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OPTIMICATION OF SCHEMES FOR REPRODUCTION OF STEVIA VARIETY 'STELA'

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Abstract

In Agricultural Institute - Shumen it has been carried out maintenance and multiplication of the first Bulgarian stevia variety 'Stela', certified in 2017. In vitro technics for maintenance and micropropagation are used, as well as in vivo development of seedlings from cuttings by preservation and reproduction of rhizomes. During the last years are often the conditions of warm and continuous autumn with a regime of the short day from the end of September, which favors the reproductive development of seeds. The results for the measured indices of height, number of stems, fresh and dry mass and the calculated randeman and dry leaves yield of seed off-springs are indicative for the stabilization of comparatively high levels of productivity of elite clones of the variety 'Stela'. The assessment shows that the most effective scheme for practical realization of seedlings is by sowing seeds in controlled conditions in January-February, diving off the germinated and stabilized plants in April-May, transplanting in June-July and receipt of formed rhizomes in September-October, which, after preservation to be realized during the next period.

Key words*: stevia, variety maintenance, reproduction.*

INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is a perennial, cross-pollinating plant from the *Asteraceae* family. It is native to the highlands of Paraguay and Brazil (Lewis, 1992). In recent years, there has been an increased interest in its cultivation as a source of natural, non-caloric sweeteners. They belong to the family of diterpene glucosides such as rebaudiside A, B, C, E and stevioside and are up to 300 times sweeter than sucrose (Geuns, 2004).

Due to its sensitivity to low temperatures in countries with a temperate climate, it is grown as an annual and can be maintained by storing the rhizomes (Lankes & Pude, 2008). Propagation by rooting cuttings from rhizomes stored in the winter is widely used. An alternative method is propagation and maintenance by in vitro methods, in which the original genotype is also preserved (Krumov et al., 1984).

In natural conditions, it is propagated by seeds. Ste via is a short-day plant and fertilization requires a certain light and temperature regime (Zaidan et al., 1980). As a cross-pollinated crop, self-incompatibility mechanisms prevent self-pollination and the production of homozygous offspring (Nakamura et al., 1985). Flowering is non-simultaneous and long-lasting. Already in the 1980s at the Sugar Beet Institute - Shumen, research was conducted on the introduction and cultivation of stevia, and the optimal technological conditions were established and methods for in vitro propagation were developed (Varbanov et al., 1996).

With the renewal of interest, the Shumen Agricultural Institute is working on the selection of new forms of stevia. In 2017, the first Bulgarian variety was recognized, with high economic qualities and adapted to the conditions of Bulgaria (Uchkunov, 2006).

Some aspects of obtaining and germinating seeds in vitro and under external conditions have been studied (Bozhimirov & Slavova, 2011; Kikindonov and Enchev, 2012). Under our conditions from propagated in vitro and after prolonged adaptation in spring in greenhouse conditions, individual plants begin to flower at the end of July. The mass flowering is from September until the harvesting of the rhizomes before the formation of autumn frosts (Bozhimirov & Slavova, 2011). The great genetic diversity of the seed progeny is the basis for the enrichment of the gene pool for the selection of forms with high productivity and stevioside content (Caneiro et al., 1997). The research presents the results of

attempts to optimize the schemes for the reproduction of seedlings of the stevia variety Stella in the conditions of Bulgaria.

MATERIALS AND METHODS

The data for the survey are from researches in 2017-2021 at the Agricultural Institute - Shumen. Field trials of seedlings from elite branches were conducted on carbonate chernozem, with 50 cm inter-row spacing and 20 cm within-row spacing. Optimum moisture storage is provided with drip irrigation. Pesticide treatment and mineral fertilization are not applied.

Seedlings from rhizomes, cuttings and seeds are planted after 25.04 to avoid frosts. Plants for economic evaluation are measured for height and number of main stems and individually mowed at the appearance of flower buds, before flowering. After separating the leaves from the stems, their mass was measured in the fresh state and after drying. Thus, the weight of the fresh and dry mass, the dry content and the yield as a ratio of the dry leaves to the total fresh mass are determined.

Selected elite plants are left to flower and produce seeds. Individual plants begin to bloom at the end of July. Mass flowering is from September until the harvesting of the rhizomes before the formation of autumn frosts. In our research, two ways of obtaining seeds are used. In the first, individual ripe seeds are harvested from plants in the field. In the second case, whole plants are harvested at the end of the growing season and dried whole. Later, the seeds are threshed without separating the empty and unripe ones.

After taking into account the diseased and dead plants, the rhizomes are harvested at the end of the growing season - usually the beginning of November and stored in a dark room at 0-4°C. In the spring before planting, the surviving rhizomes with the presence of viable stem primordia are counted.

Elite rhizomes are placed under optimal conditions of lighting, humidification and temperature of 25°C for the growth of stems. Cuttings are cut from them into 2-4 times and planted in a soil mixture under the same conditions for rooting.

To determine the laboratory germination, the seeds were planted in harmonica filter paper, in four replicates of 20 seeds, for each origin. They are counted on the seventh day. The mass of 1000 seeds was determined. In a thermostatic room at 20-25°C and lighting for 18 hours in pots with a soil mixture 4/1/1 of peat, perlite and sand, 50 mg of seeds are sown in 4 repetitions for each origin. The seeds are distributed evenly on the surface of the pre-moistened mixture and pressed tightly against it. Optimum humidification is maintained with a polyethylene chamber during the first days, avoiding over moistening.

Rooted plants from seeds and cuttings, usually 30 days after planting and sowing, are plucked - replanted 1 in a pot. Usually, it takes up to 45 days to get a seedling suitable for planting in outdoor conditions.

Statistical data processing included analysis of variance for mean, coefficient of variation, mean error, and trial accuracy.

RESULTS AND DISCUSSIONS

The main method of reproducing stevia in the conditions of Bulgaria with a moderate climate and alternation of seasons is to harvest the developed rhizomes in the fall, store them in the winter and plant them in the spring of the fall year. To maintain a base population of the Stella variety, up to 500 rhizomes are planted annually. During the period 2017-2019, the number of healthy rhizomes that survived from the stored rhizomes in the spring, planted in the field, harvested and removed in the autumn was recorded consecutively. In this way, the proportion of survival during storage and cultivation of perennial rhizomes of elite branches of the variety was calculated (Table 1).

On average over the three years, 15% of the planted rhizomes fall off during the growing season and are not harvested for foliage and storage. During winter storage and after removal and spring harvesting due to decay and lack of viable stems, an average of 26% is lost.

Propagation by rhizomes leads to an accumulation of soil pathogens over the years and increases the incidence of foliar diseases. This necessitates a mandatory cyclical

alternation of propagation by rhizomes with *in vitro* and *in vivo* propagation for the production of elite seedlings.

Table 1. Results of assessment for survival during storage and vegetation of rhizomes from elite branches of stevia variety Stela, 2017-2019

Variants	Stored rhizomes		Planted rhizomes	
	Number	Survived, %	Number	Harvested, %
203/2	150	56.2	78	95.8
A 8	148	75.0	108	89.3
9 E	149	50.0	69	97.4
97 ШЗ	240	50.0	126	100.0
10 ШЗ	273	68.0	95	91.4
7 E	156	76.0	111	93.9
№ 3	150	74.5	101	72.7
№ 5	150	61.8	50	66.0
75 ШЗ	145	83.3	99	87.2
214/2	140	65.0	120	80.0
A 9	239	79.5	125	92.0
A 10	151	80.0	121	86.7
Total	4045		2688	
Mean		73.8		85.8

In 2020, 12 selected rhizomes were placed in sand and under optimal conditions for development with 25°C and regular humidification. After the development of shaped stems of 10-15 cm, the upper parts with up to 4-6 leaves are cut off and planted 8-12 in pots with soil mixture for rooting. Within a month, the branches from the cut rhizome stems form new stems suitable for cuttings. On average, 96 cuttings with 26, 45 and 26 pieces from each cutting were obtained from a rhizome with three cuttings. After 30 days, on average, 85.8% of the cuttings are rooted and plucked - 1 plant is replanted in a pot and after 30-45 days of adaptation to external conditions, seedlings. Suitable for planting under field conditions are obtained.

The effect of the method of propagation by cuttings can be greatly increased by cutting off the tops of the rooted plants to obtain additional cuttings, which, however, is associated with an extension of the period for the production of seedlings.

Table 2. Results of the assessment for reproductive potential of cuttings from stored rhizomes from elite branches of stevia variety Stela, 2021

Variants	Planted cuttings:			Rooted cuttings %
	First dare	Second date	Third date	
9 E	30	45	20	93.8
75 ШЗ	28	46	25	73.5
№ 5	18	42	15	85.0
10 ШЗ	25	35	15	84.1
A 8	31	47	24	88.5
28 СР	20	24	17	91.1
97 ШЗ	15	25	15	86.7
62 ШЗ	36	45	23	90.5
10 ШЗ	33	26	14	94.4
№ 3	1K2	24	12	86.3
№ 4	17	25	15	85.1
A 10	40	43	24	75.7
X	26.7	44.6	26.2	84.9
VC	22.1	18.9	25.3	8.67
P%	4.22	3.11	5.73	2.71

The trend for a longer and warmer autumn in our area in recent years is an opportunity to cover the conditions of the stevia for the accumulated temperature sum and the short day at the end of the growing season. This is a condition for obtaining a larger number of viable seeds. In our research in 2020, two ways of obtaining seeds are used. In the first, individual ripe seeds are harvested from plants in the field. In the second case, whole plants are harvested at the end of the growing season and dried whole. Later, the seeds are threshed without separating the empty and unripe ones.

The seeds obtained in both ways were evaluated for germination under laboratory conditions (Table 3). The difference in germination is significant in favor of the selected single seeds during the growing season, which have an average of 55.7% germination. The postharvest chaff of whole plants at the end of the growing season was 21.8% on average, and with a lower degree of

variation VC - 22.6%. The advantage of the second method is obtaining more seeds with significantly fewer resources. This allows more mass use of seeds for reproduction in practice. For this reason, after determining the mass of the seeds, they are sown in a soil mixture of 50 mg per repetition, taking into account the germinated sprouts. With an average mass of 400 mg per 1000 seeds and relatively little variation, an average of 125 seeds per set replicate is obtained and the exact germination rate is easily calculated - an average of 36%.

The results presented are positive for the use of seeds to increase the potential for reproduction in practice. But a strict selection of the elite plants for seed reproduction must be observed to preserve the genotype of the base population of the variety.

Table 3. Results of assessment for qualities of seeds from elite plants of stevia variety Stela, 2021

Variants	Laboratory germination of selected seeds, %	Laboratory germination of seeds after threshing, %	Mass of 1000 seeds, mg	Germination in soil substrate, number of seedlings/50 mg seeds
1. 20-20	81.3	6.70	350	54.3
2. 20 -9	66.7	26.7	354	47.5
3. 20 -6	53.3	33.3	320	51.2
4. 20 - 11	66.7	26.3	350	57.0
5. 20 - 14	40.0	13.7	360	38.0
6. 20 - 15	46.7	13.3	450	39.2
7. 20 - 10	19.8	10.0	430	17.0
8. 20 - 18	73.3	33.3	490	50.0
9. 20- 16	46.3	20.0	400	42.1
10.20 - 19	80.0	33.3	510	66.0
11.20 - 12	53.3	26.7	390	47.3
12.20-5	40.0	20.0	350	31.0
Mean	55.7	21.9	403,6	45.01
SE+/-	12.6	14.8	78.9	12.7
VC%	29.3	22.6	8.73	27.8

In 2020, rhizome seedlings, cuttings and seeds were planted under field conditions. Table 4 shows the results of the analysis of individual assessment by morphological and economic indicators. The rhizomes show the strongest development of the vegetative mass, reflected in taller and more branched stems and a greater accumulation of fresh and dry biomass. Seedlings from cuttings are more vigorous in development than seed progeny, which is due to the slower initial development from germination to obtaining the adapted seedling. According to economic indicators such as dry content and yield, the differences are insignificant. The identical and relatively low levels of variation are also impressive. This is an indicator that the implementation of breeding schemes does not affect the stability of the phenotype of the variety.

Table 4. Results of variation analysis of morphological and productive parameters of harvested plants of elite branches from rhizomes and cuttings, and seeds progenies of Stela variety, 2020

Variants	Height	Stems	Fresh mass from		Dry mass from		Dry matter, total %	Randeman, %
			Leaves	Stems	Leaves	Stems		
	cm	number	g	g	g	g	%	%
640 rhizomes								
Mean	78,2	9,55	274,5	201,8	76,8	56,3	28,1	16,3
max	95,0	19,00	360,0	280,0	105,0	85,0	32,6	21,7
min	65,0	3,00	180,0	120,0	55,0	35,0	20,9	11,9
vc	11,8	29,4	18,8	25,3	20,6	19,9	11,7	17,5
p%	3,56	8,89	5,69	7,63	6,22	9,04	3,55	5,29
515 pcs seedlings from cuttings								
Mean	72,3	5,27	255,9	160,4	70,0	46,8	27,6	16,8
max	85,0	12,0	380,0	260,0	115,0	70,0	31,6	21,4
min	65,00	5,00	150,0	85,00	35,0	25,0	23,9	14,0
vc	9,00	35,0	20,5	22,4	24,4	24,8	9,7	14,7
p%	2,71	10,5	9,21	9,78	10,3	10,5	2,9	4,44
550 pcs of seedlings from seeds								
Mean	71,9	5,91	182,2	126,3	54,0	36,8	30,0	17,8
max	94,0	9,00	210,0	155,0	65,0	50,0	46,8	25,5
min	60,0	3,00	125,0	100,0	45,0	25,0	22,7	13,6
vc	13,0	26,7	14,6	17,2	19,5	22,1	10,9	19,3
p%	3,94	8,05	4,43	5,20	3,49	6,67	6,32	5,82

The possibility of obtaining developed seedlings from seed to compensate for slower initial development has been investigated. For this purpose, non-pricked seedlings from seeds were planted later on 15. 06. densely at an average of 50 plants per square meter. It was harvested and the leaf mass yield was calculated. At the end of the growing season, 1,800 rhizomes with a weight of 10-15 g were obtained from 35 square meters, suitable after storage for planting the following year. The harvested dry leaf mass from this area is 9.17 kg. which equates to 2700 kg per hectare and significant additional income.

Table 5. Results of the evaluation of yielding annual rhizomes from seed for seedlings and the additional production in dense cropping

Variants	Area	Number of plants	Economic Indices	
			Dry leaves Total	Dry leaves Yield
	Sq.m.	number	g	g/m2
1. 20-20	3,90	174	1395	356
2. 20 -9	1,75	97	510	292
3. 20 -6	2,75	126	1490	542
4. 20 - 11	3,25	154	675	208
5. 20 - 14	5,05	277	500	100
6. 20 - 15	3,50	185	615	175
7. 20 - 10	3,15	161	760	241
8. 20 - 18	6,30	315	1245	198
9. 20- 16	2,00	119	620	310
10.20 - 19	2,65	115	490	185
11.20 - 12	2,60	78	685	263
12.20-5	0,75	15	185	247
Total	34.95	1816	9170	-
Mean of m^2	1.00	52	-	269.2

CONCLUSIONS

Propagation by rhizomes leads to an accumulation of soil pathogens over the years and increases the incidence of foliar diseases. This necessitates a mandatory cyclical alternation of propagation by rhizomes with *in vitro* and *in vivo* propagation for the production of elite seedlings.

Propagation by cuttings is an effective method of mass production of seedlings, but it is associated with necessary resources for implementation.

The evaluation shows that the most effective scheme for the practical implementation of seedlings is by sowing seeds under controlled conditions in January-February, growing the sprouted and stabilized plants in April-May, transplanting in June-July and obtaining formed rhizomes in October, which after storage to be realized in the next period.

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