

Agrobiological evaluation of new apple clonal rootstocks of the Michurinsk State Agrarian University selection using different breeding methods

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Abstract. The results of many years of research on propagation of promising forms of clonal stocks of apple trees of the Michurinsk State Agrarian University selection in the mother plantation by stooling, by herbaceous cuttings and by clonal micropropagation are presented. According to the complex of economic and biological features, promising clonal rootstocks of an apple tree with high shoot-forming capacity in the mother plant were identified: dwarf apple trees 2-3-49, 2-15-2, 2-15-15, 3-4-7, 5-21-27; semi dwarf apple trees 2-3-14, 2-9-49, 2-9-102, 2-12-10, 5-24-1, 5-27-1. The rootstocks that are promising for propagation by herbaceous cuttings – 2-3-14, 2-3-49, 2-12-10, 2-15-15, 3-4-7, 5-21-93; at the same time, treatment with exogenous promoters such as IBA and NAA at a concentration of 50 mg/l increases the yield and quality of stock material. A modified MSO nutrient medium with the addition of 0.5-1.0 mg/l of 6-BAP and 0.1-0.2 mg/l of IBA is selected for the clonal micropropagation of apple stocks.

1 Introduction

Currently, a lot of attention is paid to production and consumption of fruits and berries as the most important components of a healthy diet. In Russia, the area of new intensive plantations of fruit and berry crops has significantly increased [1-4]. In this regard, creation and expanded introduction of new varieties with high-quality fruits is the main goal of many breeding programs [5].

The main fruit crop for the middle gardening zone of Russia is an apple tree, which is associated with extensive experience in growing, a detailed study of the biology and high ecological plasticity of this crop [6, 7]. Measures for significant acceleration of introduction

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of new apple tree plantings during the period of mass fruiting are needed [1, 3, 8]. The maximum degree of intensification is possible only with the use of highly adaptive and easily propagated apple tree clonal rootstocks, which provide short stature and even growth habit of grafted trees, the possibility of a high density planting, adaptation to the soil-climatic growth conditions, resistance to salinization and carbonating of soil, responsiveness to agricultural activities, high winter hardiness, drought tolerance and the ability to withstand biotic stressors [8-14]. Previously, short life cycle of 12-15 years of dwarf trees was considered a significant drawback, but today, with an accelerated updating of the assortment and, accordingly, scion-rootstock combinations, the criterions for assessing is the yield, quality and the prime cost of fruits. Upon receiving and further study of new varieties and clonal stocks, molecular genetic methods of analysis and biotechnology methods are increasingly used, which significantly accelerate the process of selection and reproduction of valuable genotypes [15-18]. Obtaining of the same total yield in the intensive garden in fifteen years as in the tall stand gardens for forty or more years, removes all doubts about the use of dwarf rootstocks [8, 10, 19]. Of particular relevance and importance is not only the selection of dwarf clonal stocks of the apple tree, but also their accelerated reproduction in various ways acceptable for each particular zone and farm.

In this regard, the objective was to evaluate new clonal stocks of the apple tree when they are propagated by stooling in the mother plantation, by the method of herbaceous cuttings and using a sterile culture *in vitro*.

2 Materials and methods

The studies were conducted in 2012-2018. The biological objects were the forms of clonal rootstocks of the apple tree obtained as a result of selection in 2002-2005 at Michurinsk State Agrarian University. We used regional 62-396 (dwarf growth force) and 54-118 (semi dwarf growth force) rootstocks from the State Register of Selection Achievements of the Russian Federation as a control experiment.

The studies were carried out according to generally accepted methods for studying fruit crops [19]. The propagation of apple stocks *in vitro* was carried out according to the recommendations of Muratova S.A. et al. [20].

The mother plant of clonal stocks is located in the Michurinsk district of the Tambov Oblast. The climate is temperate continental. Soil is medium-leached chernozem with a humus content of up to 6.2%. The planting scheme of the mother plantation is 150x30 cm. The experiment was repeated three times with 10 experimental plants. The experimental data were statistically processed in the Microsoft Office Excel and Statistica.

3 Results and discussion

Growth force of new rootstocks in the mother plantation was screened according to the methodological recommendations taking into account clear correlative relationships between the growth force and wood brittleness [21]. According to this indicator, the studied forms 2-3-49, 2-15-2, 2-15-15, 3-4-7, 5-21-27, 5-21-93 were previously assigned to the group of dwarf gene types, and forms 2-3-14, 2-9-49, 2-9-94, 2-9-102, 2-12-10, 5-27-1 to semi dwarf ones.

When assessing the quality of rootstock planting material, the presence of branching of their shoots is taken into account, which is ambiguously evaluated by scientists and practitioners. Thus, the bud awakening at a one-year growth of the stock can indicate the early maturation of grafted plants, and the presence of burrknots in the lower part of the shoot indicates a tendency to rapid root formation [14]. However, in production, the removal of

branches located in the grafting zone leads to additional manual labor costs and difficulties in shield budding.

The shoots of mother plant bushes in 94% of the studied forms were non-branching or weakly branching. The most even, practical for technological operations were cuttings of 62-396, 54-118, 2-3-49, 2-9-94, 2-9-102, 2-12-10, 2-15-2, 2-15-15 rootstocks, in which few branches were found in the upper part of the shoot, previously removed in preparation for planting in the nursery.

Modern intensive gardening technologies place high demands on planting stock – the presence of a powerful, branched root system and occurring of generative buds already in the plant nursery. In the complex of factors affecting the yield of quality seedlings, the individual biological characteristics of the stock determined by the genotype are fundamental. The remaining indicators, to one degree or another, can be controlled by agricultural techniques.

All studied stocks were cultivated under the same conditions, subject to uniform technological operations. By the time of separation of layering (September-October) on the shoots of 2-9-49, 2-9-94, 2-3-14, 2-12-10, 2-15-15, 3-4-7, 5-21-27 rootstocks, a large number of well-developed and matured roots were formed. The rooting of these forms over the observation period ranged on average from 2.8 to 3.3 points (Table 1). In a year with the most favorable weather conditions for the root formation process (2016), rooting of 2-15-2 and 2-15-15 stocks was estimated at 4.0 points, which indicates the great production potential of these forms.

The total yield of layering formed by mother plantation bushes varied widely – from 95.53 thousand units per ha at the 5-27-1 rootstock to 629.3 thousand units per ha of the 2-9-49 rootstock. All dwarf rootstocks on this basis exceeded the control experiment. Among the semi dwarf ones, the largest number of shoots was formed by 2-3-14, 2-9-49, 2-9-94, 2-9-102, 2-12-10, 2-12-27 rootstocks – from 13.3 to 28.3 units from a single bush (295.3-628.3 thousand units/ha). The greatest yield of standard layering, suitable for planting in the plant nursery, was noted for 5-21-27, 2-15-2, 2-15-15 dwarf rootstocks; 5-24-1, 5-27-1 semi dwarf rootstocks.

Table 1. Productivity of mother plantations of apple clonal rootstocks.

Clonal rootstock	Total layering output, thousand units/ha	Standard layering output		Average rooting score
		thousand units/ha	%	
<i>Dwarf forms</i>				
62-396 (control)	121.1 ± 7.6	44.4 ± 3.8	36.7	3.0
2-3-49	222.0 ± 12.8	77.7 ± 6.9	35.0	3.0
2-15-2	324.1 ± 17.3	155.4 ± 12.4	47.9	3.1
2-15-15	230.9 ± 14.6	113.2 ± 10.8	49.0	3.3
3-4-7	313.0 ± 21.7	133.2 ± 11.6	42.6	2.7
5-21-27	164.3 ± 7.2	135.4 ± 6.7	82.4	3.1
5-21-93	197.6 ± 10.4	53.3 ± 4.6	26.9	3.1
<i>Semi dwarf forms</i>				
54-118 (control)	157.6 ± 11.7	77.7 ± 5.8	49.3	2.9
2-3-14	366.3 ± 19.2	117.7 ± 8.6	32.1	3.1
2-9-49	628.3 ± 40.8	168.7 ± 11.4	26.9	2.9
2-9-94	295.3 ± 16.3	86.6 ± 7.2	29.3	2.3
2-9-102	324.1 ± 28.2	133.2 ± 9.8	41.1	2.4
2-12-10	503.9 ± 36.8	166.5 ± 14.3	33.0	2.4
2-12-27	477.3 ± 31.7	59.9 ± 4.8	12.5	2.1
5-24-1	111.0 ± 9.3	91.0 ± 7.6	81.9	2.8
5-27-1	95.5 ± 7.2	88.8 ± 7.1	92.9	2.6

The fast and productive method of propagation of fruit crops is the use of non-lignified herbaceous cuttings [8]. Cuttings of 62-396, 2-3-14, 2-3-49, 2-12-10, 2-15-15, 5-21-27, 5-21-93 stocks rooted in the range of 50.2-93.8%, even without treatment with growth promoters, which indicates a high content of endogenous phytohormones, making it possible to consider these forms as the most promising among the studied stocks for propagation by herbaceous cuttings (Table 2).

Table 2. Rooting of herbaceous cuttings of stock-grown apple trees.

Clonal rootstock	Growth promoters	Growth length, cm	Root length, cm	Root development, score	Rooting of cuttings, %
<i>Dwarf forms</i>					
62-396 (control)	control	0.3 ± 0.1	10.5 ± 2.5	2.1	50.2
	IBA	0.7 ± 0.3	14.9 ± 2.8	3.3	100.0
	IAA	0.1 ± 0.1	0.2 ± 0.1	1.0	22.2
	NAA	0.2 ± 0.1	13.6 ± 1.5	3.5	75.0
2-3-49	control	6.7 ± 0.7	6.3 ± 0.3	3.5	93.8
	IBA	1.9 ± 0.6	12.9 ± 2.0	3.6	100.0
	IAA	2.8 ± 0.7	7.5 ± 1.0	2.4	46.7
	NAA	3.7 ± 0.8	13.9 ± 1.0	3.7	100.0
2-15-2	control	1.4 ± 0.6	5.7 ± 0.4	2.5	16.7
	IBA	3.6 ± 0.5	10.7 ± 1.2	4.0	66.7
	IAA	5.4 ± 0.3	7.7 ± 1.1	2.7	35.3
	NAA	2.5 ± 1.0	12.5 ± 1.5	3.5	60.5
2-15-15	control	4.2 ± 0.2	11.5 ± 2.0	4.0	68.8
	IBA	9.1 ± 0.8	12.7 ± 3.0	4.8	100.0
	IAA	8.6 ± 0.4	8.6 ± 1.0	2.5	57.9
	NAA	10.2 ± 0.6	18.5 ± 1.5	4.0	78.6
3-4-7	control	0.8 ± 0.3	10.2 ± 2.5	2.0	20.3
	IBA	2.3 ± 0.8	17.2 ± 2.1	4.4	100.0
	IAA	5.6 ± 0.2	10.7 ± 2.1	2.3	53.9
	NAA	1.2 ± 0.4	10.9 ± 1.5	3.9	71.3
5-21-27	control	0.5 ± 0.2	4.6 ± 0.5	3.6	62.5
	IBA	2.2 ± 0.6	13.0 ± 1.1	4.6	75.0
	IAA	1.3 ± 0.2	8.9 ± 1.1	2.8	55.0
	NAA	4.0 ± 0.8	12.5 ± 1.5	4.0	72.7
5-21-93	control	0.6 ± 0.4	0.6 ± 0.1	1.9	50.3
	IBA	3.7 ± 0.9	10.4 ± 2.5	3.2	83.3
	IAA	4.0 ± 0.1	3.3 ± 0.2	2.4	57.2
	NAA	0.6 ± 0.1	8.5 ± 1.1	3.6	83.3
<i>Semi dwarf forms</i>					
54-118 (control)	control	0.2 ± 0.1	3.1 ± 0.3	2.0	25.8
	IBA	1.9 ± 0.5	10.0 ± 2.0	4.8	85.7
	IAA	0.5 ± 0.2	9.3 ± 1.0	2.7	55.4
	NAA	1.0 ± 0.3	11.8 ± 1.0	4.6	71.4
2-3-14	control	1.8 ± 0.6	9.0 ± 0.1	3.6	68.2
	IBA	0.8 ± 0.2	14.2 ± 1.9	3.7	85.7
	IAA	2.2 ± 0.5	11.2 ± 1.7	2.6	37.5
	NAA	0.2 ± 0.1	11.8 ± 1.0	3.7	70.6

2-9-49	control	2.8 ± 0.5	8.2 ± 0.7	2.8	18.2
	IBA	0.6 ± 0.2	13.0 ± 2.0	3.3	73.8
	IAA	2.5 ± 0.8	10.9 ± 0.9	1.5	16.3
	NAA	0.1 ± 0.1	13.8 ± 1.4	3.8	64.3
2-12-10	control	2.9 ± 0.8	5.5 ± 2.0	3.4	57.2
	IBA	1.9 ± 0.3	12.9 ± 1.5	3.6	73.3
	IAA	2.8 ± 0.6	6.3 ± 0.1	2.0	58.8
	NAA	3.8 ± 0.2	7.5 ± 0.1	3.5	71.4
5-27-1	control	0.5 ± 0.2	7.3 ± 1.1	2.0	23.5
	IBA	1.1 ± 0.1	8.9 ± 1.2	3.4	61.5
	IAA	0.1 ± 0.1	6.0 ± 0.6	1.5	18.2
	NAA	0.8 ± 0.5	8.0 ± 1.1	3.5	66.2

The use of indole-butyric (IBA) and naphthaleneacetic (NAA) acids in experiments at a concentration of 50 mg/l increased the rooting rate of many forms to 100%. At the same time, the use of indole-acetic acid (IAA) led to a decrease in root development compared with the control experiment. Treatment with auxins also influenced the length of the growth of herbaceous cuttings, and stimulation of shoot growth was noted in a number of forms.

Clonal micropropagation is increasingly being used for propagation of many cultures, which allows to obtain a high multiplication factor; reduce the duration of breeding process; to multiply hard-rooted plants; work throughout the year and optimize space, as well as long-term storage of valuable biological material in vitro [16].

In the case of microclonal propagation of apple rootstock of the Michurinsk SAU selection, it is most effective to use the Murashige and Skoog medium (MSO) with the addition of 6-benzylaminopurine (6-BAP), gibberellic (GA) and β-indole-3-butyric (IBA) acids [21]. It has been established that the reproduction rate of rootstock shoots depends primarily on the genotype and concentration of growth regulators in the nutrient medium. It is recommended to use sucrose at a concentration of 30 g/l as a source of carbohydrate nutrition. The highest breeding rate was observed for 2-3-49, 2-9-49, 2-9-102, 2-12-10, 2-15-15, 3-4-7 rootstocks (from 3.2 to 5.7 shoots per passage) using 0.5-1.5 mg/l of 6-BAP and 0.1-0.2 mg/l of IBA, but without adding of GA (Table 3). The greatest number of microshoots on the MSO medium at a 0.5 or 1.0 mg/l concentration of 6-BAP and 0.1 mg/l of IBA forms the 2-15-15 – from 4.4 to 5.7 pcs for one passage, with their average length of 2.6-2.9 cm.

Table 3. Effect of the MSO hormonal composition on the microclonal propagation of apple tree rootstocks.

Clonal rootstock	Hormone concentration, mg/l (+ 0.1 mg/l of IBA in all cases)						
	6-BAP 0.5	6-BAP 0.5 + GA 0.25	6-BAP 0.5 + GA 0.5	6-BAP 1.0	6-BAP 1.0 + GA 0.5	6-BAP 1.5	6-BAP 1.5 + GA 1.5
<i>Shoots, pcs.</i>							
2-3-49	2.0 ± 0.2	2.0 ± 0.2	1.8 ± 0.2	1.7 ± 0.2	2.6 ± 0.1	4.3 ± 0.1	4.9 ± 0.2
2-9-49	2.5 ± 0.2	4.4 ± 0.2	2.6 ± 0.2	4.2 ± 0.2	4.0 ± 0.2	4.8 ± 0.3	3.0 ± 0.2
2-9-102	3.4 ± 0.3	2.0 ± 0.5	1.8 ± 0.5	2.7 ± 0.4	2.6 ± 0.4	5.2 ± 0.3	3.5 ± 0.4
2-12-10	1.8 ± 0.5	1.7 ± 0.4	2.4 ± 0.4	4.0 ± 0.2	1.1 ± 0.4	4.0 ± 0.3	2.0 ± 0.2
2-15-15	4.4 ± 0.3	1.0 ± 0.5	3.2 ± 0.4	5.7 ± 0.2	3.3 ± 0.3	2.5 ± 0.5	3.4 ± 0.3
3-4-7	3.8 ± 0.2	1.7 ± 0.4	4.2 ± 0.2	3.3 ± 0.2	4.0 ± 0.2	5.0 ± 0.4	1.4 ± 0.6
<i>Shoot length, cm</i>							
2-3-49	1.4 ± 0.5	0.9 ± 0.5	1.2 ± 0.5	0.9 ± 0.3	0.9 ± 0.9	1.1 ± 1.3	0.9 ± 1.6
2-9-49	1.8 ± 0.6	1.3 ± 0.4	1.1 ± 0.5	1.4 ± 0.9	1.3 ± 0.6	1.7 ± 0.7	1.2 ± 0.3
2-9-102	2.1 ± 0.4	2.4 ± 0.6	4.0 ± 0.5	2.2 ± 0.3	2.4 ± 0.8	1.9 ± 1.5	2.5 ± 0.4
2-12-10	2.6 ± 0.4	2.1 ± 0.4	2.3 ± 1.2	2.8 ± 0.1	2.7 ± 1.2	2.1 ± 0.3	2.3 ± 0.2
2-15-15	2.9 ± 1.3	5.3 ± 0.1	4.0 ± 0.4	2.6 ± 0.9	2.4 ± 0.3	3.4 ± 0.8	2.2 ± 0.5
3-4-7	2.0 ± 1.0	4.4 ± 0.2	2.0 ± 0.4	2.6 ± 0.5	2.0 ± 0.6	2.1 ± 1.5	3.7 ± 0.2

4 Conclusion

According to the complex of economic and biological features, promising clonal stocks of apple trees of the Michurinsk State Agrarian University selection are distinguished in the mother plantation of the vertical layers: dwarf apple trees 2-3-49, 2-15-2, 2-15-15, 3-4-7, 5-21-27; semi dwarf apple trees 2-3-14, 2-9-49, 2-9-102, 2-12-10, 5-24-1, 5-27-1.

The most promising forms for propagation by herbaceous cuttings among the studied stocks are the 2-3-14, 2-3-49, 2-12-10, 2-15-15, 3-4-7, 5-21-93 rootstocks. At the same time, treatment with exogenous promoters such as IBA and NAA at a concentration of 50 mg/l allows to increase the yield and quality of stock material.

It is recommended to use the Murashige and Skoog medium containing 0.5-1.5 mg/l of 6-BAP in combination with 0.1-0.2 mg/l of IBA, as well as 30 g/l of sucrose as a source of carbohydrate nutrition, for clonal micropropagation of apple stocks.

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