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ASSESSMENT OF STEVIA (*STEVIA REBAUDIANA* B.) SEEDS VITALITY IN THE CONDITIONS OF BULGARIA

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Abstract

In the conditions of Bulgaria stevia multiplies by storage of the rhizomes and obtainment of seedlings of cuttings and from in vitro maintained clones. Seeds are obtained episodically in conditions of short day at the end of vegetation with a warm and continuous autumn. Aiming art gene fund enrichment, in 2018-2019 are harvested seeds by gathering and cleaning of tied seeds from selected plants of the Selection Field of Agricultural Institute - Shumen. For the seeds progenies in laboratory conditions the germination energy on the 4th day reaches up to 46%. On the seventh day the mean germination increases to 40.9%. In a soil mixture the average value for the seventh day falls back insignificantly to that in laboratory conditions, and the variation between the origins is significantly higher. On the 14-th day the germination reaches mean of 47.5%, as for separate genotypes it exceeds 75%. The results of the seeds obtained from regenerants show a decrease of the mean values of germination to 36.2%in laboratory, and to 31.1% - in external conditions. A bigger variation is registered in the germination energy in laboratory conditions. The one year storage of the seeds decreases the germination to the average of 15.8%. It is impressive the influence of the genotype for the germination preservation, the variation increases to 52.1%, and the lack of significant correlation between the germination before and after storage.

Key words*: stevia, seeds, germina.tion, gene fund*

INTRODUCTION

Stevia (*Stevia rebadudiana* Bertoni) is a perennial, cross pollinated plant from the Asteraea (Compositae) family. It originates from the mountains of Paraguay and Brasil (Lewis, 1992). In the last years there is an increased interest in its growing as a source for natural, non-caloric sweeteners. They are from the group of the diterpene glycosides like Rebaudioside A, B, C, E and stevioside and have up to 300 times the sweetness of sugcrose (Geuns, 2004).

Because of its sensitivity to low temperature in the moderate climate countries stevia is grown as an annual plant, and it could be maintained by storage of the rhizomes (Lankes and Pude, 2008). The *in vitro* propagation and maintenance are alternative methods, whichallow the preservation of the initial genotype (Krumov et al., 1984).

Back in the 80's in the Sugar beet Institute - Shumen started researches on the introduction and growing of stevia, and they have been established the optimal technological conditions and methods of in vitro propagation (Varbanov et al.,1996). Now in the Agricultural Institute - Shumen the researches are directed in the selection of new forms of stevia (Kikindonov, 2012). In 2012 the first Bulgarian variety of Stevia is certified, with excellent economical qualities and adopted to the conditions of Bulgaria (Uchkunov, 2016). Some aspects of the obtainment and sprouting of seeds *in vitro* and in external conditions (Bojimirov and Slavova, 2011; Kikindonov and Enchev, 2012).

In natural conditions it is propagated by seeds. Stevia is a short-day plant, and a certain light and temperature regime is required for insemination (Zaidan et al., 1980). As a cross-pollinated culture, mechanisms of self-incompatibility impede the self-pollination and the production of homozygous off springs (Nakamura et al., 1985). The flowering is not simultaneous and is long lasting. In our conditions, after *in vitro* propagation and continuous adaptation in greenhouse individual origins and plants begin to bloom in late July. The mass flowering is from September to the harvesting of the rhizomes before the formation of autumn frosts (Bojimirov and Slavova,

2011). The mass flowering is from September to the harvesting of the rhizomes before the formation of autumn frosts (Bojimirov and Slavova, 2011). The great genetic diversity of the seeds progeny is the basis for enriching the gene pool for the selection of high-productivity forms and content of stevioside (Caneiro et al., 1997; Costel et al., 2019).
The study reports some results from the assessment of seeds germination of some origins as initial material for the selection.

MATERIALS AND METHODS

The survey was conducted in 2018-2019 at Shumen Agricultural Institute. The seeds were harvested from selected plants of certain origins from the breeding program of the Institute. Individual seed progeny that are cloned in vitro as well as regenerants from isolated in vitro meristems are included. The plants obtained from the Laboratory for Tissue Culture are rooted and adapted initially in a thermostatic room and then exported to the greenhouse from the end of February to the end of April. At least 10 plants of each origin are planted in the field in early May.
Individual origins and plants begin to bloom in late July. The mass flowering is from September to the harvesting of the rhizomes before the formation of autumn frosts. In our study, unlike previous practice, plants with mature seeds are harvested and dried whole. Later the seeds are separated without the empty and parachute shoots being dropped out.
To determine laboratory germination, seeds are plated in harmonic filter paper, in four replicates of 20 each, for each origin. For preliminary determination of the optimum dates, a mixed sample of all origins was plotted in 6 repetitions and germinated sprouts were counted on days 3, 5, 7, 10, and 14. For origin assessment, 4 and 7 days for laboratory germination were selected.
In January, in a thermostatic room at 20-25°C and illumination for 18 hours in pots (repetition) with 10 cm diameter, with a mixture of 4/1/1 of peat, perlite and sand, 50 seeds are sown in 4 repetitions for each origin. Seeds are evenly distributed over the surface of the pre-moistened mixture and pressed tightly against it. Optimal humidification is maintained with a polyethylene chamber for the first 4-7 days, avoiding over moisturizing.
For estimation of the viability is studied the germination after harvest of the 2018 crop seeds and after one year of storage of seeds from the same crop. The statistical processing involves variational analysis to determine mean, mean error, coefficient of variation, and accuracy of the experiment.

RESULTS AND DISCUSSIONS

The impossibility of propagation of stevia by seeds in our severe for the crop conditions is a limiting factor for the actual entry of stevia into practice. The study assesses the germination of seeds harvested under real mass production conditions.
On Figure 1 could be traced the dynamics of seed germination under laboratory and controlled external conditions. As early as on the fifth day, the germinating energy of the seeds is manifested in optimal laboratory conditions, and between the seventh and the tenth day the final germination parameters of the seeds are reached. The later germinated sprouts are of low vitality and usually do not develop normal plants. A slight delay in germination processes is observed in the soil mixture. Optimal for germination and energy of initial growth readings are those made on the 7^{th} and on the 14^{th} day.
From 85 of more than 212 planted origins in 2017 are harvested seeds. Tables 1 and 2 show the results of a total of 48 origins, of which there were sufficient seeds to place four replicates under laboratory and controlled external conditions. This is a testament to the fact that more than half of the genotypes with harvested seeds undergo normal binding and ripening of the seeds. Over 22% of the tested origins give viable seed progeny.
The results are interpreted separately for the offspring and for the regenerants in order to determine the effect of the way of reproduction. For seed offspring under laboratory conditions, germination energy on the fourth day reaches 46%. The variation is relatively high with a CV of 25.8%. On the seventh day, the average germination is increased to 40.9% with an identical high variation of 16.6 to 66.6% and CV-31.7%.

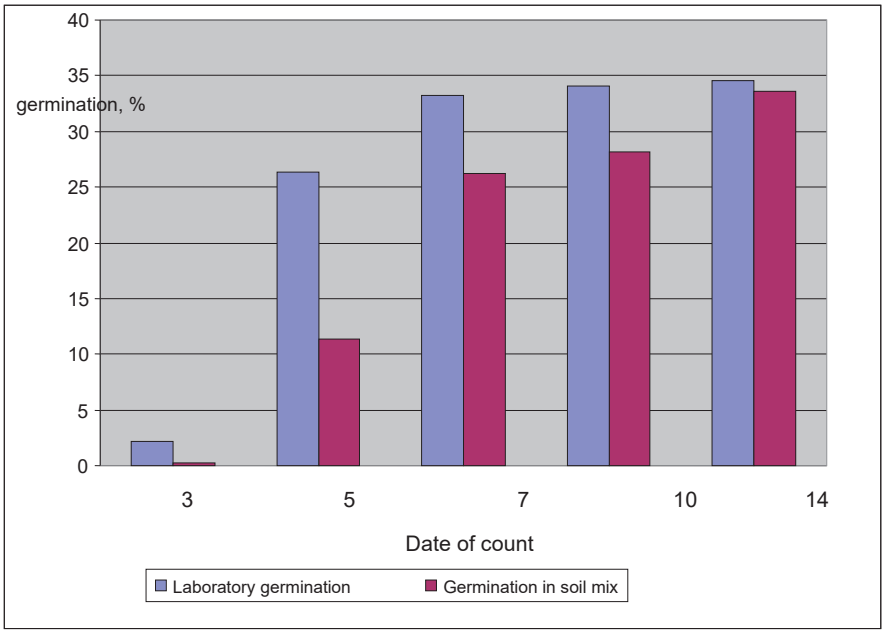

Figure 1. Dynamics of germination of stevia (*Stevia rebaudiana*) seeds in laboratory conditions and in soil mix under controlled conditions, 2018

Table 1. Germination of seeds from seed progeny plants of stevia (*Stevia rebaudiana*), 2018

Variant	Germination (%) in:			
	Laboratory conditions		External controlled conditions	
	4th day	7th day	7th day	14th day
1. IV-30	22.0	33.1	20.0	34.0
2. IV-39	28.0	50.0	28.0	44.1
3. IV-43	32.4	48.0	70.3	78.7
4. IV-46	23.7	43.0	34.2	40.0
5. IV-50	28.0	62.1	40.4	50.1
6. IV-64	26.1	45.0	32.0	36.3
7. IV-65	28.2	66.6	68.0	78.3
8. IV-66	6.6	16.6	6.0	6.0
9. V-79	13.0	22.0	28.3	50.0
10. V-91	18.5	40.3	40.0	50.6
11. V-102	33.3	50.0	46.6	72.0
12. V-116	20.2	52.0	62.0	82.0
13. V-124	23.3	45.5	36.0	44.0
14. IV-66	15.5	21.2	10.7	24.0
15. V-96	15.0	31.6	24.0	26.6
16. IV-67	26.6	45.0	30.0	44.0
17. IV-16	45.7	48.3	38.1	46.9
18. IV-32	46.2	50.0	50.0	52.1
19. IV-54	21.6	38.3	32.0	42.0
20. V-84	30.0	41.6	36.3	46.7
21. VI-20	33.7	38.3	34.0	42.3
22. IV-44	20.0	20.0	28.8	54.0
23. IV-83	26.7	40.1	38.0	44.0
24. IV-33	33.3	33.3	40.0	46.7
$\bar{X}$	**25.8**	**40.9**	**36.8**	**47.5**
Sx	**1.88**	**1.67**	**3.11**	**3.57**
CV	**35.6**	**31.7**	**41.4**	**36.8**
P %	**4.73**	**3.64**	**6.85**	**3.75**

In soil mixture, the average value for the seventh day is slightly inferior to those under laboratory conditions, with the variation between the origins being much higher - from 6.0% to 70.3% and CV - 41.4%. With the increase of the weight of environmental factors in external conditions, the genotypic differences appear to be stronger. The germination on day 14 is increased to 47.5% on average, reaching over 75% for some of the genotypes.

Table 2. Germination of seeds from stevia (*Stevia rebaudiana*) plants regenerated *in vitro*, 2018

Variant	Germination (%) in:			
	Laboratory conditions		External controlled conditions	
	4th day	7th day	7th day	14th day
1. VI-17	33.0	48.0	32.3	38.0
2. V-137	13.3	33.3	24.0	34.6
3. V-150	25.0	36.0	17.6	23.0
4. VI-18	32.3	35.0	28.0	34.0
5. V-143	25.0	33.3	14.6	16.0
6. III-168	35.3	38.6	30.0	46.0
7. III-165	36.0	46.6	30.6	36.6
8. III-180	21.6	30.0	12.0	20.0
9. III-192	3.3	21.6	14.7	32.0
10. III-162	46.7	50.0	34.1	42.7
11. V-153	13.3	31.7	20.0	34.3
12. III-166	20.7	66.0	14.7	14.0
13. III-163	13.3	40.0	22.3	32.0
14. V-147	13.0	33.0	24.7	27.7
15. III-167	8.3	36.6	32.0	52.3
16. III-170	13.0	13.0	4.0	8.7
17. V-158	20.7	20.7	17.8	23.4
18. III-191	13.0	13.0	30.3	38.0
19. III-161	13.3	53.0	30.7	34.0
20. III-175	13.3	20.0	14.7	32.3
21. III-185	40.0	40.0	24.3	24.3
22. III-195	26.7	60.0	42.3	54.0
23. V-173	32.0	35.3	28.0	34.7
24. VI-20	25.1	33.0	12.7	16.0
X	**22.5**	**36.2**	**23.2**	**31.1**
Sx	**2.28**	**7.39**	**4.74**	**2.36**
CV	**49.4**	**36.8**	**38.2**	**37.0**
P %	**4.01**	**7.04**	**5.84**	**3.76**

The results for regenerants' seeds indicate a decrease in the average germination values of 36.2% in laboratory and 31.1% in external conditions. Greater variation is recorded in the germination energy under laboratory conditions. The correlation between laboratory germination and the germinated plants in soil mixture is r = 0.688, which makes the laboratory germination a reliable parameter for assessing the viability of seed progeny for the stevia.

Table 3 shows the results of laboratory germination of seeds of 12 origins immediately upon their harvest in the fall of 2018 and after one year of storage. The vitality parameters of seeds harvested in 2018 are down compared to those in 2017. The average laboratory germination on day 7 was 33.1%, with a variation of 45.2%. The one-year seed storage reduces germination by half to 15.8% on average. Obvious is the influence of the genotype on the retention of germination, and the variation is increased to 52.1%. It is impressive also that there is no significant correlation between germination before and after storage.

Table 3. Seeds germination of stevia in laboratory conditions and after one-year storage, 2019

Variant	Laboratory conditions, %		After 1 year storage, %	
	4thday	7thday	4th day	7th day
1. IV-30	6.7	26.7	12.0	12.0
2. IV-39	15.0	15.0	10.0	20.7
3. IV-43	13.3	33.3	12.7	20.1
4. IV-46	13.3	26.6	10.0	20.2
5. IV-50	6.7	6.7	10.0	24.0
6. IV-64	53.3	53.3	0	0
7. III-165	6.7	33.7	0	12.7
8. III-180	20.0	20.0	21.0	30.0
9. III-192	53.3	60.0	0	0
10. III-162	33.7	33.7	21.7	21.0
11. V-153	46.7	46.7	40.6	42.0
12. III-166	26.7	40.0	0	0
X	**24.7**	**33.1**	**10.9**	**15.8**
Sx	2.32	2.62	3.21	3.42
CV	41.0	45.2	48.7	52.1
P %	5.21	5.72	7.21	8.72

Figure 2. Blooming plants

Figure 3. Stevia seeds

Figure 4. Germination of Stevia seeds

CONCLUSIONS

For seed offspring under laboratory conditions, the germination energy on the fourth day reaches 46%. On the seventh day, the average germination is increased to 40.9%. In the soil mixture, the average value for the seventh day was slightly inferior to those under laboratory conditions, with the variation between the origins being much higher. The germination on day 14 is increased to 47.5% on average, reaching over 75% for individual genotypes.

The results for regenerants' seeds show a decrease in average germination values of up to 36.2% in laboratory and up to 31.1% in external conditions. Greater variation is recorded in the germination energy under laboratory conditions

One-year seed storage reduces germination by half to 15.8% on average. The influence of the genotype on germination retention is impressive and the variation is increased to 52.1%. Impressive is also the lack of significant correlation between germination before and after storage.

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