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**(*Stevia rebaudiana* B.)**

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**Stela – the first Bulgarian variety of stevia  
(*Stevia rebaudiana* B.)**

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**SUMMARY**

(Stevia rebaudiana B.) -204. 2013-2015 . 12-5/07.04.2016 . " " 2-6 60 90 m. e , 250 kg/da 11-13 g/100g . :	-   The new variety of the stevia plant ( <i>Stevia rebaudiana</i> B.) is a diploid population of the C-204 line. The variety is tested during the period between 2013 and 2015 in the system of IASAS and was approved by Ordinance RD 12-5/07.04.2016 by the minister of Agriculture and Foods of the Republic of Bulgaria. Stella is a multi-stem upright bush with 2-6 main stems and branches to the third order. The height of the plants varies from 60 to 90cm. The shape of the bush is cylindrical pyramid. The leaves are intensely green and spirally arranged. The studies carried out in Shumen Agricultural Institute have shown that the yield of dry mass leaves is over 250 kg/da and the total content of sugary substances – 11-13 g/100g. -   <b>Key words:</b> stevia, diploid, variety, yield
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## INTRODUCTION

The application of sweeteners in the manufacture of various food products, such as foods and beverages is growing steadily, which is why they occupy an increasingly important place in the food industry. Increasingly great attention is paid to the influence of various food additives, including artificial sweeteners on the health of consumers in relation to the provision of greater safety.

In this regard too many studies were carried out that led to the banning of some of them, like substances such as salts of cyclamate acid, aultine, etc. The question of the use of saccharine, etc. The saccharine usage is under discussion discussion in many countries of Europe, America and Japan (Chaturvedula et al., 2011; Munish, 2012; Ibrahim et al., 2014).

At the end of last century a number of Researchers' attention is attracted to the South American stevia plant (*Stevia rebaudiana* B.) (Aranda-González et al., 2014).

To this date more than 30 steviol glycosides have been identified, which are described in the literature. For now of interest are two of them – stevioside and rebaudioside A. The stevioside is approximately 200 times sweeter than sugar and the rebaudioside A about 250 times. The stevioside is

(Chaturvedula et al., 2011; Munish, 2012; Ibrahim et al., 2014).

(*Stevia rebaudiana* B.) (Aranda-González et al., 2014).

30

200

250

(Wölwer-Rieck, 2012).

(Ishiba et al., 1982; Tanova, 2008).

(Geuns Jan, 2004).

sweeter in flavor with a slightly astringent taste, while in the rebaudioside A this tartness lacks (Wölwer-Rieck, 2012).

The increased consumer interest in natural food supplements means that products such as stevia will be the subject of attention from the users (Uchkunov et al., 2012). The new stevia origins must possess certain qualities and specific resistance to disease (Ishiba et al., 1982, Tanova, 2008).

It is expected that the high-quality sweeteners global growth demand, especially with the new practice of mixing different sweeteners (Geuns Jan, 2004).

The purpose of this study is to present some of the morphological and economic traits of the first Bulgarian stevia variety.

(*Stevia rebaudiana* B.) -204.

2013-2015 .

16  
(210/2; 210/9; 203/2; 404/7; 214/2; 302/8; 204/2; 1 ; 3 ; 4 ; 7 ; 8 ; 9 ; 10 ; 1 4)

## MATERIAL AND METHODS

The new Bulgarian stevia variety (*Stevia rebaudiana* B.) is a diploid population from the line -204.

The variety has been tested in the period 2013-2015 in the experimental fields of the Agricultural Institute with the participation of 16 different stevia origins (210/2; 210/9; 203/2; 404/7; 214/2; 302/8; 204/2; 1 ; 3 ; 4 ; 7 ; 8 ; 9 ; 10 ; 1 and 4) from the breeding program of the

<p>in vitro,</p>	<p>Institutes, sources of which are Japan, USA, as well as origins obtained by somaclonal variation by <i>in vitro</i>, individual and clonal selection.</p>
<p>07.04.2016 .</p>	<p>The results of the technical testing final report in IASAS and a decision of the Experts Committee is that the variety is distinct from other varieties, sufficiently uniform and stable.</p>
<p>12-5</p> <p>RD 12-5 from 07.04.2016 of the Minister of agriculture and foods of the Republic of Bulgaria, the variety stevia "Stella" is affirmed in Article 38 para. 8 of the Law on Protection of New Plants and Animals (RHS).</p>	<p>By Order RD 12-5 from 07.04.2016 of the Minister of agriculture and foods of the Republic of Bulgaria, the variety stevia "Stella" is affirmed in Article 38 para. 8 of the Law on Protection of New Plants and Animals (RHS).</p>
<p>2-6</p> <p>90 m.</p> <p>60</p>	<p>"Stella" represents a very upright multi-stem shrub with 2-6 main stems and branches to the third order. The height of the plants varies from 60 to 90 cm. The shape of the bush is cylindrical piramidal. The leafage is very good. The leaves are green, spirally arranged.</p>
<p>1000</p> <p>0.45 g.</p> <p>48-59,3%.</p> <p>50%</p>	<p>The "Stella" seeds of are small with fluffy outer casing. The mass of 1000 seeds is of 0.30 to 0.45 g. The laboratory germination is 48-59.3%. The seedling growth is often weak, with levels of less than 50% of the total quantity.</p>
<p>-10):</p>	<p>Distinctive marks compared to other origins ( -3, -10): the bush of the "Stella" variety is upright, the bush shape is of the cylindrical piramidal type, with larger leaves, the flowerhead is longer.</p>

Under the conditions of the Republic of Bulgaria, the "Stella" variety multiplies through vegetation. The variety shows good ecological plasticity and can be grown in all regions of the country under irrigated conditions. It has relatively good resistance to fungal diseases.

## RESULTS AND DISCUSSION

The results of the study show (Table 1) that the average height of the basic stems reached 89.3 cm. Existent is a demonstrated difference in comparison with the rest of the studied origins.

In reporting the number of the main stems of one plant was established that there are no substantial differences with the other origins. Such are also the results of the study about the indicator on the number of the lateral branches (12.4-12.1).

A review of the data on the weight of the green mass indicates that the new variety proved exceed the weight of other origins of stevia. Overall, the amount of green leaves under the "Stella" variety reaches 779.3 kg/da, the difference with the other origins is 5.2%, which difference is significant for the limit value of GD - 5%.

The most important economic indicators in the cultivation of the stevia plant is the dry mass of leaves and sweet substances content in them. The survey found that the average amount of dry

( 1),  
89.3 cm.  
  
(12.4-12.1).  
  
779.3  
5.2%,  
GD - 5%.

leaves of the studied stevia origins reaches approximately 240 kg/da at an output 16.8%.

In reporting the attack of the alternaria disease (*Alternaria steviae*), it was established that the average number of diseased plants is within the limits of 3.8%-3.9%.

1.  
**Table 1. Productivity and economic qualities of stevia origins**

Indicators	For "Stella"	Mean for experiment	Relative %	GD - 5%	Significance
Length of stems, cm	89,3	73,5	121,2	10,8	++
Main stems, pcs.	3,6	3,4	105,9	6,9	-
Lateral branches, pcs.	12,4	12,1	102,5	7,1	-
Total weight, kg/da	1488,3	1369,3	108,7	7,7	+
Fresh leaves, kg/da	779,3	741,3	105,2	5,1	+
Dry leaves, kg/da	253,8	225,4	112,2	7,1	+
Output, %	17,0	16,5	103,0	4,1	-
Diseased plants, %	3,8	3,9	97,4	5,1	-

1. The contents of the diterpene glycosides are presented on Figure 1. Analysis of the results showed that "Stella" has a relatively equal content of the stevioside with the other investigated breeding origins. The differences are in the content of Rebaudioside A, where the new

29.8%.  
0.63.  
13.42 mg/100g

variety exceeds other origins with 29.8%.

The relation value between stevioside and rebaudioside A reached 0.63. The total quantity of sweet substances is 13.42 mg/100g dry mass.

The trends in the stevia breeding is the increasing the rebaudizide A share, whose taste qualities in the pure product are preferred.



. 1.

Fig. 1. Diterpen glycosides content

## CONCLUSIONS

❖ (Stevia rebaudiana Bertoni)  
” ”

❖ The new stevia variety (Stevia rebaudiana Bertoni), "Stella" is a distinctive, homogeneous and

kg/da.	240	stable. The yield of dry leaves is over 240 kg/da.
❖		❖ The total amount of sweet substances is 13.42 mg/100g dry mass. The content of stevioside is 7.61 mg/100g, and a rebaudioside A– 4.81 mg/100g.
mg/100g	13.42	
	-	
mg/100g,	7.61	
4.81 mg/100g.	-	

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## Microbiological analysis of waters of river Morava Binqës during summer season 2013

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### SUMMARY

The main objective was to investigate microbiologically the waters of river Morava Binqës (Kosovo). The water samples were collected from different stations of river Morav Binqës, during the summer season in 2013.

The isolates were characterized and identified as: total mesophylic bacteria, total coliform, streptococcus bacteria, salmonella and Shigella, and fungi. The study therefore, stresses on the need to control the fecal pollution of water bodies.

**Key words:** microbiology, analysis, river, water

## INTRODUCTION

The importance of water is underscored by the fact that many great civilizations in the past sprang up along or near water bodies. The development of water resources has often been used as a yardstick for socioeconomic and health status of many nations worldwide.

However, pollution of waters often negates the benefits obtained from the development of these water resources.

Water is one of the most essential needs for the continued existence of all living organisms on earth. The day-to-day activities of all living organisms require water in some form.

It is effectively and efficiently put into use by plants, animals, microorganisms and man. In the microbial world, no single microorganism has been discovered to be active at the extreme lack of water for the singular reason that man cannot exist without water, it is of paramount importance to monitor domestic water supply (Sofola and Lawal, 1983).

and Lawal, 1983).

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## MATERIAL AND METHODS

### Collection of water samples

4 Samples were collected from 4 different sites on the river Morava Binqës, during summer season 2013.

2013 .

Water samples from the water of river were brought to the

500 ml. laboratory in 500 ml sterile glass bottles. In order to inactivate chlorine, sterile sodium thiosulphate solution was added (13.2 mg/l). The samples were immediately stored under ice-cold conditions and microbiological analyses were performed within 2h of collection.

**Isolation and identification of the strains**

Violet Red Bile Agar (VRBA) use for coliform bacteria counts, SS agar for salmonella and shigella bacteria, Nutrient Count Agar (PCA) for total aerobic mesophilic bacteria counts, Bile aesculin agar for streptococcus bacteria and Potato Dextrose Agar (PDA) of pH 3.5 adjusted with 10% tartaric acid (PDA) for fungi.

Alter incubation at 37, 22, 32 and 35 °C for 24 h, 48h and 72h colonies with characteristic properties were counted for coliform, yeast-mould, total aerobic mesophilic and lactic bacteria counts respectively.

In this technique, 100 ml of water sample filtered through a membrane filter. After incubation, the number of coliform colonies is counted.

## RESULTS AND DISCUSSION

The obtained results presented in Table 1, show that the number of coliform bacteria was higher at second location with 240.000, while the other locality has lower number.

	60.000.	Coliform bacteria at third locality was with 60.000.
	,	The presence of coliforms group in this water samples generally suggests that a certain selection of water may have been contaminated with faeces either of human or animal origin. Other more dangerous microorganisms could be present (Richman, 1997).
	,	
	.	
	-	
	(Richman, 1997).	
	150.000	The higher number of Streptococcus faecalis detected at first locality with 150.000, while the lower number was detected at third locality with 110.000.
	-	
	110.000	
	.	
	-	
	310.000	Also the number of Salmonella and shigella was higher at locality two with 310.000, while the lower number was detected at locality one with 60.000.
	-	
60.000	,	
	.	
	(30.000-80.000)	The present investigations have rendered the values (30.000-80.000) of fungi per 100 ml of water, which have exceeded the prescribed limit.
	100 ml	
	,	
	.	
	-	
	560.000	The highest number of aerobic heterotrophic mezophilic bacteria was detected at third locality with 560.000, while the lower number was detected at second locality (130.000).
	-	
	,	
	(130.000).	
	-	
	,	
	.	
Buchanan	Gibbons (1974)	The isolated bacteria species were identified to be same with those commonly encountered in water and aquatic environments as it was also reported in a study on streams surface water in Wyoming in U.S.A. reported by Buchanan and Gibbons (1974).
	-	
	.	
		The poor microbiological quality might be due to

- contamination caused by human activities and livestock. It is a common practice for people living along the river catchment to discharge their domestic and agricultural wastes as well as human excreta/wastes into rivers. In addition to using the river as a source of drinking water people use the source for bathing, washing of clothes and for recreational purposes such as swimming. Wild and domestic animals seeking drinking water can also contaminate the water through direct defecation and urination.

1.

2013

**Table 1. Results of microbiological analysis of water of river Morav Binqës during summer season 2013**

Group of bacteria	Amount of analysed water	Locality 1	Locality 2	Locality 3	Locality 4
Aerobic heterotrophic mezophilic bacteria	100 ml	260.000	130.000	560.000	350.000
Total coliform	100 ml	210.000	240.000	60.000	120.000
Streptococcus faecalis	100 ml	150.000	130.000	110.000	140.000
Salmonella and shigella	100 ml	60.000	310.000	30.000	120.000
/ Fungi	100 ml	40.000	60.000	30.000	80.000

All water samples were contaminated with total coliform, Streptococcus, aerobic heterotrophic mesophilic bacteria, salmonella and Shigella, and fungi. These bacteria cause water borne diseases like intestinal infections, dysentery, typhoid

(Bharti and Katyal, 2011).  
 (Sohani and Sanjeeda, 2012).  
 E. coli  
 (Juhna et al., 2007; Ewing, 1986).  
 (Meays et al., 2004).

fever, cholera, and other illnesses (Bharti and Katyal, 2011).

Treatment of water by Municipal Corporation should be such that impurities as well as removal of pathogenic organisms done completely or minimize to such extent that it should not be hazer done to humans and animals (Sohani and Sanjeeda, 2012).

E. coli causes diarrhoea, urinary tract and kidney infections, and peritonitis septicaemia. The isolation of this pathogen from the lake is worrisome because the lake water is collected (Juhna et al., 2007; Ewing, 1986). It is not impossible to assume that this pathogen or fecal contamination of the lake's water was introduced into the production process by healthy human carriers through handling. This is because the lake's water was collected prior to contact with the external environment (Meays et al., 2004).

## CONCLUSIONS

The present investigation has led us to conclude that the quality of water samples subjected to study of bacteriological standards the water needs to be treated before using it in domestic applications.

Based on achieving results led us to conclude:

- the waters of river "Morava Binqës" is polluted by bacteria at all locality;

