Winter hardiness of apple clonal rootstocks in Southern Cisbaikalia

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Abstract. The paper presents the results of a study of apple clonal rootstocks carried out in 2017-2022 in the trial plots located in the Irkutsk region. The objects of the study were clonal rootstocks from various scientific institutions. The conducted fieldwork allows us to state that the ecological conditions of the Southern Cisbaikalia are generally favorable for the use of clonal rootstocks in the regional horticulture. It was found that the most adaptive forms of all the studied clonal rootstocks are the rootstocks of the Ural selection (Ural and Ural 2) and the rootstock of the Estonian selection (E-56). The clonal rootstocks 70-6-8, Arm18, and K-2 withstood winter worst of all. According to the fieldwork results, the rest of the rootstocks showed medium winter hardiness. As a result of laboratory freezing experiments, E-56 high winter hardiness was confirmed. The study of cultivar-rootstock combinations showed good compatibility of clonal rootstocks with semi-cultivated apple trees. The variety and type of the rootstock were statistically proven to affect the degree of temperature damage to fruit trees. The clonal rootstocks with high winter hardiness and those with medium winter hardiness and high recovery ability were selected for further breeding work.

1 Introduction

Siberian horticulture development is impossible without the use of modern approaches and technologies. Despite the vast territory, industrial cultivation of fruit crops (namely apple trees) is possible in Siberia in orchards of 2 to 10 hectares, solely based on the microclimatic zoning of the region [1]. For this reason, an intensive approach in Siberian horticulture is essential.

The culture of low-growing fruit trees is the most pronounced form of intensive fruit growing since dwarf and semi-dwarf trees are perfectly adapted to intensive cultivation [2]. First of all, they ensure the use of limited space of fruit plantations, which is important in case of a shortage of sites suitable for planting orchards. The clonal rootstocks used in this process determine the early maturity of fruit trees, which is important for maximum production in extreme climatic conditions and ensures the economic efficiency of cultivation [3]. Thanks to the use of clonal rootstocks, reducing the time of entry into commercial apple

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fruiting will increase the productive age of fruit trees (Siberian varieties of apple trees on seedling rootstocks enter commercial fruiting in 5-6 years, the total life of an apple tree in the orchard is up to 20 years).

Low-growing clonal rootstocks, the root system of which can withstand negative temperatures of -15 ... -16°C, are currently used; recently bred rootstock forms can withstand temperature drops to -18 ... -20°C [4, 5]. According to long-term observations of the ‘Irkutsk Observatory’ meteorological station, the soil temperature in the study area at a depth of 20 cm during the winter periods 2004-2016 did not fall below -15.2°C (data from the Federal State Budget Institution “Irkutsk Department for Hydrometeorology and Environmental Monitoring”). That fact significantly increases the chances of these rootstock forms to be used in Siberian horticulture.

The purpose of this paper was to study the possibility of using low-growing clonal rootstocks in the conditions of the Southern Cisbaikalia, the territory most suitable for horticulture in the Irkutsk region. The solution of the following tasks promoted the purpose:

- to characterize the field winter hardiness of available clonal rootstocks according to long-term observations;
- to evaluate the resistance of wood of different clonal rootstock genotypes to the four components of winter hardiness under the artificial freezing conditions;
- to identify promising scion-rootstock combinations of apple varieties successfully grown in the study region.
- to identify forms of clonal rootstocks promising for breeding.

2 Materials and methods

A collection of the following clonal rootstocks was assembled: six varieties of clonal rootstocks bred by Michurinsk State Agrarian University (62-396, 54-118, 70-20-20, 70-6-8, 64-143, 62-223), rootstock bred by A.P. Apoyan (Armenian Research Institute of Viticulture, Winemaking and Fruit Growing) (Arm18), rootstock of Estonian selection (E56), eight rootstocks of Orenburg Experimental Station of Horticulture and Viticulture selection (Ural, Ural2, Ural5, 18-7, B-3-4, 4-12, 8-2, OB), rootstock of the Krymsk Experimental Breeding Station of VIR (K-2).

The studies were carried out in 2017-2022 in the trial plots located in the Irkutsk district of the Irkutsk region and in the territory of Siberian Institute of Plant Physiology and Biochemistry of the Siberian Branch of the Russian Academy of Sciences (Irkutsk).

The clonal rootstock collection was planted in 2017 in the plot of the Irkutsk district. 2-16 plants of each genotype were planted. Siberian berry apple trees (vegetatively propagated clone) were planted as a control. The plantings were randomized.

The trial plot for studying scion-rootstock combinations was planted in the spring of 2017. 50-100 plants of each genotype were planted: 62-396, 70-20-20, 54-118, 70-6-8. Siberian berry apple tree seedlings were used as a control rootstock. The rootstock survival rate and the yield of seedlings were taken into account during the studies.

The studies of winter hardiness in the field and laboratory conditions were carried out according to the Program and Methods for Variety Studies of Fruit, Berry and Nut Crops [6].

The laboratory experiments on artificial freezing of rootstocks were carried out in Siberian Institute of Plant Physiology and Biochemistry of the Siberian Branch of the Russian Academy of Sciences. To create a freezing temperature, the low-temperature Binder chamber with a range of negative temperatures from -10 to -80°C was used. Thaw conditions (+5°C) were simulated in the Binder chamber. The time of freezing ranged from 8 to 24 hours.

The visual method was used to determine the compatibility of scion and rootstock.

The statistical processing of the results on the winter hardiness of apple varieties on different clonal rootstock genotypes was carried out using nonparametric indicators (the

Mann-Whitney U-test, the null hypothesis (H0), the Kruskal-Wallis H test (the non-parametric alternative to ANOVA), the Kruskal-Wallis's analysis-of-variance-by-ranks test. The statistical processing was carried out using the Statistica12 program.

3 Results and discussion

The study period climatic conditions were unstable. The smallest frost-free period was in 2017 (100 days). During the other years, it varied from 120 to 128 days (Table 1).

Table 1. Setting fields. Number of frost-free days in 2017-2021.

Years	Frost-free period, days
2017	100
2018	128
2019	120
2020	124
2021	122

Note. The table was compiled according to the data of the Federal State Budgetary Institution "Irkutsk Department for Hydrometeorology and Environmental Monitoring", Irkutsk.

According to long-term data, periods of unfavorable temperatures in winter and growing seasons relative to the average long-term indicators were identified. 2018-2019 climatic conditions were of particular severity. Temperatures below -30°C were observed in December. Late January and early February were marked by long periods of temperatures below -40°C. The absolute maximum (-2.2°C) was recorded in January 2017. 2019-2020 climatic conditions were relatively mild. The critical temperature (-30°C) was observed for a short period of time only in early February. In 2020, December was the coldest month. The average temperature was 3°C lower than in January. The temperature drop in 2021 to -30°C and below was observed for a short period of time in January, February, and March.

In 2021, the lowest temperatures were recorded during the growing season May – September. In 2017, September was colder than usual (9.2°C). In 2018, the decrease in average temperatures was observed in July (18.0°C), in 2019 – in June (16.7°C). Compared with long-term data, the highest average monthly temperatures during the growing season were recorded in 2020. In terms of temperature, 2018 and 2020 were the most favorable for the plant development.

The level of snow cover in the cold season is the most important factor contributing to the good wintering of perennial plants. The amount of snow did not differ significantly over the years of observation. However, the time of the snow cover settlement did not always precede the first critical frosts. The required snow level (more than 15 cm) was observed by the beginning of December 2017 and 2018. In 2019, 2020, and 2021 that amount of snow was only by mid-December. The snow cover usually melted at the end of March – beginning of April.

Winter hardiness is the main characteristic of fruit crops, which determines the longevity of a fruit tree and thus the economic efficiency of its cultivation. The entire history of Siberian horticulture is associated with Siberian berry apple tree, the seedlings of which are used as rootstocks. In this case, winter hardiness is a determining factor. Depending on the variety, trees grafted with berry apple trees turn out to be medium-grown or vigorous, the size is not even, fruiting entry is not constant even within the same variety (usually it is in 3-4 years, commercial fruiting starts in 5-6 years).

The instability of the climatic conditions of the Siberian region as a whole and of the Southern Cisbaikalia in particular does not allow speaking about the long-term use of perennial apple plantations. The maximum life span of a semi-cultivated apple tree is no more than 20 years (no more than 15 years of commercial fruiting). Considering that the majority of winter-hardy semi-cultivated apple trees have the periodicity of fruiting, this period is halved.

The use of clonal rootstocks allows obtaining a marketable yield in a short period of time (2-3 years). The yield can be used both for fresh produce consumption and for obtaining processed products. Reducing the time from obtaining a seedling to obtaining a yield of full value as well as increasing the yield capacity per unit area due to the introduction of clonal rootstocks will partially provide residents with fruit grown in our region [7-9].

Phenological rhythms are one of the indicators of species acclimatization. The adaptation of plants to certain environmental conditions can be evaluated by the passage of phenological phases as well as by the features and completeness of the passage of seasonal and ontogenetic development cycles.

Phenological studies are one of the most accessible and effective methods for studying the degree of plant adaptation [10, 11]. According to our observations, almost all the types of rootstocks passed all phenological phases during the frost-free period. Siberian berry apple tree clone and E-56 were the fastest to reach dormancy. The rest of the rootstock genotypes ended the growing season at about the same time. The exception was 2018, when the temperature dropped below -10°C in early October. Due to that, the natural leaf fall of most clonal rootstock genotypes did not have time to end.

Observation of the growth of the clonal rootstocks on the collection plot for a long time (2018-2022) made it possible to rank the collection genotypes according to winter hardiness. Siberian berry apple tree clone, the rootstocks of the Ural selection (Ural and Ural 2), the rootstock of the Estonian selection (E-56) showed high rates of winter hardiness throughout all the years of studies (Table 2).

According to the damage degree and the ability to regenerate, these varieties can be classified as highly winter-hardy. The clonal rootstocks 70-6-8, Arm18, K-2 were classified as low winter-hardy. The rest of the rootstocks showed average winter hardiness over the years of the study. The rootstocks 62-396, 54-118, 70-20-20, 18-7, 4-12, and 62-22 showed the high regenerative capacity and therefore the root system preservation.

V.I. Budagovsky [12] found that the winter hardiness of the root system of clonal rootstocks correlates well with the winter hardiness of their aerial parts. Therefore, the method of artificial freezing of one-year shoots makes it possible to identify highly winter-hardy and frost-resistant clonal rootstock forms, the main indicator in cultivation of which is the root system.

In the experiments, we evaluated the resistance of wood of the different clonal rootstock genotypes to the four components of winter hardiness (Table 3).

The evaluation of the results (Table 3) for components 1 and 2 of winter hardiness during artificial freezing in mid-December at -35°C and in January at -45°C showed that not all the studied apple trees withstand early winter frosts and retain high frost resistance in the hardened state. No damage was noted in rootstock cuttings Ural5, 54-118, E56, 4-12, 62-396, 62-223, 64-143, 18-7, and in the cuttings of the control Siberian berry apple clone.

Table 2. The degree (mean score) of damage to the clonal rootstocks in the collection plot, 2018-2022.

Clonal rootstock	**2018**	**2019**	**2020**	**2021**	**2022**
62-396	1	3	2	1	2
54-118	1	3	2	1	1
70-20-20	0	3	2	1	3
70-6-8	1	3	2	1	3
64-143	0	3	2	1	1
62-223	1.3	3	2	1.1	2
Arm18	2	3	3	3	3.5
E-56	1	2	1	0.5	0
Ural	1	2	1	0	2
Ural-2	1	2	1	0	2
Ural-5	0	3	2	1	1
18-7	0	3	2	1	2
B-3-4	1	3	2	1	2
4-12	1	2	1	0.4	1
8-2	0	3	2	1	1
OB	1.3	3.5	2.8	1.8	1.5
K-2	1.5	3.5	2.5	2.5	3
Berry apple tree (clone)	0	0	0	0	0

The ability to maintain frost resistance during the thaw period (3rd component of winter hardiness) was shown by almost all the rootstock varieties, with the exception of Ural, Ural2 and Arm 18. Ural and Ural2 had reversible damage of 2-2.3 points. Arm18 showed significant wood browning (3 points).

Siberian berry apple tree, E56, 8-2, and 62-396 showed the ability to restore frost resistance during re-hardening after a thaw period (component 4). Arm18 rootstock cuttings had significant damage (4.3 points). In the rest of the rootstocks, the damage was reversible and ranged from 0.7 to 2.3 points.

The regrowth test (Table 4) showed that Arm18 had the weakest tissue state (from 1.8 to 2 points). Less critical was the condition of cuttings B-3-4 (3.7-4 points), OB (3-3.3 points), 70-20-20 (from 3 to 5 points), 62-396 (from 3 to 4 points), and 54-118 (3-4 points). Despite the damage during freezing, the Ural and Ural2 rootstocks showed good recovery ability during the regrowth (4-5 points). Siberian berry apple tree and E56 showed the absence of damage.

Table 3. The degree of damage to the wood of the clonal rootstocks during artificial freezing, points.

Rootstock genotype	-35	-45	+5,-25	+5,-25,-35
62-396	0	1	0.7	0
54-118	0	0.7	1	1
70-20-20	1	2	1	1.3
62-223	0	0.3	0.7	1
64-143	0	0.2	1	1
Arm18	3	4.3	3	4.3
E56	0	0	0	0
Ural	2	3	2.3	2.3
Ural2	2	1.2	2	1.8
Ural5	0	0	0.7	1.2
4-12	0	1	0.3	0.3
B-3-4	2	2.3	1.3	1.3
8-2	1	2	0	0
18-7	0	1	0.7	0.7
OB	2	1	0.7	0.7
Berry apple tree (clone)	0	0	0	0

One of the most important characteristics of a rootstock is its compatibility with the main cultivated varieties. The main properties of fruit trees depend on the quality of the obtained planting material: winter hardiness, vigor, precocity, productivity, fruit quality [13-15]. To determine the compatibility of the clonal rootstocks and the main varieties of apple trees used in the horticulture of the region, a series of experiments on different combinations of scions and rootstocks was carried out in the nursery:

- 62-396: semi-cultivated apple trees Katyusha, Rayskoye, Prevoskhodnoye, Lada;
- 54-118: semi-cultivated apple trees Katyusha, Rayskoye, Prevoskhodnoye, Lada;
- 70-6-8: semi-cultivated apple trees Katyusha, Rayskoye, Prevoskhodnoye;
- 70-20-20: semi-cultivated apple trees Katyusha, Rayskoye, Prevoskhodnoye, Lada, Zavetnoye, Purpurovaya crab apple;
- 64-143: semi-cultivated apple trees Krasnoyarskiy snegirek, Podarok sadovodam, Sokovoye, Lada, Zavetnoye, Purpurovaya crab apple;
- E56: semi-cultivated apple trees Lada, Zavetnoye, Purpurovaya crab apple;
- K-2: semi-cultivated apple trees Zavetnoe, Lada, Purpurovaya crab apple;
- Ural5: semi-cultivated apple trees Lada, Zavetnoe, Purpurovaya crab apple. Either Siberian berry apple tree clone or its seedlings were used as a control.

Table 4. General condition of cuttings in regrowth samples after artificial freezing, points.

Rootstock genotype	-35	-45	+5,-25	+5,-25,-35
62-396	3	4	4	4
54-118	3	4	4	4
70-20-20	3	3.3	5	4.3
64-143	5	4.3	4.3	4.3
62-223	4	4	4.3	4
Arm18	2	1.8	2	2
E56	5	5	5	5
Ural	4	4	5	4.7
Ural2	4	5	4	4
Ural5	4	4	4.3	4
4-12	4	4	4	4
B-3-4	4	3.7	3.7	3.7
18-7	4	5	4	4
8-2	5	4.7	4	4
OB	3	3.3	3.3	3
Berry apple tree (clone)	5	5	5	5

Strong scion-rootstock graft union, active growth, well-developed leaf apparatus, the absence of any signs of starvation or color disturbances, etc. were observed in all the cultivar-rootstock combinations.

The yield of seedlings ranged from 81 to 100% on the different rootstock genotypes. The seedlings then were planted in the orchard, where their study continued. Over four years of observations (2018-2021), the grafted on Siberian berry apple tree semi-cultivated apple varieties were found to receive minimal damage (Table 5).

When grafted on the clonal rootstocks, Katyusha had the least damage and Prevoskhodnoye was damaged most of all. Lada was the exception. This variety had approximately the same damage when grafted on Siberian berry apple tree and on clonal rootstocks.That was also confirmed by the statistical evaluation (Table 6), according to the results of which no significant differences were found between plants on different rootstocks (the degree of damage to Lada and the control variety) ($P>0.05$).

In all the other cases (Katyusha, Prevoskhodnoye, Rayskoye, and rootstocks 2, 3, 4, and 5), significant differences between the compared samples were noted. That suggests that the variety and type of rootstock affect the degree of thermal damage. The same conclusion can be drawn owing to the overall evaluation of the samples by the Kruskal-Wallis's test (H): Katyusha H = 30.194** ($P<0.01$); Prevoskhodnoye H = 29.146**; Rayskoye H = 44.908**; Lada H = 1.243 ($P>0.05$).

Table 5. The degree of damage (mean score) of different apple varieties on clonal rootstocks in the nursery and orchard.

Rootstock	Grafted variety	2018	2019	2020	2021
70-6-8 (K-2) (semi-dwarf)	Katyusha	1	2	1	1
	Prevoskhodnoye	2.5	2.5	2	2
	Rayskoye	2	2	1	0.75
70-20-20 (K-3) (medium)	Katyusha	1.7	2	1.2	0.5
	Prevoskhodnoye	2	2.5	2.3	2
	Rayskoye	2	2.5	2.5	2
62-396 (K-4) (dwarf)	Katyusha	1	2	1.3	1.3
	Prevoskhodnoye	2	2.5	2.2	2
	Rayskoye	2.5	2	1.2	1
	Lada	2	2	1.7	2
54-118 (K-5) (law)	Katyusha	1	2	1	1.5
	Prevoskhodnoye	2.5	2.5	2	2
	Rayskoye	2.5	2.5	2	2
	Lada	2.5	2	1.2	1.25
Control (Siberian berry apple seedling)	Katyusha	1	0	0	0
	Prevoskhodnoye	1	2	1.5	1.25
	Rayskoye	1	1	0.2	0.5
	Lada	1	2.5	2	2.25

Table 6. Evaluation of the damage degree to the apple varieties in accordance with the type of rootstock in comparison with the control (the Mann-Whitney U test).

Variety	K-2	K-3	K-4	K-5
Katyusha	**24****	**34****	**14****	**20****
Prevoskhodnoye	**28****	**28****	**39****	**21****
Rayskoye	**52****	**0**	**38.5****	**0**
Lada	-	-	117.5 $P>0.05$	113.5 $P>0.05$

Note (hereinafter): * - $P<0.05$; ** - $P<0.01$; Significant indicators are in bold.

4 Conclusion

Long-term observations suggest that clonal rootstocks can be used in horticulture of the Southern Cisbaikalia. According to the results, the rootstocks of the Ural selection (Ural and Ural 2) and the rootstock of the Estonian selection (E-56) showed the highest winter hardiness of all the studied clonal rootstocks. 70-6-8, Arm18, and K-2 showed low winter-hardiness. The rest of the rootstocks showed medium winter hardiness. The laboratory results confirmed high winter hardiness of E-56. The study of the cultivar-rootstock combinations showed good compatibility of the clonal rootstocks with the semi-cultivated apple trees. The variety and type of rootstock were statistically proven to affect the degree of temperature damage to fruit trees. The clonal rootstocks with high winter hardiness and those with medium winter hardiness and high recovery ability were selected as promising for breeding.

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