Initial and post-cryogenic assessment of viability of russian plum cultivars pollen (*Prunus rossica* Erem.) in the conditions of the North-West of Russia

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Abstract. The viability of pollen of 5 collectible varieties of Russian plum of the Pushkinskiye and Pavlovskiye VIR Laboratories SPB before and after cryopreservation was studied. It was found that a significant factor for the level of pollen viability is "variety" (p=0.07), the factors " cryopreservation" (p=0.457), "year" (p=0.221), as well as the interaction of the factors "variety× cryopreservation" (p=0.172), were not significant at the p<0.05 level of significance. For long-term storage of Russian plum pollen in liquid nitrogen, the year of pollen collection is not a significant factor. For most genotypes, no negative impact of cryoconservation on pollen viability was revealed. The varieties 'Aureus', 'Podarok Sankt-Peterburgu' and 'Exotica' after cryopreservation for 1 year showed a tendency to increase the pollen viability indicators.

1 Introduction

Diploid plum species, 2n=16, which also include a new cultigenic species - the Russian plum (Prunus rossica Erem.), have a complex of valuable biological traits: immunity, early fruitfulness, yield, frost resistance, early ripening, decorative effect. [1, 2]. In the Leningrad region, the entire assortment of diploid plum species was either attracted from other regions, or obtained in the process of methodical crosses on the basis of the field collection of the Pushkinskiye and Pavlovskiye VIR Laboratories (Table 1). The significant polymorphism and ecological plasticity of the Russian plum suggests the selection of adaptive and stable genotypes. [2] One of the main components that determine the productivity of the variety is the viability or germination of pollen on the stigma of the pistil. In some varieties, especially those related to Chinese plum and American plum, pollen is not sufficiently viable [3]. There are significant differences in the level of pollen viability in varieties of Russian plum, in different genotypes of the species, this level was in the range of 1.6-39.0%. [4]. The high pollen viability on artificial growing medium was observed in the varieties of Russian plum in Belarus - from 47.0 to 77.0%, however, in this region, genotypes with low pollen viability were also identified, from 1.0 to15. 0%. [5] In

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the Crimea (Nikitsky Botanical Garden), pollen germination on artificial growing medium in the varieties of the "hybrid" group of alycha - "Aromatnaya" and "Zemlyanichnaya", pollen viability was in the range of 1.6-39.0%. 4]. In Latvia, the Chinese plum variety ‘Skoroplodnaya’ (one of the parent forms of many Russian plum varieties) had pollen viability ranging from 0.2 to 25.6% depending on the year of research. [6].

Researchers note the dependence of pollen germination on both the weather conditions of the year and the genotype [3].

The objective of the research was to compare the pollen viability level of new varieties of Russian plum under unstable weather conditions in the North-West of Russia during the maturation of the male gametophyte, as well as to determine the degree of influence on the pollen viability of ultra-low temperatures during cryopreservation.

2 Materials and methods

In the conditions of the North-Western region in the collection garden of the scientific and production base Pushkinskiye and Pavlovskiye VIR Laboratories (Saint Petersburg, Pavlovsk), in 2017-2019, a study of the pollen viability of 5 new varieties of Russian plum originating from two geographical regions of the Russian Federation was conducted (Table 1).

Pollen of the studied samples was collected in the collection garden in the II-III decades of May. Pollen collection from trees of each variety was carried out in dry weather in the amount of 200-250 well-developed buds. Anthers were separated with a preparation needle, dried in a light room for two to three days at a temperature of +21°C to a loose state. To determine the initial viability of the pollen, it was placed in Petri dishes on a growing medium containing 0.8% agar-agar and 10% sucrose. A suspension of pollen in distilled water was applied to the surface of the growing medium. Petri dishes were placed in a thermostat at a temperature of +21°C. Pollen germination was observed for 1-5 days. Pollen with a length of the pollen tube exceeding the diameter of the pollen grain was considered as sprouted. The number of sprouted pollen grains was calculated in the field of a Motik 100M microscope at a 100-fold magnification in 30-50 random fields of view and 6-8 drops of pollen suspension [7]. After determining the initial viability, the pollen was placed in cryoprobes and immersed in liquid nitrogen. Then, after cryopreservation of pollen for 1 year, it was thawed at an air temperature of +21°C for 5-10 minutes, then they were placed on a growing medium and its viability was determined [8, 9].

The indicators of weather conditions, the daily course of air temperature and precipitation, were obtained in 2017-2019 in the Department of Automatic Information Systems of Plant Genetic Resources (AIS PGR) of the VIR. The most unfavorable weather conditions for the flowering of the Russian plum were formed in April and May of 2017 and 2019. In April 2017, negative night air temperatures were observed for 18 days (in 2019 – 15 days) (Fig. 1).

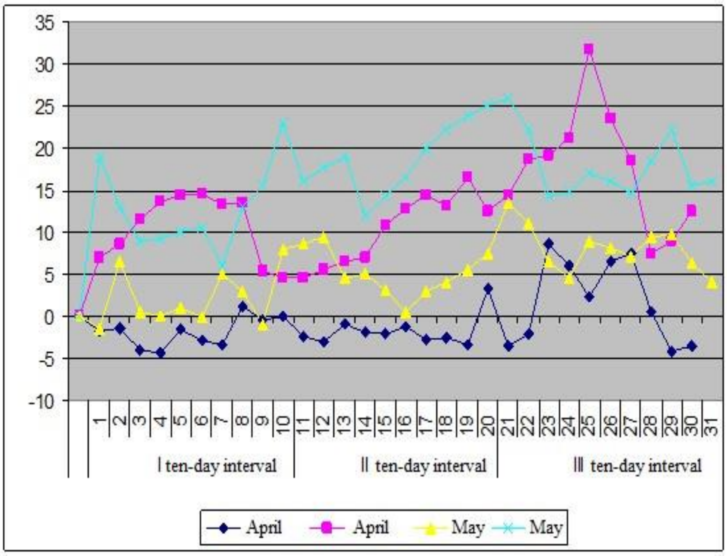

Fig. 1. Daily changes in air temperature in April-May (minimum and maximum t˚C; data from the VIR weather station; city of Pushkin, 2019)

In the first and second decades of May 2017 and in the first decade of May 2019, the minimum air temperatures fell below biological zero, i.e. below +5° C. The diurnal amplitudes were 5° C... 10°C. The weather conditions in 2018 were more favorable for plum pollen maturation: in April, negative values of night temperatures were observed for 6 days, and in May, 5 days with a minimum temperature of 0 to +1°C were noted. In 2019, we observed a warmer temperature regime compared to 2017, however, the minimum air temperatures in April were low negative. During the flowering period, in the third decade of May, there were significant fluctuations in air temperature.

Table 1. Origin of new varieties of Prunus rossica Erem.

VIR catalog No.	Varieties	Genetic origin	Originator
43068	Aureus	'Podarok Sankt-Peterburgu' × 'Zolotaya sliva'	VIR
41445	Podarok SanktPeterburgu	'Skoroplodnaya' × 'Pionerka'	VIR
43069	Spuravaya	Seedling from free pollination of the variety 'Спуровая'	VIR
43047	Exotica	«	VIR
43041	Timiryazevskaya	Seedling from free pollination of the Kubanskaya kometa variety	RSAU-MACA

The 'Timiryazevskaya' variety is a seedling from free pollination of the Russian plum 'Kubanskaya kometa' (Table 1). Among the varieties of VIR selection, the pollen viability was analyzed in the variety 'Podarok Sankt-Peterburgu' and in two more varieties - its descendants, where this variety was one of the parent forms.

All varieties of Russian plum that were studied are breeding, new, and are being tested for the first time in the conditions of the North-Western region of Russia. Trees planted in 2012, entered the fruiting season in 2014. The significance of the differences in the compared samples was assessed using the Student's t-test after checking the normality of the distribution [10], the variance analysis of the two-factor experience based on the results of 3 years was performed using the StatSoft Statistika 13.0 software package.

3 Results and discussion

In 2017, most of the genotypes had the lowest initial pollen viability over all the years of observations. (fig. 2).

Only the variety ‘Timiryazevskaya’ (RSAU-MACA) had 29% of pollen germination, which is approximately the average level of germination (Table 2). Other genotypes had a very low level of pollen grains germination: from 5.6% in the varieties 'Aureus' and 'Podarok Sankt-Peterburgu'; to 1.9%, respectively, in the variety 'Spurovaya'.

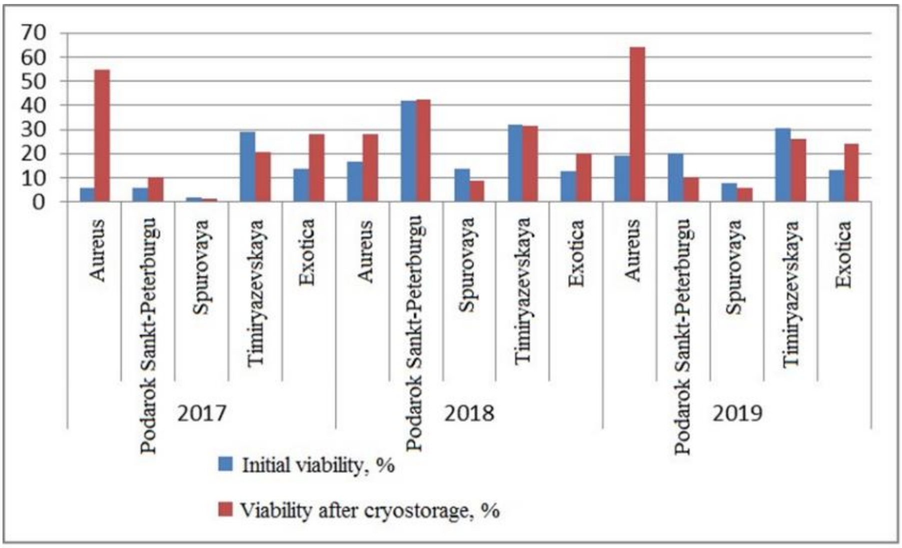

Fig. 2. Viability of Russian plum pollen before and after cryostorage in the North-West region in 2017-2019.

The highest level of initial pollen viability in the studied Russian plum genotypes was observed in 2018-2019. The minimum level of pollen viability in this period of time was observed in the variety 'Spurovaya' (13.9%), the maximum – in the variety 'Podarok Sankt-Peterburgu' - 42.1% and 'Timiryazevskaya' - 32%, and from 16.8 to 19.4% - in the variety 'Aureus'. The value of the initial viability of the varieties varied over the years, depending on the temperature conditions, with a positive reaction, to an increase in air temperature.

After pollen cryopreservation in liquid nitrogen, both an increase and a decrease in pollen viability indicators were observed in the studied genotypes. A significant increase in the level of pollen grains germination was observed in the varieties 'Aureus' and 'Podarok Sankt-Peterburgu', (VIR) compared to the control. In these varieties, the pollen viability increased after cryopreservation to 54.8-64% and 10.5-42.5%, depending on the year of observation. A decrease in pollen viability was observed in the varieties ‘Timiryazevskaya’ up to 20.5% and ‘Spurovaya ' - up to 1.4%. Change the pollen viability after cryopreservation depending on the year of observation varied like the version with the original vitality: after laying for the cryopreservation of pollen in 2018-2019 level of viability has mostly grown in varieties ‘Aureus’ (from 16.8 to 28.3%; and from 19.4 to 64%) in the variety ‘Podarok Sankt-Peterburgu' and ‘Timiryazevskaya’ it have not changed, and in varieties ‘Spurovaya’ weakly decreased, but was higher than in other years of observations. (Fig. 2)

Table 2 . The viability of Russian plum pollen before and after cryostorage in years 2017-2019 (Pushkinskiye and Pavlovskiye VIR Laboratories SPB)

Name of variety	Year of study	Initial pollen viability, %	Pollen viability after cryostorage, %	Significance of differences, p
Aureus	2017	5.6±2.8	54.8±4.9	<0.001*
	2018	16.79±1.43	28.3±3.74	0.47
	2019	19.38±1.86	64±3.63	0.16
Podarok Sankt-Peterburgu	2017	5.6±1.2	10.5±2.4	0.034*
	2018	42.1±4.8	42.54±3.3	0.519
	2019	20.06±2.14	10.26±3.4	0.04*
Spuravaya	2017	1.9±0.9	1.4±0.5	0.650
	2019	13.89±2.04	8.74±2.78	0.04*
Timiryazevskaya	2017	29±3.8	20.5±1.1	0.024*
	2018	31.95±2.86	31.44±1.38	0.022*
Exotica	2017	13.6±1.7	28.1±2.6	0.000*
	2018	12.54±1.35	20.24±2.21	0.590

Note: *The differences are statistically significant compared to the control, $p<0.05$

In unfavorable years (2017, 2019) for pollen maturation in the varieties of Russian plum, ultra-low temperatures had a significant impact on pollen viability: in the varieties ‘Aureus’, ‘Podarok Sankt-Peterburgu' and ’Exotica‘, pollen viability significantly increased compared to the initial one; in the varieties ’Spurovaya‘ and ’Timiryazevskaya', the viability decreased after the experiment. In 2018, with more favorable conditions for pollen maturation, the ' cryopreservation ' factor did not have a significant role, it was insignificant. In the varieties 'Aureus' and 'Exotica', the pollen viability increased, in the varieties ‘Timiryazevskaya’ and ‘Podarok Sankt-Peterburgu’ either decreased slightly or did not change, and in the ‘Spurovaya’ variety it significantly decreased. (Table 2)

The variance analysis of the two-factor experiment based on the results of 3 years of study showed that the significant factor for pollen viability is 'variety' (p=0.07); the 'cryopreservation' factor (p=0.457), 'year' factor (p=0.221.), as well as the interaction of the factors 'variety×cryopreservation' (p=0.172), were not significant at the significance level of $p<0.05$. Graphic images of the averages of the studied genotypes indicate a distinct positive reaction to the cryopreservation of pollen in the variety ‘Aureus’. Nevertheless, there are genotypes with low initial viability and with a negative reaction to cryopreservation , but this reaction in our experiments was not 'fatal' for the pollen of the studied genotypes.

The data obtained by the viability of Russian plum pollen are consistent with the results on the cryopreservation of blackcurrant and black cherry pollen in the conditions of the North-West of Russia [8,9], a significant factor for the pollen viability is the 'variety'. Nevertheless, the weather conditions of April-May 2017 and 2019 negatively affected the initial pollen viability.

For the purposes of long-term pollen storage at ultra-low temperatures, the year of pollen deposition in liquid nitrogen is not a significant factor.

4 Conclusions

Unfavorable weather conditions in April and May of 2017 and 2019 affected the decline in pollen viability indicators. Most of the studied Russian plum genotypes had low and very low initial pollen viability. Less dependent on changes in air temperature during the maturation of the male gametophyte was the variety ‘Timiryazevskaya', in which the initial pollen viability was at a medium level (29.0-32%).

After cryogenic storage, the viability of pollen significantly increased in the varieties ‘Aureus', 'Exotica', 'Podarok Sankt-Peterburgu’ - up to 64, 28.1 and 42.5%. The varieties ‘Timiryazevskaya’ and 'Spurovaya' viability decreased to 31.4% and 1.4%, respectively.

The method of pollen cryopreservation , as well as the assessment (before and after cryopreservation) of the morphological and physiological features of germinating pollen, will allow identifying adaptive genotypes that are potentially suitable as a pollinator, which should be confirmed by further field studies.

Acknowledgments

The work was carried out within the framework of the state assignment according to the VIR thematic plan on the topic No. 0662-2019-0004 "Collections of vegetatively propagated crops (potatoes, fruit, berry, ornamental, grapes) and their wild VIR relatives - study and rational use"

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