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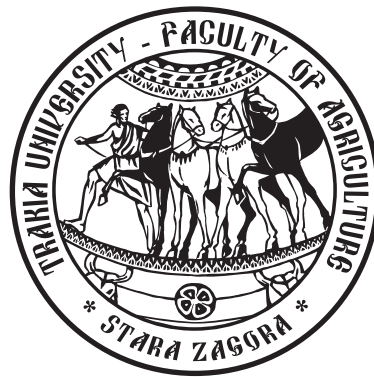
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Assessment of initial material for stevia (*Stevia rebaudiana* B.) breeding

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Abstract. There is an increased interest in growing stevia (*Stevia rebaudiana* Bertoni) as a source of natural, non-caloric sweeteners in recent years. In the 1980's, in the former Sugar beet Institute near Shumen our researches on the introduction and growth of stevia have been carried and the optimal technological conditions and in vitro propagation methods have been established. In the present research some results are given of the assessment of origins made in 2011 as initial material for breeding. The productiveness of fresh mass of the whole plants, dry matter after drying, yield as a ratio between dry leaves weight and the fresh mass, the height, the number of shoots or nodes and the percentage of plants with visible signs of *Alternaria* and *Fusarium* diseases have been measured.

Keywords: stevia, fresh mass, dry matter, productiveness

Introduction

Stevia (*Stevia rebaudiana* Bertoni) is a perennial, cross-pollination plant of the short day from the *Compositae* family. It comes from the mountains of Paraguay and Brasil. In recent years there is a strong interest in its growing as a source of natural low-caloric sweeteners. They are of the type of the diterpene glucosides as rebaudiside A, B, C, E and stevioside and are 300 times sweeter than sucrose (Lewis, 1992).

Because of its sensitivity to low temperatures in the countries with moderate climate like Bulgaria stevia is grown as an annual plant, which is maintained by preservation of the roots. An alternative successful method is the propagation and maintenance of Stevia by in vitro methods (Bojimirov and Slavova, 2011), by which the initial genotype is preserved. For the seed formation a certain light and temperature regime is necessary (Zaidan et al., 1980). The low germination of the seeds is an obstacle for their mass usage for growing stevia in our conditions (Varbanov et al., 1996). The great genetic variability of the seed progeny is a basis for enrichment of the gene pool for selection of forms with high productivity and steviosides content. In the 1980's, in the former Sugar beet Institute near Shumen our researches have been carried on the introduction and growth of stevia and the optimal technological conditions have been established and in vitro methods for propagation have been developed. With the current interest a project for breeding new forms of stevia has been started in the Agricultural Institute–Shumen. In the research some results are given of the 2011 assessment of origins as an initial breeding material.

Material and methods

Materials of the breeding program of Agricultural Institute–Shumen has been used, and also origins from Japan and the USA. Seeds progeny from 2010 harvest were sown and plantings in different phases were obtained. At the same time plants from adopted regenerants from seed and somaclonal progeny from

in vitro propagation and cultivation of immature embryos and meristems were received. Totally 800 plants from seeds and 1000 from multiplied plants were planted. For assessment were planted also 680 roots from 140 origins.

Measurements of the fresh mass productiveness of the whole plants, of the dry matter after drying, the yield as a ratio between the weight of the dry leaves and the fresh mass, were made on 10 plants of each origin in a phase before button formation. The productiveness was recalculated in kilograms per da with 6666 plants per da for the 1 year plantings and 50000 plants per ha for the roots. The height is measured from the place of cutting to the peak of the plant. The number of shoots or nodes is counted before the swath. The percentage of the plants with visible signs of *Alternaria* and *Fusarium* infection was counted too.

A variation analysis of these indices of 61 origins, grown from roots, and of 149 origins of adopted plants from seeds, embryos and meristems regenerants was made according to Lidanski (1988). 2-A8, 2-17, 2-19 and 3-53 are selected origins, tested in the second year of development from roots preserved in the winter. In first year of development from seeds are 4-49FM, 6-11FM, 6-19FM, 5-76FM and the adopted to external conditions propagated regenerants from in vitro isolated meristems, embryos and immature seeds are 5-137RM, 3-168RM, 3-170RM, 3-171RM.

Results and discussion

The results of the variation analysis, given in Table 1, are indicative of the presence of a great variability of the studied gene fund. The height of the plants from roots during the cutting varies from 55 cm to 100 cm with mean of 75 cm, while the plants from 1 year plantings are higher and reach 105 cm. The number of shoots is strongly influenced by the genotype, and by the planting itself and the way of planting. The infected plants among the plants from roots are significantly more and reach 24% of the counted plants, which is explained with the accumulation of pathogens there. The use of regenerants of in vitro developed seeds, embryos and especially

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Table 1. Variation analysis of stevia origins traits, 2011

Variant	Parameters of variability				
	Mean $\pm$ SEM	Min	Max	CV %	P%
Roots II year – 61 pcs					
Height, cm	75.7 $\pm$ 0.81	55	100.0	13.2	1.69
Number of shoots	12.6 $\pm$ 1.77	1	30.0	30.9	1.39
Part of infected, %	24.4 $\pm$ 2.35	0	90.0	75.0	9.94
Fresh mass yield, kg/ha	16083 $\pm$ 646	7556	35835	31.1	3.99
Dry matter content, %	24.4 $\pm$ 0.72	2.09	54.0	22.9	2.94
Output, %	15.4 $\pm$ 0.47	9.69	35.7	23.8	3.05
Dry mass yield, kg/ha	2438 $\pm$ 115	978	5556	36.7	4.71
Plantings I year – 149 pcs					
Height, cm	77.1 $\pm$ 12.4	45	105	13.5	1.11
Number of shoots	2.72 $\pm$ 0.12	1	19	23.5	4.39
Part of infected, %	9.95 $\pm$ 1.19	0	83.3	45.7	11.9
Fresh mass yield, kg/ha	14870 $\pm$ 386	5667	32223	31.7	2.59
Dry matter content, %	24.2 $\pm$ 0.32	13.6	41.2	15.9	1.31
Output, %	13.6 $\pm$ 0.27	8.2	38.9	22.0	1.80
Dry mass yield, kg/ha	1987 $\pm$ 496	912	4110	30.3	2.84

meristems, is an effective method for healing the planting. The fresh mass productiveness of stems and leaves is strongly dependent on the genotype and the variation coefficient reaches 31%. The plants from roots are more productive than the I year planting and their yield is an average of 16000 kg/ha, and there are origins with more than 30000 kg/ha. The dry matter varies less – from 10% to 40%, mean of

24% and output varies from 9,69% to 35,7%. The part of the leaves of the total weight varies for the different origins, that is why the output is also between 8 and 38 percents. The final yield of dry mass is a resultative index and is with a high degree of variation.

Data for selected origins, shown on Table 2, are also indicative for the stevia productive potential. The USA origin 2-6A8 is used as a

Table 2. Productiveness of selected stevia origins, 2011

Variant	Fresh mass kg/ha	Output %	Dry leaves mass kg/ha	Second grow up			Total yield of dry leaves mass	
				Fresh mass kg/ha	Output %	Dry leaves mass kg/ha	kg/ha	Rel. %
Roots II-year								
2-6 A8	11660	15.4	1800	9260	17.8	1650	3450	100
2-17	19490	13.7	2070	13210	16.8	2220	4290	124
2-19	16250	13.9	2250	11630	17.1	1990	4240	123
3-53	13110	11.8	1530	7100	18.3	1300	2830	82
I-year from seeds								
4-49 FM	10000	15.6	1560	7750	16.5	1280	2840	82
6-11 FM	17300	13.3	2300	11340	11.9	1350	3650	106
6-19 FM	14030	12.4	1730	7080	14.4	1020	2750	80
5-76 FM	25370	12.6	3197	21480	12.1	2600	5800	168
I-year regenerants								
5-137 RM	27040	12.6	3400	18150	13.5	2450	5850	170
3-168 RM	21670	9.1	1970	14290	14.2	2030	4000	116
3-170 RM	15250	8.2	1250	13610	15.8	2150	3400	96
3-171 RM	18290	8.1	1490	13330	13.8	1840	3330	97

Standard. The most productive in the second year of development from roots origins reach 19490 kg fresh mass yield, and up to 2250 kg dry leave mass during the main harvest before button formation. Also high is the regenerative productive potential – up to 13210 kg fresh mass and 2220 kg dry leaves. The yield for both cuttings is not over 18.3%. The total yield increases with the possibility for a second grow up, and reaches 5000 kg/ha.

From the tested annual plants the highest productivity manifested the adopted in vitro regenerants of meristems and immature embryos. The total dry leaves yield reaches 5850 kg for 5-137RM. The lower productivity for the seeds progenies is combined with a higher output as a result of the higher dry matter content and the better leaf forming of the plants. The maintained by in vitro micropropagation regenerants keep unchanged the genotype of the selected for productivity origins. With the seeds progenies in result of the sexual reproduction appears a break-up directed to the initial wild genotype, which has a lower productivity.

Conclusion

A rich gene fund for stevia breeding has been created. The tested origins are characterized by high productiveness of fresh and dry mass and resistance to leaf diseases. The methods of in vitro propagation allow maintaining selected genotypes with high

productivity. With the roots reproduction there is an accumulation of pathogenic factors, which have negative effects on productivity. The propagation via seeds and regeneration from immature embryos enriches the gene pool, and with totally decreasing productivity the potential for selection of genotypes with higher dry matter content and number of leaves is increased.

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