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Preliminary Studies on *Stevia Rebaudiana* Bertoni Plants Cultivated Under the Field Conditions of Southern Poland

Agriculture

KEYWORDS : herb mass, phenolics, rebaudioside, stevioside

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ABSTRACT

Three forms of Stevia rebaudiana were grown in the open field of University of Agriculture in Kraków from mid May to the beginning of October. Some parameters of plant growth and development such as height, width, weight, share of leaves, and number of shoots, were determined. The quality of collected herb was compared taking into consideration the content of steviol glycosides, phenolics, carotenoids, chlorophylls and ascorbic acid.

Introduction

Stevia rebaudiana leaves have been used for many centuries as a source of natural sweetener in food products (Ramesh, Singh & Mageji, 2006). This is related with the content of steviol glycosides, which, are not metabolized in the human body and are 250-300 times sweeter than sucrose. Low caloric content, resistance to acids and high temperature, and reports of positive influence of *Stevia* products on diabetes have increased the interest in this plant (Midmore & Rank, 2002). In November 2011, the European Commission approved the use of steviosides in food industry as a sweetener. Therefore in the near future demand for *Stevia* herb in the European Union will certainly increase. So far, raw material of this plant in Europe is mostly imported.

Stevia rebaudiana Bertoni is a herbaceous, perennial plant of the *Asteraceae* family. It originates from subtropical regions of Paraguay, where it grows wild in sandy soils near streams (Brandle, Starrat & Gijzen, 1998). In moderate climate during the growth season it may successfully develop. In Poland stevia is known only in the amateur cultivation, since no detailed studies were conducted on its growth and yield in the field conditions.

The aim of this study was to observe the development of three forms of *Stevia rebaudiana* Bertoni in the open field in Southern Poland, in order to determine the effects of their cultivation in these conditions.

Material and methods

The investigation was conducted on three forms of *Stevia rebaudiana* Bertoni marked as AX, X4, and X6. Rooted cuttings with two side shoots were planted in the open field (brown alluvial soil) at the Experimental Station of the University of Agriculture in Krakow on 18 May 2012. Analysis of soil taken directly before planting showed the following results pH - 6.5; salinity - 0.57 g NaCl·l^{-1}; the nutrient content (mg·l^{-1}) N-NH_4- 3.5; N-NO_3- 52.5; P - 104; K - 188; Ca - 1324; Mg - 189. Before planting the side shoots were trimmed above the second pair of leaves. The plant spacing was 60 x 50 cm.

During growth season the dates of flower buds appearing and blooming were noted. The harvest of AX was carried out in the phase of full blooming on 28 September 2012. The other two forms were harvested at the beginning of blooming on 10 October 2012. At the day of harvest the measurements of plant height, width, and number of shoots were done. After harvest, the herb was weighed and then dried in 20-22°C. After drying, the share of leaves in the herb was determined.

The content of ascorbic acid, chlorophylls a and b, and carotenoids was determined in fresh herb, while that of phenolic compounds and steviosides - in air-dried herb. The content of L-ascorbic acid was determined by the iodometric method of Tillmans (Krełowska-Kułas, 1993), that of chlorophyll and carotenoids – spectrophotometrically, after the extraction of plant material in 80% acetone, basing on the equations by Lichtenthaler and Wellburn (1983). The amount of total phenolics was determined by a photometric method with the Folin–Ciocalteu reagent using gallic acid (GA) as a standard (Zheng & Wang, 2001, Singh, Chidambara Murthy & Jayaprakasha, 2002), after herb extraction in 80% methanol. Concentration of stevioside and rebaudioside A was measured by High Performance Liquid Chromatography (HPLC, Agilent Technologies 1260), with diode array detector (DAD).

Obtained results were subjected to the analysis of variance and Tukey's test at p=0.05. Homogeneous groups were marked by the same letters.

Results and discussion

The characteristics of *Stevia rebaudiana* plants at harvest time is shown in Table 1. The first signs of AX form blooming were observed on 13 July. Two weeks later every plant of this form had flowers. X4 and X6 plants developed flower buds in the beginning of October, shortly before harvest. The plant height did not differ in dependence of *Stevia* form. The greatest plant width and number of shoots were observed in X4. The weight of both fresh and dried herb per plant from X4 and AX was higher than from X6, but the share of leaves in herb of X6 was the greatest.

Table 1.

	AX	X4	X6
State of plant development	full blooming	flower bud formation	flower bud formation
Plant height [cm]	69.0 a	70.5 a	62.7 a
Plant width [cm]	61.2 ab	69.0 b	53.5 a
Number of shoots	48.4 ab	52.1 b	31.2 a

	AX	X4	X6
Weight of fresh herb per plant [g]	567 b	586 b	251 a
Weight of dried herb per plant [g]	185 b	144 b	79 a
Share of leaves in herb [%]	52.7 b	47.0 a	64.7 c

The results of chemical analysis of *Stevia* herb (table 2) show that he highest level of ascorbic acid featured AX. In the case of chlorophyll and carotenoids the greatest amount was found in X4 and AX form. The content of phenolic compounds and rebaudioside-A for X6 was the greatest. This form also showed the highest rebaudioside-A to stevioside ratio. The leaves of X4 had the greatest amount of stevioside.

Table 2. The content of some compounds in the herb of three *Stevia rebaudiana* forms

	AX	X4	X6
Ascorbic acid [mg·100 g^{-1} f.w.]	101 c	72.3 b	60.6 a
Phenolics [g·100 g^{-1} d.w.]	7.20 a	7.43 a	8.61 b
Chlorophylls a+b [mg·g^{-1} f.w.]	5.4 b	6.1 b	3.0 a
Carotenoids [mg·g^{-1} f.w.]	1.4 b	1.6 b	0.8 a
Stevioside [µg·100 mg^{-1} d.w.]	31.2 a	85.0 c	48.3 b
Rebaudioside A [µg·100 mg^{-1} d.w.]	8.69 a	24.6 b	120.0 c
Rebaudioside-A to stevioside ratio	0.28 a	0.29 a	2.48 b

In the cultivation under field conditions of Southern Poland the plants of three *Stevia* forms reached a similar height. According Brandle and Rosa (1992) *Stevia* plants grew to 50-60 cm, while Shock (1982) and Dwivedi (1999) indicated that they could reach 100-120 cm. The plants in cultivation were usually smaller (60-80 cm) than in the natural habitat (over 100 cm). Moreover, the average plant height depended on the region and plants cultivated in moderate climate were lower.

Slight differences between the forms were found for the width of plants. The X4 plants grew the widest mostly due to their growth pattern and ability to form very thick shoots. This could also be justified by the number of shoots, which were most numerous in X4 form.

This growth parameter probably affected the yield of herb. The largest amount of herb was obtained from X4 and AX form. Both fresh and dry herb mass of these plants were more than twice greater than from X6. According to Ramesh, Singh, and Megeji (2006) in Palambur the *Stevia* yield per ha was 15-20 t of fresh herb and 1.7 t of dried leaf. In the natural habitat in Paraguay average dried leaf yield was 1.5-2.5 t·ha^{-1}. Taiariol (2004) obtained 3.0-3.5 t·ha^{-1} in Japan, while Brandle and Rosa (1992) 3 t·ha^{-1} in Canada. The dried leaf yield from the present study, calculated theoretically on the basis of herb mass per plant and plant spacing, was 3.25, 2.26, and 1.66 t·ha^{-1} for AX, X4, and X6, respectively. This suggests that in the climate of Southern Poland, the cultivation of *Stevia* could bring satisfactory results.

The X4 and X6 herb was collected at the time of flower bud appearance, two weeks later than AX, which was cut at full blooming. Dwivedi (1999) claimed that plants should be collected at the time of first flower appearance. Barathi (2003) advised harvesting before appearance of any flowers, because during flowering the stevioside content diminished. The results of present experiment indicated that plants of X4 and X6 were collected at right time, but of AX too late.

Morita, Fujita, and Iwamura (1978) determined 60 mg of ascorbic acid in 100 g of fresh herb. In the present study, similar results were observed only in the case of X6 form. X4 and AX contained more vitamin C. Abou-Arab, Abou-Arab, and Abu-Salem (2010) showed that the content of chlorophylls was 161.7 mg·100 g^{-1} and the content of carotenoids 39.8 mg·100 g^{-1}. In the present study, the leaves of the AX and X4 contained about 3.5 times more of those compounds. Midmore and Rank (2006) claimed that rebaudioside A had a superior taste to stevioside, without metallic after-taste. In plants with the rebaudioside-A to stevioside ratio higher than 1 the bitter after-taste was weaker. Thus plants with higher rebaudioside-A content are preferred. In this work the herb of AX, due to its harvesting in full blooming, had lower stevioside level than X4 and X6. Considering the two latter, better results concerned X6 with very high rebaudioside-A content and optimum rebaudioside-A to stevioside ratio. Unfortunately this form was characterized by the lowest leaf yield. The highest level of stevioside was noted in X4 which also gave a good leaf yield.

Conclusions

1. The yield of the three *Stevia rebaudiana* forms studied under the cultivation in the open field in Southern Poland was comparable to the results obtained in warmer climates, where the cultivation of this plant is common.
2. The AX and X4 plants produced nearly twice the weight of herb as compared to the X6 plants, however the latter had significantly higher share of leaves in herb.
3. The highest ascorbic acid content was found in AX herb, but on account of its early blooming this herb had the lowest level of steviosides.
4. The X6 herb was found to have the highest rebaudioside-A to stevioside ratio.

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