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THE ADAPTIVE POTENTIAL OF THE *Rosa canina* L. ROOTSTOCK OBTAINED in vitro IN THE CONDITIONS OF THE SOUTH OF WESTERN SIBERIA

O.Yu. VASILYEVA✉, E.V. AMBROS, M.V. KOZLOVA

Central Siberian Botanical Garden, Siberian Branch RAS, 101, ul. Zolotodolinskaya, Novosibirsk, 630090 Russia, e-mail vasil.flowers@ramber.ru (✉ corresponding author), ambros_ev@mail.ru, margareta23@inbox.ru
ORCID:
Vasilyeva O.Yu. orcid.org/0000-0003-0730-3365
Kozlova M.V. orcid.org/0000-0002-3347-5948
Ambros E.V. orcid.org/0000-0002-2119-6503
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Abstract

Garden roses are reproduced by grafting cultivars on resistant rootstocks, predominantly intraspecific forms of *Rosa canina* L. The underground part of these rootstocks is winter-hardy even in the forest-steppe and southern taiga of Western Siberia. However, their shoot systems of formation (SSF), on which generative shoots are formed, related to the type of shortened fruit, are damaged during the wintering period. After extreme wintering, *R. canina* plants of ontogenetic states g1-g3 for one year can pass into the state of "temporarily non-flowering". In the Central Siberian Botanical Garden of the SB RAS (CSBG, Novosibirsk), long-term studies of seasonal development, ontogenesis, biomorphology, reproductive biology and winter hardiness of wild rose species from the *Caninae* Crėp sections are carried out. For the first time, the results of a long-term study of winter hardiness and seed productivity of the selected form of *R. canina* were presented, and an assessment of the SSF after extreme wintering, characterized by various damaging factors, were carried out. The aims of the present study were to establish in vitro conditions and propagate through direct organogenesis a promising winter-hardy form of *R. canina* used as a rootstock for garden roses, as well as to assess the adaptive potential of plants obtained by micropropagation under continental climate. In vitro experiments were carried out on a winter-hardy form selected in the CSBG from F_3 plants of local reproduction. The primary explants were meristems with two leaf primordia isolated from axillary buds of annual vegetative shoots. At the establishment stage the explants were cultured on hormone-free modified liquid Murashige and Skoog's medium (MS) supplemented with 100.0 mg/l glutathione and 30.0 g/l glucose. Direct organogenesis was induced on MS medium supplemented with 2.0 mg/l 6-benzylaminopurine (BAP) and 1.0 mg/l 3-indoleacetic acid (IAA). MS medium with 1.0 mg/l BAP was used for shoot multiplication. A hormone-free half-strength MS medium supplemented with 1.0 mg/l IAA was used to root the obtained microshoots. The regenerants were grown in containers with sterile sand, then in pots with a substrate consisting of a mixture of peat with perlite, humus, sand, and coconut substrate (1:1:0.5:0.5) and transferred to soil culture. Further studies were carried out in 2015-2021 on the experimental plot of the CSBG, located in the forest-steppe zone of the south of Western Siberia (Novosibirsk, Akademgorodok), which is characterized by a continental climate. In the study of morphogenesis, classical and modern biomorphological approaches were used, which consider the shrub form as a complex shoot systems of formation in space and the change of these systems in time. The seasonal dynamics of starch in the shoots was studied using a reaction with iodine in potassium iodide. Seed productivity was determined. At the multiplication stage, 8±1 microshoots per explant on MS media with 1.0 mg/l BAP was obtained. The in vitro rooting frequency was 60.0 % with a mean number of 2.0±1.0 roots per microshoot on half-strength MS with 1.0 mg/l IAA. It was revealed that the pregenerative period in vitro-derived *R. canina* plants is reduced by a year, compared with ones of seed origin. Plants enter the ontogenetic state g1 in the third year, and the formation of partial bushes, which can be used to seed plantations, begins in the fourth year of vegetation. Under the conditions of the continental climate of the forest-steppe of Western Siberia, all in vitro-derived specimens of the selected form had annual fruiting on shoots above the snow cover. The exception was 2020-2021, however, even after a severe wintering, hypanthia formed in the lower part of the SSF. A prolonged decrease in temperature to –30 °C in December led to partial damage to the middle part of the SSF, while a short-term decrease in air temperature to –28 °C in January did not cause serious damage even to the middle part of the SSF. The selected form was also resistant to strong spring frosts in the second decade of May. With favorable wintering and the preservation of terminal buds, the SSF of mature generative plants (g2) continued to increase in height. The combination of two favorable wintering periods in a series led to the formation and preservation of powerful SSF up to 2 m tall, as well as to the formation of mainly 2-3 hypanthia with a high number of completed seeds on short fruit shoots. Starch hydrolysis in those shoots of *R. canina*, in which complete leaf fall was observed in October (phenophase L5), was almost completed in November. However, single starch grains were still in single-row and multi-row medullary rays, as well as in the perimedullary zone.

Keywords: *Rosa canina*, clonal micropropagation, ontogenesis, partial bush, seed productivity, histochemical studies, forest-steppe of Western Siberia

Garden roses are traditionally grown in grafted culture [1-4]. In Siberia, there are no natural habitats for the most widely used rootstock *Rosa canina* L. which grows in Europe, Western and Central Asia, North Africa, the Crimea and the Caucasus. The eastern border of the range of this species does not reach the Kama [5].

The main problem is that the underground part of the *R. canina* rootstock has a high winter hardiness, while the aboveground shoot system, even in the forest-steppe of Western Siberia, often receives serious damage because of wintering. It is possible to successfully grow *R. canina* under-howling seedlings from seeds collected in natural habitats with a milder climate, but the production of seeds of local reproduction on an industrial scale in the region is not guaranteed.

Repeated attempts to use wild roses of the local flora (R. acicularis Lindl., R. majalis Herrm.) as rootstocks in the Urals and Western Siberia, as well as the most winter-hardy introduced wild roses (*R. rugosa* Thunb.) have shown that these species are unpromising. All of them are characterized by the presence of a large number of thorns and small spines on the shoots, which make grafting and budding extremely difficult, as well as high overgrowth, which further inhibits the varietal graft. *R. acicularis* and *R. majalis* also have a short period of good bark separation associated with sap flow and thin, bursting bark in the grafting zone [6].

According to foreign taxonomic and molecular genetic studies [7-9], *R. canina* is an extremely polymorphic species with a wide range, which allows the search and selection of ex situ forms that are promising in specific ecological and geographical conditions both as a rootstock and and as a medicinal plant [10-12]. Outside the natural range, in conditions of a more comfortable than continental monsoon climate of the Russian Far East, cases of naturalization of the introducent R. canina were noted [13].

Long-term studies of seasonal development, ontogeny, biomorphology, reproductive biology and winter hardiness of wild rose species from the sections *Caninae* Crėp., *Indicae* Thory, *Synstylae* DC have been carried out in the Central Siberian Botanical Garden of the Siberian Branch RAS (CSBG, Novosibirsk). and *Cinnamomeae* DC. Due to the special type of *Caninae* meiosis [14], as well as the tendency of *R. canina* and *R. corymbifera* to autogamy and facultative apomixis [15], the seed progeny of selected forms of these species is characterized by matroclinity. The most effective way to solve the problem of accelerated reproduction of winter-hardy highly productive forms is by using clonal micropropagation. Foreign biotechnologists described a positive experience of in vitro propagation of some species [16-18] and cultivars [19-21] of roses.

In this paper, for the first time, the results of a long-term study of winter hardiness and seed productivity of a selective highly winter-hardy form of *Rosa* *canina* propagated in vitro are presented, and an assessment is made of the state of the formation shoot system after extreme wintering. It was revealed that after extreme wintering plants of the selected form do not pass into the category of “temporarily not blooming”.

The aim of the work was to assess the adaptive potential of a promising winter-hardy form of *Rosa canina*, introduced into culture in vitro.

Materials and methods. Reproduction of the winter-hardy form of *R. canina*, used as a rootstock for garden roses, was carried out using microcloning.

Plants of the initial introduction populations of *R. canina* were grown from seeds collected in natural habitats in Kabardino-Balkaria. First, the selection of the best two-year-old individuals of seed origin was carried out according to the growth energy in the pregenerative period, winter hardiness, moderate formation of renewal shoots, and resistance to fungal diseases. Further, samples with the highest yield of hypanthium fruits and regular fruiting were noted. The selected winter-hardy forms (in particular, *R. canina* no. 23 and *R. canina* no. 39) were used for comparison during further selections. To analyze the rhythms of growth and development, the degree of readiness of *R. canina* plants for wintering, the species of the local flora *R. majalis* Herrm was also involved in comparative studies.

Experiments in vitro were carried out on a selected winter-hardy form isolated in CSBG from F_3 plants of local reproduction. The primary explants were meristems with two leaf primordia isolated from axillary buds of annual vegetative shoots. At the stage of introduction into in vitro culture, the explants were cultured for 3 days in a liquid nutrient medium according to the Murashige-Skoog (MS) prescription, supplemented with 100.0 mg/l glutathione and 30.0 g/l glucose [22, 23]. To induce direct organogenesis, MS medium with 2.0 mg/l 6-benzylaminopurine (6-BAP) and 1.0 mg/l 3-indoleacetic acid (IAA) was used. Actually micropropagation of regenerants was carried out on MS medium with 1.0 mg/l BAP. Microplants were rooted on MS medium with half the content of micro- and macroelements, supplemented with 1.0 mg/l IAA. The transfer of regenerants to soil culture was preceded by cultivation in containers with sterile sand, then in pots with a substrate consisting of a mixture of peat with perlite, humus, sand and coco substrate (1:1:0.5:0.5) [24].

Further studies were carried out in 2015-2021 at the CSBG experimental site located in the forest-steppe zone of the south of Western Siberia (Novosibirsk, Akademgorodok) which is characterized by a continental climate. The meteorological conditions of vegetation and wintering were analyzed on the basis of data from the GMS closest to the CSBG (Ogurtsovo settlement, Novosibirsk).

Plants from pots with a substrate were transplanted into open ground. Phenological observations were carried out according to the described method [25].

When studying morphogenesis, classical and modern biomorphological approaches were used [26, 27], which consider the shrub form of growth as a set of shoot systems of formation (SSF) in space and the change of these systems in time. In the above-ground part of the bush, tillering shoots, stem and rhizome, were isolated, in the underground part, the xylopodia and xylorizome [28]. The dynamics of shoot formation was evaluated on 15 plants. Qualitative features of ontogenetic states were described on the basis of the ontogeny periodization scale [29].

The seasonal dynamics of the starch content in shoots was studied using a reaction with iodine in potassium iodide. The composition of the reagent was as follows: 2 g of potassium iodide, 0.2 g of crystalline iodine and 100 ml of distilled water [30]. Micropreparations were prepared using an MC-2 sledge microtome

(Spectro Lab, Ukraine) with a TOC-II thermal cooling table (Tochmedpribor, Ukraine), a Carl Zeiss Axioscop-40 light microscope (Carl Zeiss, Germany), and an AxioCam MRc-5 video camera (Carl Zeiss, Germany) with AxioVision 4.8 image acquisition and processing software (Carl Zeiss, Germany, https://carl-zeiss-axiovision-rel.software.informer.com). With regard to seed productivity, methodological guidelines for seed breeding of introducers [31] and our own developments [32] were used. Seed productivity in the studied forms of *R. canina* was assessed by 20 hypanthia fruits.

Statistical processing was carried out according to B.A. Dospekhov [33] in Microsoft Excel 2003. Formulas were used to calculate the statistical characteristics of the sample with quantitative variability of traits: arithmetic means (*M*), mean errors (±SEM), and coefficients of variation (*Cv*) were calculated.

Results. The features of morphogenesis of the selective winter-hardy form of *R. canina* which is characterized by a technologically valuable small number of thorns on shoots (Fig. 1, a) were studied in vitro in buds taken in September-October, since they had the maximum frequency of shoot formation (62.0 %).

Combination of 2.0 mg/l 6-BAP and 1.0 mg/l IAA with glutathione in the zero passage provided obtaining viable microshoots by the end of the first passage (after 6 weeks of culture). Regeneration of microshoots from the meristems of axillary buds occurred along the periphery of their base (see Fig. 1, b). On average, approx. three shoots per explant were formed. At the stage of actual micropropagation on media with 1.0 mg/L 6-BAP, the number of microshoots per explant was 8±1 (see Fig. 1, c). When rooting regenerants on MS medium with half the content of micro- and macronutrients and 1.0 mg/l IAA with a rhizogenesis frequency of 60%, each microshoot produced an average of 2 1 roots (see Fig. 1, d, e).

The survival rate of regenerated plants ex vitro was 95-100%, the plants were characterized by good developed aboveground part and root system, e.g., 2-4 roots of 2.5-7.5 cm in length (see Fig. 1, f, g). Plants with such morphological characteristics (see Fig. 1, h) were studied for their ontomorphogenesis and reproductive biology in vivo.

Initially, during a short time, a highly winter-hardy form was propagated in the amount necessary to create an experimental seed plantation with minimal use of shoot material. The pregenerative period in *R. canina* of microclonal origin was reduced by one year, individuals entered the ontogenetic state g1 in the third year, and the formation of partial bushes that can be used to replenish seed plantations began in the fourth year of vegetation. With seed propagation, it would take another 1-2 years to stratify the seeds.

The second stage of work was necessary since the meteorological conditions and damaging factors during almost half-yearly wintering in the conditions of the forest-steppe of Western Siberia vary greatly from year to year. This zone is characterized by a sharp decrease in temperature in November to –20... –25 °C, prolonged December and January frosts below –35 °C, insufficient accumulation of solid precipitation in the first half of wintering. Long-term studies carried out by us made it possible to cover all the problematic wintering periods and form the most complete picture of the adaptive potential of the selective winter-hardy form of *R. canina*. Particular attention was paid to the development and preservation of shoot systems after wintering, depending on meteorological conditions. In addition, hydrothermal conditions from the 2nd decade of September to the 3rd decade of October were important to achieve the end of the linear growth of the shoots and the initiation of the terminal bud.

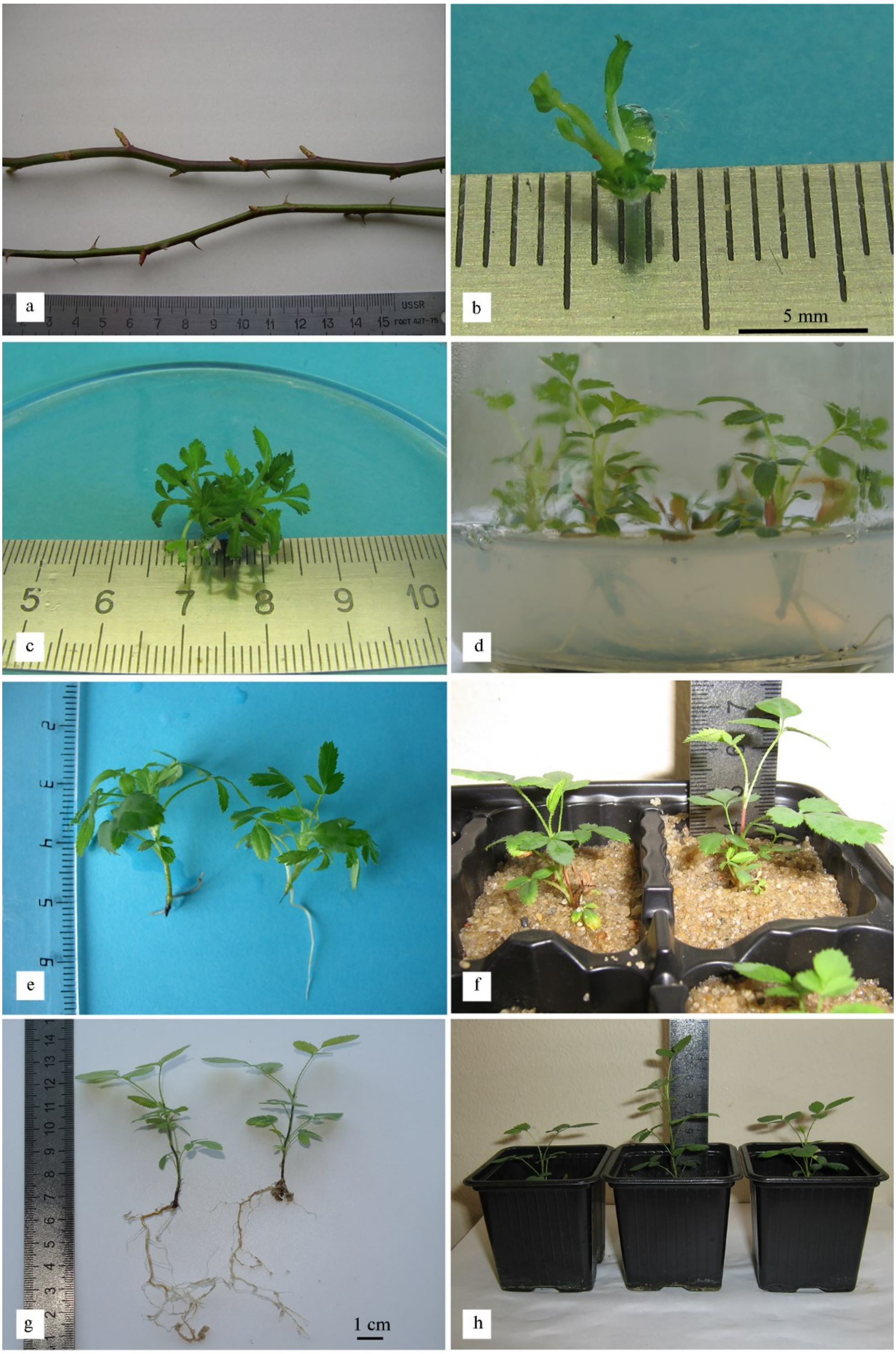

Fig. 1. Clonal micropropagation of the selected winter-hardy form *Rosa canina* L.: a — annual vegetative shoots, starting material for introduction into in vitro culture, b — formation of microshoots from the meristem with 2,.0 mg/l of 6-benzylaminopurine (6-BAP) and 1.0 mg/l of 3-indolylacetic acid, c — microshoots after 6 weeks of micropropagation on MS medium with 1.0 mg/l of 6-BAP, d — rooting of regenerants on medium 1/2 MS with 1.0 mg/l IAA, d — micro-plants after 6 weeks of cultivation on a rooting medium; sand and coco substrate (1:1:0.5:0.5) after 4-week adaptation.

1. Meteorological characteristics of the 2015-2021winter periods in the conditions of the continental climate of the forest-steppe of Western Siberia (Novosibirsk)

Month	Air temperature, °C								Precipitation, mm		
	decade						average per month	deviation from the norm	decade		
	I		II		III				I	II	III
	mean	min	mean	min	mean	min					
2015											
November	–2.0	–10.8	–14.3	–21.1	–8.4	–24.9	–8.2	–0.6	6.0	0.0	30.0
December	–4.2	–15.1	–6.4	–17.7	–7.3	–27.1	–6.0	7.9	9.0	7.0	32.0
2016											
January	–20.2	–29.3	–20.2	–34.8	–18.2	–29.7	–19.5	–2.7	1.0	5.0	0.0
February	–6.8	–19.6	–14.8	–26.3	–6.3	–20.3	–9.3	6.3	10.0	4.0	0.5
November	–6.1	–20.8	–22.3	–33.2	–10.2	–31.7	–12.9	–5.3	21.0	6.0	19.0
December	–10.4	–26.5	–9.4	–22.0	–16.6	–36.1	–12.1	1.8	23.0	19.0	11.0
2017											
January	–8.9	–29.2	–19.2	–29.5	–15.1	–31.9	–14.4	2.4	13.0	6.0	5.0
February	–12.7	–21.0	–20.1	–32.8	–5.0	–16.4	–12.6	3.0	5.0	8.0	4.0
November	0.7	–6.9	–7.6	–22.9	–8.8	–24.8	–5.2	2.4	16.0	11.0	7.0
December	–9.3	–18.1	–17.9	–27.8	–9.7	–24.3	–12.3	1.6	5.0	1.0	33.0
2018											
January	–20.2	–31.5	–15.7	–31.2	–26.8	–37.4	–20.9	–4.1	3.0	21.0	3.0
February	–14.9	–26.5	–15.4	–27.1	–15.5	–28.3	–15.3	0.3	0.8	3.0	1.0
November	–5.7	–22.5	–11.7	–23.0	–7.1	–17.1	–8.2	–0.6	27.0	16.0	27.0
December	–21.9	–36.5	–12.8	–28.1	–23.5	–35.4	–19.4	–5.5	6.0	11.0	5.0
2019											
January	–15.5	–27.9	–13.8	–26.6	–14.7	–28.2	–14.7	2.1	3.0	2.0	7.0
February	–30.0	–40.1	–10.4	–25.8	–7.0	–14.7	–15.8	–0.2	0.0	4.0	4.0
November	–1.3	–14.8	–13.8	–29.5	–15.2	–28.5	–10.1	–2.5	14.0	14.0	8.0
December	–6.1	–22.5	–8.2	–13.9	–13.6	–31.6	–9.3	4.6	14.0	14.0	28.0
2020 год											
January	–7.9	–22.4	–11.9	–25.8	–11.7	–28.8	–10.5	6.3	11.0	16.0	17.0
February	–7.9	–27.4	–10.8	–29.8	–4.9	–17.2	–7.9	7.7	19.0	7.0	9.0
November	2.2	–2.8	–7.0	–20.8	–9.3	–19.4	–4.7	2.9	11.0	18.0	0.2
December	–16.2	–25.3	–10.5	–21.5	–21.4	–40.0	–16.0	–2.1	10.0	10.0	20.0
2021											
January	–27.2	–39.3	–19.0	–32.1	–19.1	–41.0	–21.8	–5.0	3.0	6.0	19.0
February	–11.2	–26.3	–16.4	–36.1	–21.5	–32.6	–16.4	–0.8	13.0	12.0	6.0

As our studies have shown, to more accurately predict the degree of SSF damage, it is necessary to take into account not only the average ten-day air temperature and the total amount of solid precipitation per decade, but also the minimum ten-day temperature (Table 1). Thus, the average air temperature in the third decade of November 2016 was –10.2 °C, and the minimum dropped to –31.7 °C. This beginning of wintering is considered extreme, since during the entire second decade of November the minimum air temperatures were below –20 °C, and during 6 days they even dropped to –25 °C. The deviation of the average monthly air temperature from the norm was 5.3 °C. The end of the growing season in 2016 with a sharp drop in temperature in October also did not contribute to the completion of the growth of annual shoots and the establishment of terminal buds.

2. The structure of a bush of young (g1) **and mature** (g2) **generative plants of the selected winter-hardy form of *Rosa canina* L. derived from clonal micropropagation in the conditions of the continental climate of the forest-steppe of Western Siberia** (n = 15, M±SEM; Novosibirsk, Akademgorodok)

Year, status	SSF		Bushing shoots		Stem shoots		Partial bushes	
	number	height, cm	number	height, cm	number	height, cm	number	height, cm
2016, g1	2.13±0.19	128.73±1.52	2.07±0.21	122.60±3.75	0		0	
2017, g2	3.47±0.13	143.13±1.83	2.60±0.67	155.80±2.88	7.33±0.43	45.93±1.42	0.67±0.13	92.80±2.23
2018, g2	5.33±0.32	175.93±3.32	4.13±0.26	159.27±1.12	5.87±0.24	23.33±2.18	2.13±0.09	96.20±1.05
2019, g2	2.13±0.09	119.00±1.50	2.13±0.09	119.00±1.50	2.13±0.09	119.00±1.50	2.13±0.09	119.00±1.50

N o t e. SSF — shoot system of formation.

After extreme wintering, young generative (g1) plants of the selected form did not go into the state of "temporarily not blooming", although the SSF was damaged in the upper and partially middle parts of the shoots. The fruits were formed in the lower and middle tiers of the SSF.

Intensive formation of stem shoots during the growing season of 2017 (Table 2) was the result of the death of the upper and damage to the middle parts of the axial shoot due to extreme wintering. The most favorable beginning of the winter period was observed in November 2017 and November 2018.

The combination of two favorable winterings in a row led to the formation and preservation of powerful SSF up to 2 m tall, as well as to the formation of mainly 2-3 hypanthia with a high number of completed seeds on short fruit shoots (Table 3). In nursery, rosehip nuts, which are formed inside the hypanthium, are traditionally called seeds, and the hypanthium itself (overgrown receptacle) is called the fruit. In order to predict the yield of high-quality rootstock seeds from the harvested fruits, we counted the completed seeds and then correlated these indicators with the yield of harvested fruits in various selected forms, including the most winter-hardy.

3. Seed productivity of the selected winter-hardy form of *Rosa canina* L. derived from clonal micropropagation in the conditions of the continental climate of the forest-steppe of Western Siberia (n = 15; Novosibirsk, Akademgorodok)

Plant age, years	Seeds per fruit				Weight of filled seeds per fruit, g	
	filled		unfilled			
	M±SEM	Cv, %	M±SEM	Cv, %	M±SEM	Cv, %
			R. canina № 23			
4	36.32±0.90	24.3	6.35±0.45	34.2	0.82±0.02	14.3
5	29.72±1.05	35.6	11.40±0.62	42.0	0.86±0.02	11.7
6	32.95±1.16	22.1	7.82±0.56	39.4	0.95±0.02	9.8
			R. canina № 39			
4	38.05±1.23	29.7	5.42±0.40	44.5	0.88±0.03	12.0
5	35.21±1.37	19.2	8.10±0.63	32.1	0.94±0.02	8.4
6	32.71±0.90	18.6	7.30±0.47	37.3	0.97±0.03	9.3
			R. canina, selected form			
4	31.75±1.43	20.5	4.90±0.43	39.6	0.69±0.03	20.3
5	35.30±1.48	18.7	5.20±0.49	42.5	0.84±0.04	21.4
6	34.85±0.95	12.2	5.75±0.44	34.3	0.91±0.04	19.8

In the winter-hardy form, with favorable wintering and the preservation of terminal buds, the SSF of mature generative plants (g2) continued to increase in height. Long-term studies have shown that the SSF of the selected form of *R. canina* were the most unstable to low negative temperatures in November, when the process of transition to the state of winter dormancy has not yet been completed.

Histochemical express diagnostics of the readiness of wild roses for wintering was carried out taking into account the fact that by the beginning of leaf fall, the tissues of the shoots contain the autumn maximum of starch. In October-November, in winter-hardy species and forms, starch hydrolysis occurs most completely, and in tissues, this has a centripetal character. There are a single-row and multi-row medullary rays, perimedullary zone and core, but is already absent in the cortical parenchyma, phloem, cambium and xylem [6].

In those shoots of *R. canina*, in which complete leaf fall was observed in October, starch hydrolysis was almost completed in November, single starch grains were still in single-row and multi-row medullary rays, as well as in the perimedullary zone (Fig. 2, a) . In the highly winter-hardy local species *R. majalis*, which is used in studies as a control, single starch grains were found only in the perimedullary zone (see Fig. 2, b). Consequently, in both species, the content of starch in the tissues was minimal and was estimated at 1 point. Histochemical studies of *R. canina* shoots in the pre-winter period [6, 34] carried out at the Central Siberian Botanical Gardens revealed differences in starch dynamics even among fairly winter-hardy forms: starch grains in single-row and multi-row medullary rays could be located in less than 50% of the cells, which corresponds to 2 points.

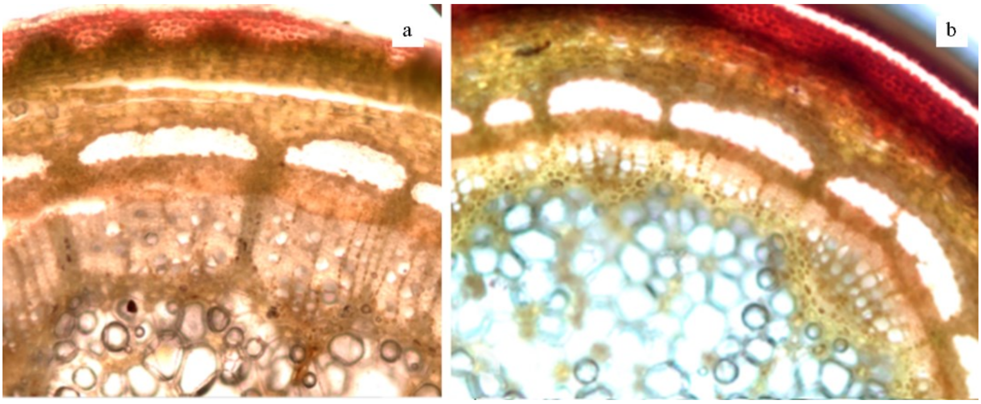

Fig. 2. The content of starch in the tissues of annual shoots of *Rosa canina* L. (a) **and *R. majalis* Herrm.** (b) **in the pre-winter state** (Novosibirsk, Akademgorodok, November 2021; iodine—potassium iodide syaining, magnification ×40, light microscope Carl Zeiss Axioscop-40, Carl Zeiss, Germany; video camera AxioCam MRc-5, Carl Zeiss, Germany, with AxioVision 4.8 imaging software, Carl Zeiss, Germany, https://carl-zeiss-axiovision-rel.software.informer.com).

A prolonged (for more than 2 days) decrease in temperature to –30 °C in December led to partial damage to the middle part of the SSF. A short-term (for several hours during the day) decrease in temperature ranged from –25 to –28 °C in January did not cause serious damage even to the middle part of the SSF (for example, in the winter of 2018-2019).

Wintering of 2020-2021 was also among the extreme ones, during which in the third decade of December, as well as in the first and third decades of January, the minimum temperature dropped to –41 °C. As a result, the upper and partially middle parts of the SSF were died. A negative impact on the development of the generative sphere of many fruit crops, as well as the death of flowers of grape varieties and actinidia at the beginning of the growing season of 2021 in the conditions of Novosibirsk, was also exerted by severe frosts on May 20 (–3.2 °C) and on May 21 (–6.0 °C). These days, the average daily temperature was 6.8 and 8.5 °C below the norm. Nevertheless, in the basal and middle parts of the SPF, on the short fruit shoots of the selected form of *R. canina*, predominantly single hypanthia were formed, which confirms the high winter hardiness and frost resistance (Fig. 3, a).

Seed material, as a rule, is intended for obtaining seedlings of wild rose rootstocks on an industrial scale, and it is promising to use partial bushes for the speedy expansion of mother plantations. The studied selective form was characterized by the accelerated formation of partial bushes (see Fig. 3, b), which served as one of the marker signs of the transition to the mature generative state (g2).

Creation of initial introduction populations of *R. canina* by two-stage selection made it possible to carry out further selection of winter-hardy highly productive forms among plants grown from seeds of local reproductions. The analysis of selected forms grown from seeds of the first local reproduction has proved the prospects of these research works. Comparison of the *R. canina* best forms in the Central Siberian Botanical Garden and in one of the nurseries of Holland [35] in terms of the yield per bush showed that individual forms of *R. canina* during the period of maximum fruiting under local conditions have the same high productivity as in Dutch highly specialized enterprise.

Fig. 3. Mature generative plants (g2) **of the *Rosa canina* L. selected form:** a — fruiting in the lower part of the formation shoot system after extreme wintering, b — partial bush (Novosibirsk, Akademgorodok, 2021).

An analysis of studies conducted with wild rose rootstocks abroad [1, 7, 10] showed that the problems of increasing the winter hardiness of rootstocks in a temperate continental and milder climate are irrelevant. The main selections are carried out for resistance to fungal diseases, and the issues of overcoming deep seed dormancy are also being addressed. Research carried out at the Central Siberian Botanical Garden and focused on the identification of winter-hardy forms of rootstocks of garden roses with high seed productivity are of great importance for regions with harsh climatic conditions and make it possible to avoid mass purchases of rootstock seedlings in the southern regions of Russia and the CIS. The features of the reproductive biology of wild roses revealed in the process of research [15] are of wider theoretical interest. From an ecological point of view, the assessment of adaptive potential will also be of importance, carried out in many respects, in particular, using histochemical studies using modern instrumentation with digital image processing.

Thus, the maximum frequency of shoot formation (62.0%) in the selective winter-hardy form *Rosa canina* was noted in buds taken in September-October. It was revealed that the pregenerative period in *R. canina* of microclonal origin is reduced by a year compared to plants of seed origin, therefore, individuals enter the ontogenetic state g1 (young generative) in the third year of vegetation. Partial shrubs that can be used to replenish seed plantations are formed in the fourth year of the growing season. Under the conditions of the continental climate of the forest-steppe of Western Siberia, in all specimens of the selected form, propagated in vitro, annual fruiting was noted on shoots above the snow cover. The exception was the wintering of 2020-2021, however, even after a severe wintering, hypanthia formed in the lower part of the shoot systems of the formation. An important feature of the phenorhythmics of the selective form of *R. canina* is the ability to complete growth and lay a terminal bud on most shoots of the current year, that is, to fully prepare for wintering, which is confirmed by histochemical studies of the dynamics of starch content.

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