eISSN: 09748369

Biological method for increasing adaptive potential of Stevia (*Stevia rebaudiana* (Bertoni) Bertoni), producer of native sugar substitute

Biology and Medicine

Research Article

Volume 6, Issue 3, Article ID: BM-048-14, 2014
Indexed by Scopus (Elsevier)

www.biolmedonline.com

Biological method for increasing adaptive potential of Stevia (*Stevia rebaudiana* (Bertoni) Bertoni), producer of native sugar substitute

Vladimir I. Trukhachev, Galina P. Starodubtseva, Alexander I. Voiskovoy*, Alla A. Krivenko, Inna A. Donets
Stavropol State Agrarian University, Zoo Technical Lane, 12, 355017, Stavropol, Russia.

***Corresponding author**

Citation: Trukhachev VI, Starodubtseva GP, Voiskovoy AI, Krivenko AA, Donets IA (2014) Biological method for increasing adaptive potential of Stevia (*Stevia rebaudiana* (Bertoni) Bertoni), producer of native sugar substitute. Biol Med 6(3), Article ID: BM-048-14, 7 pages.

Received: 5th Nov 2014; Accepted: 9th Dec 2014; Published: 20th Dec 2014

Abstract
This paper describes the possibility to increase adaptive potential of stevia, a native sugar substitute, due to Biologically Active Substances (BAS) treatment of green apical stems used for obtaining plantlets. Positive prolonged action of biologically active substances of organic and microbial nature on growth and development of aerial parts and rhizosphere of Stevia plant has been established. Active use of these growth regulators has a comprehensive effect on metabolic processes. Combined treatment with all three preparations contributed to a significant increase in accumulation of stevioside complex in leaves. The developed biological method for increasing adaptive potential of stevia plants is not only of purely scientific but also of great practical importance. Growth regulators are used in the initial stage of the growing season, thus ensuring genetic and chemical purity of the products obtained; this fact is of particular importance, since stevia leaves are used for healthful and dietary meals for people with poor health, and for children.

Keywords: Stevia; native sugar substitute; biologically active substances; leaf mass yield.

Introduction

Stevia is a producer of natural sugar substitute for diabetics and people suffering from obesity and other disorders of carbohydrate and lipid metabolism. This plant grows in the wild part of South America and is widely cultivated [1-5]. Biologically Active Substances (BAS) that regulate plant growth are widely used in plant farming. It was established that the Darina 17 and the Potassium Ligno-Humate organic fertilizers, as well as the Biophyte 1 microbiological agent, actively influence cellular metabolism of agricultural plants, and are capable of increasing yield and quality of wheat, potatoes, sugar beet, etc. [6]. This is a result of BAS effect on the nature of modification variability, where manifestation of a symptom shifts to the positive area of reaction norm, without changing plants genotype [7,8]. However, effects of these biologically active substances on adaptive potential of stevia plants remain unstudied.

The integral characteristic of the process technology effect on the adaptive potential of stevia plants is the green vegetative mass yield rate, which characterizes a set of morphological and physiological processes in the genotype *x* of environment interaction system; on the other hand, productivity of green vegetative mass determines yield of marketable products (dry leaves).

Methods

In the conditions of the Central Caucasus, stevia is cultivated on a 1-yr cycle using seedlings obtained from rooting green apical leaf cuttings from parent plants in a greenhouse. The basis of the study was a three-factor field experience according to the $2 \times 2 \times 2$ scheme; Factor A: 0 – no treatment; 1 – treatment with Potassium Ligno-Humate; Factor B: 0 – no treatment; 1 – treatment with Darina 17; Factor C: 0 – no treatment; 1 – treatment with Biophyte 1.

Green leaf cuttings were treated with BAS aqueous solutions in accordance with the original methodology [9]. Field experience was established in accordance with common procedure. Plot accounting area is 6.3 m². Planting scheme is 0.25×0.75 m. The procedure is repeated three times. Monitoring – plants obtained from green cuttings from rooted plants without treating with BAS, preceded by winter soft wheat. Experimental data were processed using the method analysis of variance for a three-factor experiment [10].

Results and Discussion

During the years of research, moisture content varied (the limiting factor) during stevia crop season. In more favorable conditions, when there was enough moisture for stevia plants during the growing season of 2007, the average yield of green vegetative mass for the experiment was 13.65 t/ha (Table 1). Treating green leaf cuttings with Potassium Ligno-Humate determined an increase in the indicator, as compared to the reference, but its effect (+1.07 t/ha) was insignificant, $LEDA_{05} = 1.09$ t/ha.

A considerable increase in stevia green vegetative mass was obtained in variants with green leaf cuttings treated with Darina 17 (+1.21 t/ha) and Biophyte 1 (+1.33 t/ha), $LEDB_{05} = LEDC_{05} = 1.09$ t/ha. Considerable excess (+1.94 … 3.92 t/ha) as compared to the reference (11.32 t/ha) was obtained in all variants of treating green cuttings with BAS, except for processing with Darina 17 (+1.31 t/ha) where indicator value exceeded the reference, but was insignificant, since LED_{05} individual variance = 1.69 t/ha. The highest yields of green vegetative mass was obtained after treating with Darina 17 + Biophyte 1 (15.24 t/ha) and three preparations (15.13 t/ha).

In the conditions of rainless 2008, there was a reduction of green vegetative mass yield, as compared to 2007. The average green vegetative mass yield in 2008 was 7.70 t/ha. Treating leaf cuttings with Potassium Ligno-Humate (+0.04 t/ha) and Darina 17 (+0.30 t/ha) determined a slight increase in the indicator, as compared to the reference, the effects were insignificant, $LEDA_{05} = LEDB_{05} = 0.66$ t/ha. In these stressful for stevia plants conditions, significant effect was preserved of green leave cuttings treatment with Biophyte 1 solution (+2.08 t/ha), $LEDC_{05} = 0.66$ t/ha.

A considerable excess (+1.46 ... 3.18 t/ha) as compared to the reference (5.82 t/ha) was obtained for all variants of green leaf cuttings BAS treatment, except for processing with Potassium Ligno-Humate + Darina 17 (+1.31 t/ha), LED_{05} individual variance = 1.01 t/ha In drought conditions of 2008 the highest yield of green vegetative mass compared to other variants was in

Table 1: BAS effect on "Ramonskaya Sweet Tooth" stevia green vegetative mass yield, t/ha.

Factor A Potassium Ligno-Humate	Factor B Darina 17	Factor C Biophyte 1		A, $LED_{05} = 1.09$	B, $LED_{05} = 1.09$
		O	B1		
2007					
O	O	11.32	13.26	13.11	13.04
	D 17	12.63	15.24		14.25
L	O	13.95	13.63	14.18	
	D 17	14.02	15.13		
C, $LED_{05} = 1.09$		12.98	14.31	$LED_{05 factors interaction} = 1.53$ t/ha $LED_{05 individual variance} = 1.69$ t/ha	
$\bar{x} = 13.65$ S_x, % = 4.07%					
2008					
Factor A Potassium Ligno-Humate	**Factor B Darina 17**	**Factor C Biophyte 1**		**A, $LED_{05} = 0.66$**	**B, $LED_{05} = 0.66$**
		O	**B1**		
O	O	5.82	8.63	7.68	7.55
	D 17	7.41	8.85		7.85
L	O	7.28	8.47	7.72	
	D 17	6.13	9.00		
C, $LED_{05} = 0.66$		6.66	8.74	$LED_{05 factors interaction} = 0.92$ t/ha $LED_{05 individual variance} = 1.01$ t/ha	

Table 2: "Ramonskaya Sweet Tooth" stevia green vegetative mass yield, main effects and effects of interaction.

Variant of treatment	Main effects and interaction effects, t/ha		Average yield for 2 yrs
	2007	2008	
Total	$13.65 = \bar{x}$	$7.70 = \bar{x}$	–
Potassium Ligno-Humate	0.54	0.02	10.65
Darina 17	0.60	0.15	10.00
Biophyte 1	0.67	1.04*	10.95
Darina 17 + Biophyte 1	−0.22	−0.30	12.05
Potassium Ligno-Humate + Biophyte 1	−0.47	−0.02	11.05
Potassium Ligno-Humate + Darina 17	0.26	0.04	10.05
Potassium Ligno-Humate + Darina 17 + Biophyte 1	0.10	0.38	12.05
Reference (no treatment)	–	–	8.55

*Significant at probability level of 95%.

plants with leaves cuttings treated with Biophyte 1 (8.63 t/ha), Darina 17 + Biophyte 1 (8.85 t/ha), Potassium Ligno-Humate + Biophyte 1 (8.47 t/ha) and in case of triple treatment (9.00 t/ha).

In the conditions of the more favorable 2007, both main and interaction effects were insignificant (see Table 2).

In drought conditions of 2008, the effect of green leaf cuttings treatment with Biophyte 1 (1.04* t/ha) was most significant. Other effects, same as in the last year, were negligible. From the assessment of the main effects and the effects of their interaction, it follows that when leaf cuttings were treated with Potassium Ligno-Humate, Darina 17, and Biophyte 1 yield of green mass was largely determined by the additivism effect.

Thus, the positive effect of treating fresh planted stevia leaf cuttings with low-molecular aqueous BAS solutions: Potassium Ligno-Humate, Darina 17, Biophyte 1 manifested itself at the coenotic level as well, this was manifested in the yield of green vegetative mass of the "Ramonskaya sweet tooth" stevia breed. It should be noted that the phenotypic effect in the ontogeny of individual preparations is largely determined by water supply during the vegetation season of Stevia plants.

In average, during the 2 yrs of the research, treatment of green leaf cuttings with BAS improved green vegetative mass yield by 16.3-40.7%. The highest yields of green vegetative mass was obtained after treatment with Darina 17 + Biophyte 1 (12.05 t/ha) and Potassium Ligno-Humate + Darina 17 + Biophyte 1 (12.05 t/ha). In the reference group (no treatment) the indicator value was 8.55 t/ha.

In the conditions of 2007, average green leaf yield during the experiment was 6.31 t/ha (Table 3). Treatment with Darina 17 (+0.39 t/ha) and Biophyte 1 (+0.53 t/ha) determined slight increase in the indicator, as compared to the reference, $LEDC_{05}$ = 0.71 t/ha. Treating green leaf cuttings with Potassium Ligno-Humate determined a significant increase in the yield of green leaves (+1.03 t/ha), $LEDA_{05}$ = 0.71 t/ha.

Considerable excess (+1.30 ... 1.92 t/ha), as compared to the reference group (5.37 t/ha) was obtained in all cases of treating with Potassium Ligno-Humate: Potassium Ligno-Humate (6.75 t/ha), Potassium Ligno-Humate + Biophyte 1 (6.82 t/ha), and Potassium Ligno-Humate + Darina 17 + Biophyte 1 (7.29 t/ha). Significant positive effect had green leaf cuttings treatment with Darina 17 + Biophyte 1 (6.67 t/ha), LED_{05}individual variance = 1.10 t/ha. Excess of green leaves yield was insignificant as compared to the reference group after treatment with Biophyte 1 (+0.13 t/ha) and Darina 17 (+0.25 t/ha).

In the conditions of rainless 2008, there was a reduction of green leaves yield, as compared to 2007. The average green leaves yield in 2008 was 3.83 t/ha. Green leaf cuttings treatment with Potassium Ligno-Humate (−0.01 t/ha) and Darina 17 (0.10 t/ha) determined slight increase in the indicator, effects were not significant, $LEDA_{05}$ = $LEDB_{05}$ = 0.36 t/ha. Significant effect appeared after treating green leaf cuttings with Biophyte 1 (+0.78 t/ha), $LEDC_{05}$ = 0.36 t/ha.

Table 3: BAS effect on "Ramonskaya Sweet Tooth" stevia green leaves yield, t/ha.

Factor A Potassium Ligno-Humate	Factor B Darina 17	Factor C Biophyte 1		A, $LED_{05} = 0.71$	B, $LED_{05} = 0.71$
		O	B1		
2007					
O	O	5.37	5.50	5.79	6.11
	D 17	5.62	6.67		6.50
L	O	6.75	6.82	6.82	
	D 17	6.43	7.29		
C, $LED_{05} = 0.71$		6.04	6.57	$LED_{05 factors interaction} = 0.99$ t/ha $LED_{05 individual variance} = 1.10$ t/ha	
$\overline{x} = 6.31 S x$, % = 5.75%					
2008					
Factor A Potassium Ligno-Humate	Factor B Darina 17	Factor C Biophyte 1		A, $LED_{05} = 0.36$	B, $LED_{05} = 0.36$
		O	B1		
O	O	2.87	4.29	3.84	3.78
	D 17	3.87	4.31		3.88
L	O	3.97	4.00	3.83	
	D 17	3.07	4.28		
C, $LED_{05} = 0.36$		3.44	4.22	$LED_{05 factors interaction} = 0.50$ t/ha $LED_{05 individual variance} = 0.56$ t/ha	
$\overline{x} = 3.83 S x$, % = 4.80%					

A considerable excess (+1.00 ... 1.41 t/ha) as compared to the reference (2.87 t/ha) was obtained for all variants of green leaf cuttings BAS treatment, except for processing with Potassium Ligno-Humate + Darina 17 (+0.20 t/ha), LED_{05} individual variance = 0.56 t/ha. In unfavorable conditions of vegetation during the rainless 2008, high yield of green leaves was formed by treating green leaf cuttings of plants with Biophyte 1 (4.29 t/ha), Darina 17 + Biophyte 1 (4.31 t/ha), Ligno-Humate + Biophyte 1 (4.00 t/ha), and Potassium Ligno-Humate + Darina 17 + Biophyte 1 (4.28 t/ha).

In conditions of 2007, significant positive effect (3.14* t/ha) appeared after treating green leaf cuttings with Potassium Ligno-Humate + Biophyte 1; LED_{05} factors interaction was 0.99 t/ha (Table 4).

Table 4: "Ramonskaya Sweet Tooth" stevia green leaves yield main effects and interaction effects.

Variant of Treatment	The main effects and interaction effects, t/ha		Average yield for 2 yrs
	2007	2008	
Total	$6.31 = \overline{x}$	$3.83 = \overline{x}$	–
Potassium Ligno-Humate	0.52	−0.002	5.40
Darina 17	0.20	0.05	4.75
Biophyte 1	0.26	0.39*	4.90
Darina 17 + Biophyte 1	−0.16	−0.21	5.50
Potassium Ligno-Humate + Biophyte 1	3.14*	−0.08	5.40
Potassium Ligno-Humate + Darina 17	0.21	0.02	4.75
Potassium Ligno-Humate + Darina 17 + Biophyte 1	−0.02	0.27	5.80
Reference (no treatment)	–	–	4.15

*Significant at probability level of 95%.

Thus, Potassium Ligno-Humate and Biophyte 1 showed synergism in the effect of green leaf cuttings treatment on green stevia leaves yield. In other cases, both main and interaction effects were insignificant, i.e., the effect of additivism was encountered

In drought conditions of 2008, the effect of green leaf cuttings treatment with Biophyte 1 (0.39* t/ha) was the most significant, HCP05 main effects was 0.36 t/ha. Insignificant were both main effects of treating with potassium Ligno-Humate and Darina 17, and interaction effects. Thus, the effect of additivism manifested itself.

In average, during the 2 yrs of the research, treatment of green leaf cuttings with BAS improved green leaves yield by 15.4-40.7%. The highest yield of green leaves was obtained after treatment with Potassium Ligno-Humate (5.40 t/ha), Potassium Ligno-Humate + Biophyte 1 (5.40 t/ha), Darina 17 + Biophyte 1 (5.50 t/ha), and treatment with three preparations: Potassium Ligno-Humate + Darina 17 + Biophyte 1 (5.80 t/ha). In the reference group (no treatment) the indicator value was 4.15 t/ha.

Thus, the effect various BAS treatment on stevia green leaf yield and on green vegetative mass yield was determined by conditions of stevia plant water supply during ontogeny. In 2007, Potassium Ligno-Humate and Biophyte 1 showed synergism in the effect of green leaf cuttings treatment on green stevia leaves yield, which is confirmed by the considerableness of the positive effect on their interaction (3.14* t/ha), LED_{05} factors interaction was 0.99 t/ha. In arid 2008, treatment with Biophyte 1 has the biggest effect. In other cases, both main and interaction effects were insignificant, i.e., the effect of additivism was encountered.

In the more favorable conditions of 2007, average dry leaf yield was 6.31 t/ha (Table 5).

Green leaf cuttings treatment with BAS caused an increase in the indicator, but their effect was insignificant: Potassium Ligno-Humate (+0.27 t/ha), Darina 17 (+0.16 t/ha), and Biophyte 1 (+0.21 t/ha), $LEDC_{05}$ = 0.28 t/ha.

A considerable excess of dry leaf yield as compared to the reference group (2.15 t/ha) was observed after green cuttings treatment with Potassium Ligno-Humate (+0.55 t/ha) + Darina 17 + Biophyte 1 (+0.52 t/ha), Potassium Ligno-Humate + Biophyte 1 (+0.58 t/ha), and Potassium Ligno-Humate + Darina17 + Biophyte 1 (+0.76 t/ha), LED_{05} individual variance = 0.44 t/ha.

In the rainless conditions of 2008, there was a reduction of green leaves yield, as compared to 2007. The average green leaves yield in 2008 was 1.34 t/ha. Treatment of green leaf cuttings with Potassium Ligno-Humate (+0.05 t/ha)

Table 5: BAS effect on "Ramonskaya Sweet Tooth" stevia dry leaves yield, t/ha.

Factor A Potassium Ligno-Humate	**Factor B Darina 17**	**Factor C Biophyte 1**		**A, LED_{05} = 0.28**	**B, LED_{05} = 0.28**
		O	**B1**		
2007					
O	O	2.15	2.20	2.32	2.44
	D 17	2.25	2.67		2.60
L	O	2.70	2.73	2.59	
	D 17	2.57	2.91		
C, LED_{05} = 0.28		2.42	2.63	$LED_{05factorsinteraction}$ = 0.39 t/ha $LED_{05individualvariance}$ = 0.44 t/ha	
$\bar{x}$ = 2.52S x, % = 5.74%					
2008					
Factor A Potassium Ligno-Humate	**Factor B Darina 17**	**Factor C Biophyte 1**		**A, LED_{05} = 0.09**	**B, LED_{05} = 0.09**
		O	**B1**		
O	O	0.99	1.27	1.32	1.20
	D 17	1.33	1.68		1.48
L	O	1.28	1.26	1.37	
	D 17	1.07	1.85		
C, LED_{05} = 0.09		1.17	1.52	$LED_{05factorsinteraction}$ = 0.13 t/ha $LED_{05individualvariance}$ = 0.14 t/ha	

Table 6: Main effects and interaction effects of the "Ramonskaya Sweet Tooth" stevia dry leaves yield.

Variant of treatment	The main effects and and effects of interaction, t/ha		Average yield for 2 yrs
	2007	2008	
Total	$2.52 = \bar{x}$	$1.34 = \bar{x}$	–
Potassium Ligno-Humate	0.21	0.02	1.99
Darina 17	0.08	0.14*	1.79
Biophyte 1	0.11	0.17*	1.74
Darina 17 + Biophyte 1	−0.07	−0.05	2.18
Potassium Ligno-Humate + Biophyte 1	−0.01	0.02	2.00
Potassium Ligno-Humate + Darina 17	0.09	0.11	1.82
Potassium Ligno-Humate +Darina 17 + Biophyte 1	0.37	0.09	2.38
Reference (no treatment)	–	–	1.57

*Significant at probability level of 95%.

caused an insignificant increase, while treatment with Biophyte 1 (0.35 t/ha) and Darina 17 (+0.28 t/ha) caused a significant increase in dry leaves yield, $LEDC_{05} = 0.09$ g.

Thus, the conditions of water supply for the "Ramonskaya sweet tooth" stevia breed had strong impact on manifestations of BAS effect during dry leaf yield formation, as well.

The significant positive effects manifested itself only in arid vegetation conditions in 2008 after Biophyte 1 was used, which also manifested itself in the green vegetative mass yield and green leaves yield; and after treatment with Darina 17, which showed a specifically positive impact on this particular indicator.

A considerable excess (+0.27 ... 0.86 t/ha) as compared to the reference group (0.99 t/ha) was obtained for all variants of green leaf cuttings BAS treatment, except for processing with Potassium Ligno-Humate + Darina 17 (+0.08 t/ha), where an increase was insignificant (LED_{05} individual variance = 0.14 t/ha).

In drought conditions of 2008 the highest yield of dry vegetative mass as compared to the reference group was in plants treated with Darina 17 + Biophyte 1 (1.68 t/ha) and Potassium Ligno-Humate + Darina 17 + Biophyte 1 (1.85 t/ha). With that, dry leaves yield after treatment with three preparations was substantially higher than that after various variant of treatments (0.17 + ... +0.78 t/ha).

In the conditions of a more favorable 2007, both main and interaction effects were insignificant (see Table 6).

In drought conditions of 2008, the effect of green leaf cuttings treatment with Biophyte 1 (0.17* t/ha) and Darina 17 (0.14* t/ha) was most significant, HCP05 main effect was 0.09 t/ha. Thus, during both years of the experiment, the phenomenon of additivism prevailed. In average, during the 2 yrs of the research, treatment of green leaf cuttings with BAS improved green leaves yields by 6.2-51.6%. The highest dry leaves yield was obtained by treatment with Potassium Ligno-Humate (1.99 t/ha), Potassium Ligno-Humate + Biophyte 1 (2.00 t/ha), Darina 17 + Biophyte1 (2.18 t/ha). The greatest positive effect was obtained by treatment with a mixture of three preparations: Potassium Ligno-Humate + Darina 17 + Biophyte 1 (2.38 t/ha), the increase as compared to the reference group without treatment was +51.6%.

Conclusion

In general, treatment of green leaf cuttings with biologically active substances: Potassium Ligno-Humate, Darina 17, and Biophyte 1 had an overall positive prolonged effect on formation of green vegetative mass yield, green and dry leaves yield, yield values reached 40.9, 41.5, and 51.6%, respectively, while yield values in the reference group (no treatment) were: 8.55, 4.15, and 1.57 t/ha.

On average, 2 yrs maximum productivity of green vegetative mass of green and dry leaves was obtained by treatment with a mixture of three preparations: Potassium Ligno-Humate, Darina 17, and Biophyte 1 (12.05, 4.75, and 2.38 t/ha, respectively). The highest yield of green vegetative mass was obtained after green leaf cuttings treatment with Darina 17 + Biophyte 1 (12.05 t/ha), while yield of dry leaves was obtained after treatment with Potassium Ligno-Humate (1.99 t/ha), Potassium Ligno-Humate + Biophyte 1 (2.00 t/ha), Darina 17 + Biophyte 1 (2.18 t/ha), and a mixture of the three preparations (2.38 t/ha).

From the insignificance of the main effects, except for the effects of treatment with Biophyte 1 and Darina 17, dry leaf yield in drought 2008, and interaction effects during both years of experiment, it follows that additivism prevailed.

The significant effect of green leaf cuttings treatment with Biophyte 1 and Darina 17 in drought conditions makes it possible to recommend these preparations for green leaf cuttings treatment in order to improve stevia sustainability to the limiting environmental factor. At large, treatment of green leaf cuttings with BAS before planting promotes improving adaptive potential of this crop in the conditions of the Central Caucasus.

References

1. Sumida T (1980) Studies on *Stevia rebaudiana* (Bertoni) as a new possible crop for sweetening resource in Japan. Journal of the Central Agricultural Experiment Station 31(1): 67-71.

2. Bertoni SM (1901) Plantas Usuales del Paraguay. Brossa: Asuncion, pp. 40-68.

3. Pokhrel PR (2009) Stevia an Introduction. Nepal: Lumbini Buddha Prints, p. 26.

4. Pokhrel PR (2010) Stevia growing in Nepal. Gorkhapatra, Dec. 4.

5. Pokhrel PR (2010) Stevia cultivation in Nepal – An introduction. Nepali National Daily, Dec. 4.

6. Barabash IP (2009) Phytohormones: Growth regulators. In the Classification: Theory and Practice. Stavropol: Stavropol SAU, p. 381.

7. Donets IA, Kryvenko AA, Voiskovoy AI (2009). Use of biologically active substances for inducing economically valuable modifications of stevia. Sugar Beet 7: 38-40.

8. Pokhrel PR (2010) Russian scientist in Udayapur. Muluki Khabar National Daily, Dec. 4.

9. Kryvenko AA, Donets IA, Voiskovoy AI (2010) A Method of Treating Stevia Green Leaf Cuttings. Russia, Stavropol State Agrarian University, Patent No. 2402193.

10. Dospekhov BA (2011) Field Study Methods. Moscow: Agropromizdat, p. 351.