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Behavior of apple rootstock M9 produced by somatic organogenesis in stoolbed

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Abstract. *The growth characteristics of apple clonal rootstock M9 in stoolbed is traced. The observed variations produced by somatic organogenesis showed better productivity shoots compared with control plants produced by clonal micropropagation. This was manifested in the formation of well-rooted and greater length growth of top-quality shoots.*

Keywords: apple, rootstocks, stoolbed, somatic organogenesis

Introduction

Clonal rootstock M9 is the most used low rootstock in our fruit growing. Many researchers have worked for decades on the question of its production in stoolbed. As source plants they used root cuttings. In their experiments the authors address their attention mainly on productivity and quality of the object (Andreev, 1979; Gryazev, 1979; Mitov et al., 1979; Koval, 1980; Samus, 1983; Verobyov, 1985; Vehov and Retinskaya, 1988; Pepelyankov and Dobrevska, 1995; Dobrevska and Tabakov, 2002; Lipa and Lipecki, 2006; Dobrevska, 2010).

Today intensification enters totally fruit growing. This requires their purity. Purity is one of the key conditions brought to the rootstocks as a starting material in the production of trees. This necessitated the deployment of different methods of micro-propagation in the production of virus-free starting fruit seedlings. Various ways of experimenting have been conducted in an attempt to seek for a more efficient production system. One is somatic organogenesis. Our study has been made in this direction. The main question whose answer we seek in the publication, given the fact that the subject has not been analyzed both at home and abroad, is as follows: Does this impact on the productivity of some apple rootstocks in the stoolbed.

Material and methods

Well-rooted plants of the apple rootstock M9 were planted in the autumn of 2003 in the Learning and experimental field of the Department of Fruit Growing of Agricultural University - Plovdiv, located in the village of Brestnik municipality, Plovdiv region. The observed plants were obtained in the biotechnology laboratory of the Institute of Fruit Growing-Plovdiv. Studied variants are produced by somatic organogenesis and the control plants – through proven technology for clonal micropropagation (Ivanova, 1988). The soil in the stoolbed is leached maroon-forest. The field experiment is embedded in a block method of Fisher (Zapryanov and Marinkov,1978), with four repetitions for each variant. After planting, all agronomic techniques adopted for use stoolbed were completed (Mitov et al., 1996). The length of annual growth, number of shoots of rhizome, thickness of shoots and number of roots of shoots were taken into account.

These results were statistically processed by the method of variance analysis.

Results and discussion

Considering the average length of growth per year (Table 1), it is noteworthy that the length of both the main and the feather twigs of the investigated variants are generally greater than those of the control plants. In most of the cases the difference was not statistically significant. Much better and mathematically proven results in the differences of the main twigs were reported during the second year in all variants. Regarding the average length of annual growth, average for the three years, again all variants showed higher performance both in main and feather twigs (Figure 1). However, variants 2, 3 and 4 are clearly standing out in which differences are significant and mathematically proven. The same trend was observed for the aggregate growth (main plus feather twigs). The growth is higher in all variants and again variants 2, 3 and 4 are a proof of the difference compared to the control plants.

The results of the indicator average number of shoots of rhizome are of particular interest (Table 2). Here we can confidently talk about higher productivity of the shoots in the investigated variants. Looking at the results by year, we find that all variants during the three years, have higher productivity compared to the control. Impression, however, make variants 5, 6, 7 and 8 which show a good and very good proof of the differences in the first year. The same trend in version 5 and 6 is repeated in the third year. In respect to the average number of shoots indicator, the observed statistically significant differences in all investigated variants showed a good mathematical proof during the three years of observation.

All of the rootstocks in the observed variants are considered by the thickness standards adopted in Bulgaria and are measured at 15 cm from their base (Table 3). It is striking that in the second year the rootstocks of all variants are top quality and proven thicker than the control plants. In the first and third years, most variants produced second-rate rootstocks, but also here there are differences demonstrated in some of them. One of the reasons for the demonstrated greater thickness is probably the formation of longer

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Table 1. Average length of the main and the feather twigs for each year

Variant	2007		2008		2009	
	Main twigs	Feather twigs	Main twigs	Feather twigs	Main twigs	Feather twigs
1 – K	106.50	0.00	62.50	0.00	94.50	9.17
2	135.00	0.60	103.25	0.00	102.75	48.38
3	145.25	1.17	111.00	2.60	102.25	8.50
4	116.00	5.68	108.50	19.92	106.50	32.50
5	105.00	4.53	101.50	11.92	104.00	24.75
6	93.75	0.00	90.25	36.85	81.00	10.00
7	116.50	11.03	108.00	16.39	94.25	0.00
8	82.25	22.75	101.75	4.25	95.50	0.00
GD 5 %	33.30	15.15	21.43	20.56	18.71	27.65
1 %	45.33	20.62	29.17	27.98	25.46	37.64
0.1 %	61.14	27.82	39.35	37.75	34.34	50.77

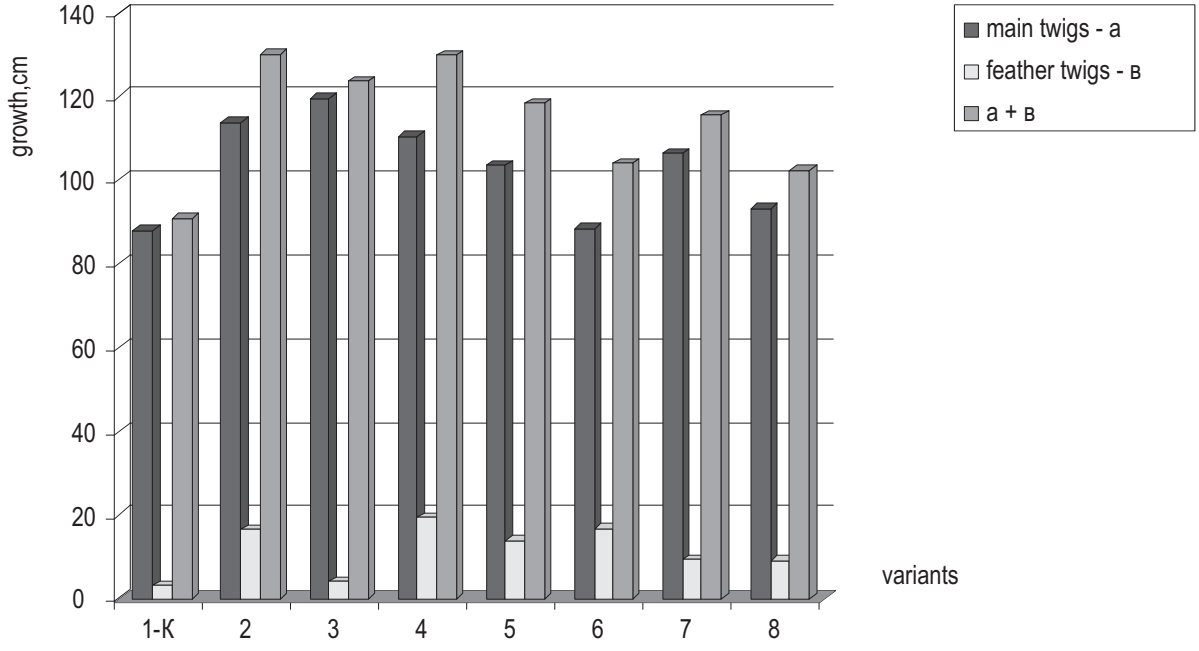

Figure 1. Average length of the annual growth for three years and total growth for the period

feather twigs on the monitored variants compared with the control ones. Analyzing the results of the same index, averaged over the three years, we find that all options except 6 and 8 are top quality shoots with proven differences compared to the control plants.

The results for the average number of roots of plants are controversial over the years (Table 4). During the first year the average number of roots of shoot was significantly larger and statistically different from the control. In the second year no significant differences were observed, and in the third year - the plants of all variants have shown fewer roots compared to the control. The average results for the three-year period showed no significant differences in this indicator between the variants and the control.

From the presented and analyzed results here, it could be said that at a young age the plants of variants produced by somatic organogenesis exhibit characteristics normally associated with non-M9 rootstock (Trachev, 1973). They exhibit stronger growth in the length increase and thickening of the surface part, rather than forming adventitious roots at the base of the shoots, compared to control plants obtained by clonal micropropagation.

Table 2. Average number of shoots of rhizome for each year and average for the entire period

Variant	2007	2008	2009	Average for the period
1 – K	0.75	3.50	3.00	2.42
2	1.88	5.25	5.13	4.09
3	1.38	4.38	5.50	3.75
4	2.00	5.25	5.25	4.17
5	2.63	5.88	6.63	5.05
6	3.13	6.63	5.88	5.21
7	3.00	6.75	4.75	4.83
8	5.00	5.63	4.00	4.88
GD 5 %	1.28	3.78	2.71	1.57
1 %	1.74	5.14	3.69	2.18
0.1 %	2.35	6.93	4.98	3.03

Table 3. Average thickness of shoots for each year and average for the entire period

Variant	2007	2008	2009	Average for the period
1 – K	6.75	5.67	8.03	6.82
2	9.09	7.43	8.30	8.27
3	9.42	8.06	7.50	8.33
4	8.91	8.59	8.05	8.52
5	8.04	7.88	7.41	7.78
6	6.66	7.19	6.16	6.67
7	7.92	8.12	6.73	7.59
8	5.91	7.44	6.69	6.68
GD 5 %	2.16	1.44	1.54	1.37
1 %	2.94	1.96	2.09	1.91
0.1 %	3.97	2.65	2.83	2.65

Table 4. Average number of roots of shoot for each year and average for the entire period

Variant	2007	2008	2009	Average for the period
1 – K	3.88	4.71	10.33	6.31
2	8.38	5.72	5.67	6.59
3	6.73	5.96	5.82	6.17
4	7.94	4.72	7.00	6.55
5	10.86	4.88	6.22	7.32
6	10.56	4.72	7.17	7.48
7	7.35	4.62	6.87	6.28
8	9.83	5.10	6.94	7.29
GD 5 %	4.42	1.85	2.40	3.23
1 %	6.01	2.52	3.27	4.49
0.1 %	8.11	3.40	4.41	6.24

Conclusion

The length of the annual growth of the mother plants from trial variants are characterized by higher values than the control plants**.** Tracking variants have leveled top quality shoots. The productivity of root shoots from the rhizomes of the tracking variants is nearly twice higher than that of the control. The root formation of the variants and the control plants did not vary significantly at a young age. Generally, the variants originating from somatic organogenesis exhibit higher productivity of well-leveled and top quality rooted shoots, compared with the control, produced by clonal micropropagation.

At this stage of the experiment, however, only the morphological manifestations of the follow-up plants in stoolbed can be observed, without being fully convinced that they may have some connection with the parent genotype. This could be determined in the future through in-depth genetic studies, especially having in mind that research in this field of science has not been done neither in Bulgaria, nor abroad.

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Thesis:

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