 100 \text{ g}^{-1}$) and greatest in the sample 0 W ($270 \text{ mg} \cdot 100 \text{ g}^{-1}$). Similar tendencies were noted in the samples gathered in September, but the total concentration of phenolic compounds in all the samples was greater at that time. In September, sample 0 W was characterised with the highest concentration of phenolic compounds ($337.88 \text{ mg} \cdot 100 \text{ g}^{-1}$), while samples NR, 2 W and 1 W were characterised with lower, similar content of phenolic compounds in the range from 241.03 (for NR) to $260.57 \text{ mg} \cdot 100 \text{ g}^{-1}$ (for 1 W). In the study by Mahajan et al. [30], stevia plants grown with a deficit of water were also characterised with a higher total concentration of phenolic compounds, which could possibly result in greater health benefits due to the consumption of these plants or products from these plants. The major phenolic compounds found in the stevia leaves are hydroxycinnamic acid and quinic acid, characterised with valuable therapeutic properties [3]. General studies about a variety of different plants, mostly from the *Asteraceae* family, have shown that increases in the production of various phenolic compounds is a typical response of many of the plant species to drought stress; therefore, it is not surprising to see these results in the studies about *Stevia rebaudiana* plants [31–33].

In July and September, the highest concentration of ascorbic acid was determined in 0 W, while lower content was detected in 1 W and NR. The concentration of ascorbic acid had not changed from July to September as much as content of phenolic compounds, but small changes in the range up to 5–6% could be noted. In July, the highest concentration of ascorbic acid was determined in sample 0 W ($139.99 \text{ mg} \cdot 100 \text{ g}^{-1}$) and the lowest was detected in sample 1 W ($121.02 \text{ mg} \cdot 100 \text{ g}^{-1}$). In September, sample 0 W was still characterised with the greatest concentration of ascorbic acid ($143.30 \text{ mg} \cdot 100 \text{ g}^{-1}$) and NR had the lowest content of ascorbic acid ($124.66 \text{ mg} \cdot 100 \text{ g}^{-1}$). It is also important to note that only in the sample NR did the concentration of ascorbic acid decrease from July to September. On the other hand, a study by Hajhashemi and Sofo [34] revealed that drought stress can decrease the total concentration of ascorbic acid in stevia leaves over two-fold, which is contrary to the results acquired in our own study in Poland. These results might indicate that possibly stevia grown in Poland, even without additional irrigation, is not under sufficient drought conditions, which can induce loss of the ascorbic acid in the stevia leaves. A high concentration of vitamin C in stevia could possibly show viability of utilising these

plants as an animal fodder, which was proven by Xiong et al. [35] and Atteh and al. [36], who have shown that stevia and production waste of stevia products can be consumed by pigs and poultry.

3.4. Concentration of Chlorophyll A, Chlorophyll B, Chlorophyll A + B and Carotenoids in *Stevia* Leaves

The concentration of various pigments (chlorophyll A, chlorophyll B, chlorophyll A + B and carotenoids) in stevia leaves varied significantly between different types of irrigation as well as the times of the stevia leaf harvest (July and September) (Figure 3).

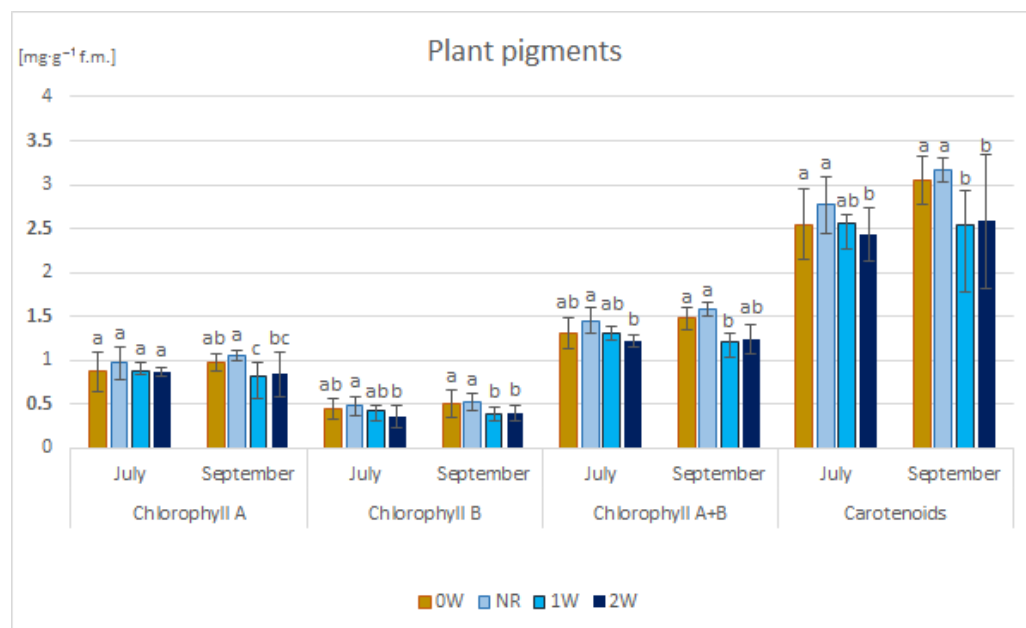


Figure 3. Influence of irrigation on the concentration of chlorophyll A, chlorophyll B, chlorophyll A + B and carotenoids in leaves of stevia measured in the years 2014–2016. $n = 9$; 0 W—not irrigated, 1 W—irrigated once a week, 2 W—irrigated two times a week, NR—irrigated only during the dry periods (no rainfall). Different letters (a, b) within one parameter (concentration of chlorophyll A in July, concentration of chlorophyll A in September, ...) indicate significantly different values according to Duncan's test.

In July, all the analysed samples were characterised with a similar concentration of chlorophyll A in the range of $0.86\text{--}0.97\text{ mg}\cdot\text{g}^{-1}$. In September, however, sample NR was characterised with the greater concentration of chlorophyll A ($1.06\text{ mg}\cdot\text{g}^{-1}$), similar to the content in sample 0 W ($0.97\text{ mg}\cdot\text{g}^{-1}$). The total concentration of this component has not increased in the samples 1 W and 2 W, in which it was even lower than in July, equal to 0.81 and $0.84\text{ mg}\cdot\text{g}^{-1}$, consecutively. The concentration of chlorophyll B varied in the analysed samples in July and September. The highest concentration of chlorophyll B in July was determined for the sample NR ($0.48\text{ mg}\cdot\text{g}^{-1}$), while the lowest was detected in sample 2 W ($0.36\text{ mg}\cdot\text{g}^{-1}$). In September, sample NR and 0 W were characterised with a higher concentration of chlorophyll B (0.52 and $0.51\text{ mg}\cdot\text{g}^{-1}$, consecutively) than samples 1 W and 2 W (0.39 and $0.40\text{ mg}\cdot\text{g}^{-1}$). The study of Mahajan et al. [30] has shown that a crucial factor influencing the concentration of chlorophyll (chlorophyll A, chlorophyll B and chlorophyll A + B) was the year of the stevia plantation, and the amount of water used to irrigate stevia plants did not have a significant effect on its concentration. However, drought induced by the polyethylene glycol in the cultivation of stevia in the study by Hajhishemi and Sofo [34] reduced total chlorophyll content in stevia plants almost two-fold. A study by Vasilakoglou et al. [37], on the other hand, has shown that chlorophyll content was almost at the same level in the stevia plants grown with adequate or deficit irrigation.

The concentration of carotenoids increased from July to September only for two of the analysed samples. In July, sample NR was characterised with the greatest concentration of carotenoids equal to $2.77 \text{ mg}\cdot\text{g}^{-1}$, while the lowest was determined in sample 2 W ($2.43 \text{ mg}\cdot\text{g}^{-1}$). In September, sample 2 W was characterised with a similar, lowest, concentration of carotenoids ($2.58 \text{ mg}\cdot\text{g}^{-1}$), while content of carotenoids in the NR increased to $3.17 \text{ mg}\cdot\text{g}^{-1}$. In the before-mentioned study of Hajihishemi and Sofo [34], a different result was detected, because leaves of the drought-induced stevia plants were characterised with lower carotenoid content than leaves of stevia that acquired an adequate amount of water [34]. Similarly, in the study of Muhealdin et al. [38], in leaves of stevia plants irrigated with a lower amount of water, a smaller quantity of carotenoids was found than in the control sample. However, it is important to note that these two studies were analysing stevia plants grown in warmer climate; therefore, the results might not be easily transferred to the characteristics of stevia plants grown in cooler climate in Poland.

3.5. Concentration of Various Elements (Calcium, Magnesium, Phosphorus and Potassium) and Nitrate Ions in Stevia Leaves

Different irrigation practices did not have as significant of an influence on the concentration of calcium, magnesium, phosphorus and potassium (Table 5) as they had on the concentration of previously discussed compounds in Sections 3.3 and 3.4.

Table 5. Influence of irrigation on the concentration of various elements and nitrates in leaves of stevia measured in the years 2014–2016.

Treatment ¹	Calcium		Potassium		Magnesium		Phosphorus		Nitrates (NO ₃ [−])	
					[mg·100 g ^{−1} of d.m.]				[mg·kg ^{−1} of d.m.]	
	July	September	July	September	July	September	July	September	July	September
0 W	900.00 ± 180.28 a	769.17 ± 207.22 a	3316.67 ± 549.15 a	2584.72 ± 358.70 a	163.33 ± 50.31 a	133.33 ± 29.79 b	128.72 ± 14.09 a	143.78 ± 53.08 a	2742.93 ± 906.75 a	1662.17 ± 553.03 a
NR	886.11 ± 138.13 a	805.56 ± 199.42 a	2813.89 ± 463.19 a	2519.44 ± 163.35 a	167.22 ± 42.51 a	157.78 ± 37.84 ab	135.30 ± 38.06 a	116.54 ± 29.30 a	1496.34 ± 612.32 a	977.44 ± 387.74 ab
1 W	897.22 ± 205.56 a	825.00 ± 203.49 a	2926.39 ± 475.91 a	2700.00 ± 241.17 a	159.44 ± 39.64 a	150.56 ± 36.52 ab	147.39 ± 32.13 a	127.66 ± 35.02 a	1548.66 ± 706.41 a	922.94 ± 238.69 ab
2 W	936.11 ± 162.07 a	894.44 ± 173.56 a	2945.83 ± 516.65 a	2737.50 ± 309.55 a	173.33 ± 66.47 a	170.00 ± 35.44 a	130.03 ± 15.63 a	129.19 ± 30.54 a	1753.97 ± 565.51 a	829.46 ± 285.17 b

¹ Values are expressed as the mean ($n = 9$) ± standard deviation. Mean values with different letters within the same column are significantly different ($\alpha = 0.05$) according to Duncan's test; 0 W—not irrigated, 1 W—irrigated once a week, 2 W—irrigated two times a week, NR—irrigated only during the dry periods (no rainfall).

The concentration of calcium in all the analysed samples was similar and it ranged from 886.11 to $936.11 \text{ mg}\cdot 100 \text{ g}^{-1}$ in July and decreased to 769.17 – $894.44 \text{ mg}\cdot 100 \text{ g}^{-1}$ in September. The concentration of calcium in the leaves of stevia grown in Poland was lower than total content of calcium in the leaves of stevia grown in warmer climates, in which it could range up to $1500 \text{ mg}\cdot 100 \text{ g}^{-1}$ [39,40]. Magnesium content ranged from 159.44 to $173.33 \text{ mg}\cdot 100 \text{ g}^{-1}$ in July and in September, it was the lowest in sample 0 W ($133.33 \text{ mg}\cdot 100 \text{ g}^{-1}$) and the greatest in sample 2 W ($170 \text{ mg}\cdot 100 \text{ g}^{-1}$). In the study of Khiraoui et al. [41], stevia plants grown in selected, more humid regions of Morocco were characterised with a greater concentration of magnesium than stevia plants grown in arid conditions. The concentration of phosphorus in samples NR and 1 W decreased from July to September, increased in the sample 0 W from July to September and remained at almost the same level in the sample 2 W. The total concentration of the phosphorus in all the samples gathered in July was statistically homogenous and it ranged from 128.72 (0 W) to $147.39 \text{ mg}\cdot 100 \text{ g}^{-1}$ (1 W). Similarly, the concentration of phosphorus was homogenous in all the samples from September and the results ranged from 116.54 (NR) to $143.78 \text{ mg}\cdot 100 \text{ g}^{-1}$ (0 W). In the study of Ucar et al. [39], a similar concentration of phosphorus was determined in the stevia samples, albeit a significant increase in phosphorus content was noted between July and September. Potassium content decreased in all the samples from July to September by 10–30%; however, in each of the time periods during which the stevia leaves were analysed, potassium content remained at a similar level de-

spite differences in the irrigation practice. Potassium content ranged from 2813.89 in NR to 3316.67 mg·100 g⁻¹ in 0 W in July, while it was in the range of 2519.44–2737.50 mg·100 g⁻¹ in September. The greatest loss of potassium in the time period between two of the analyses was determined for the sample 0 W. However, in comparison, stevia plants grown in various parts of Morocco with different annual rainfall levels have shown differences in the potassium content [41]. Stevia plants grown in semi-arid regions were characterised with the greatest concentration of potassium, while stevia plants grown in semi-humid conditions were characterised with lower content of this element. However, the stevia plants grown in arid conditions acquired the lowest concentration of potassium; therefore, it is difficult to determine whether different water levels in the stevia cultivation play a significant role in the potassium content in the stevia leaves [41]. The concentration of nitrates also changed between July and September, but differences between applications of different watering regimes could be detected only in September. In July, the concentration of nitrates was in the range of 1496.34–2742.93 mg·100 g⁻¹, while in September, the highest concentration of these ions was determined in sample 0 W (1662.17 mg·100 g⁻¹) and the lowest in sample 2 W (829.46 mg·100 g⁻¹). Similar results were acquired in the study by Mahajan and Pal [42], in which stevia plants grown in the soil with lower moisture content were characterised, at harvest, with the lowest uptake of nitrogen and nitrates. A similar level of nitrates as detected in the samples NR, 1 W and 2 W could be noted in stevia leaves grown using different nitrogen fertilisation treatment, using three different nitrogen fertilisers (urea, ammonium nitrate and ammonium sulphate) and three different doses (50 kg, 100 kg and 150 kg per ha). These results suggest that lack of irrigation can disturb the nitrate utilisation in the stevia plants [43].

4. Conclusions

Different irrigation regimes turned out to have a significant impact on the characteristics of stevia grown in Poland, which is cooler than the region where stevia originally comes from. These results have shown that different irrigation practices can be used in order to change selected properties of the stevia plants. Yield of stevia plants that were irrigated once a week or only during the drought times was the highest. Stevia plants that were not irrigated were characterised with the lowest height and lateral width. Leaves of un-irrigated stevia contained the greatest dry mass content and highest concentration of phenolic compounds. Stevia plants that were irrigated once or twice a week were characterised with a lower concentration of chlorophylls or carotenoids at the time of the harvest, but stevia plants that were not irrigated contained a greater amount of carotenoids and chlorophyll. However, as no irrigation regime improves all of the analysed parameters, which could be of interest to potential *Stevia rebaudiana* farmers, it is impossible to select one of the analysed irrigation regimes and determine it as the most optimal regime for the stevia cultivation.

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