Viability assessment of quince cuttings (*Cydonia* Mill.) and pear pollen (*Pyrus* L.) after cryoconservation in liquid nitrogen vapor (-183-185°C)

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Abstract. The viability of cuttings before and after cryopreservation in nitrogen vapor was assessed on six varieties of quince and seven varieties of pear pollen taken for study in the field gene bank of the Krymsk Experimental Breeding Station – VIR branch. Post-cryogenic evaluation showed that the viability of quince cuttings averaged 51.0±2.8%, which exceeds international standards (40%) and the requirement for specimens recovered after cryostorage. Storage of cuttings at -5°C can be used for a short period of time, up to 1-1.5 years. The viability of pear pollen revealed small initial values after collection in the garden (from 0 to 6.95%), but it increased in varieties by 4-10 times after cryopreservation in nitrogen vapor. According to the initial values, the Georgievskaya Early variety stood out - 6.95%, and after cryopreservation - 52.81% and Suksen early ripening (4.37% initial value and 47.5% after cryopreservation), Elite 12-69-37 with zero initial value after cryo pollen viability was 25.75%.

1 Introduction

The VIR field gene collection preserves samples of vegetatively propagated fruit and berry crops, which, lose the characteristics of the mother plant in the next generations, when propagated by seed. For these crops, methods of cryopreservation of vegetative parts of plants (annual shoots, buds, pollen and meristems) are used in practice, stored in liquid nitrogen or its vapor at -183 ... - 185°C [1]. Under such storage conditions, the plant material remains genetically stable, without changes [2, 3]. We used the modified Forsline method [4] for cryopreservation of cuttings in nitrogen vapor as the main one, with the addition of methods from other authors [5]. It should be noted that relatively few studies on the cryopreservation of quince vegetative shoots and buds have been carried out, mainly in southern countries and regions of cultivation. However, at present, interest in it is beginning to increase and quince

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has a great potential for using its products in the pharmaceutical and food industries due to its economic qualities [6].

One of the main components that determine the productivity of the studied varieties is the viability or germination of pollen on the stigma of the pistil. Different levels of pollen germination are observed depending on the year, variety or crop. For example, after 5 months of storage in nitrogen vapor plum pollen viability is from 3 to 36%, apple tree pollen viability in the same period of storage is 39.8% - 65.8%, strawberry pollen - 17-37.1% [7]. Pollen cryopreservation also makes possibility to preserve the genetic material of more productive and economically valuable varieties, for their crossbreeding, regardless of the amount of freshly collected pollen and weather conditions. The dried after harvesting in the garden pollen withstands the ultra-low temperature of liquid nitrogen vapor without losing its viability and fertility, and is also convenient for transportation [8].

2 Material and methods

6 varieties of quince were taken from the collection garden of the branch of the Krymsk EBS VIR for cryopreservation and determination of the viability of post-cryogenic regeneration of cuttings: 1. Rajabli No. 2 (k-18539), 2. Nivushka (k-48866), 3. Novogodnaya (k-48868), 4. Kubanochka (k-48864), 5. From the Ferghana Range No. 1 (k-48886), 6. From the Ferghana Range No. 2 (k-48887) (Table 1.).

Table 1. Brief characteristics of quince varieties taken for the study

№	Variety	VIR catalog number	Origin	Winter hardiness*	Fruit ripening period*
1	Rajabli No. 2	k-18539	Azerbaijan, NIISiV	average	early
2	Nivushka	k-48866	Russian Federation, Krasnodar, SKZNIISVV	high	late
3	Novogodnaya	k-48868	Russian Federation, Krasnodar, SKZNIISVV	high	early
4	Kubanochka	k-48864	Russian Federation, Krasnodar, SKZNIISVV	high	late
5	From the Ferghana Range No. 1	k-48886	Azerbaijan, NIISiV	average	early
6	From the Ferghana Range No. 2	k-48887	Russian Federation, Krasnodar, SKZNIISVV	average	average

Note: * winter hardiness and terms of technical ripeness of fruits were determined in the conditions of the south of the Russian Federation.

The cuttings have been done out during the winter dormancy of plants in December in the garden of the Krymsk EBS of the VIR branch. Determination of the initial viability, drying (in the Thermo 818 incubator) and cryopreservation of the cuttings (cut into segments with 2-3 buds) have been done according to a modified method, which was used to work with quince in the long-term storage laboratory of VIR genetic resources. When laying for cryopreservation, the cooling of the plant material has been done first slowly, with an interval of 1°C -2°C/min to -30°C ... -32°C, then the freezing rate was increased by 3°C - 4°C/min, lowering temperature to -48°C - 50°C and after freezing, the samples were placed in cryotanks for long-term storage in nitrogen vapor at -183°C - 185°C [9]. During cold storage (taken as control), the cuttings were kept in a refrigerator at -5°C. After 6 months of storage, the cuttings were taken out of cryotanks and a refrigerator, thawed in a water bath, and their viability was determined by germinating in a thermostatically controlled light room, at a temperature of + 22°C, illumination of 6-7 thousand lux, with a photoperiod of 16 hours a

day and 8 hours of the night. The results of assessing the viability of quince cuttings, studied in different years, are shown in tables 2 and 3.

The viability of pollen was studied on 5 pear varieties taken in the garden of the Krymsk EBS VIR branch: 1. Chinese 7 (k-6268), 2. Suxen early ripening (k-6318), 3. Elite 12-69-37 (k-6165), 4. Elite 17-62-26 (k-6168), 5. Georgievskaya early (k-6194). After collection in the garden (2022), dried pollen was delivered to the laboratory, the initial viability was determined, and then, in cryotubes, by direct immersion, they were placed for long-term storage in nitrogen vapor. After a year of storage, pollen was removed from cryotanks, thawed, and its viability was determined by germination in Petri dishes on an artificial nutrient medium containing 10% sucrose and 0.8% agar-agar. 6-8 drops of a pollen suspension in distilled water were applied to the surface of the nutrient medium, and the samples were placed in a thermostat for germination at +22°C. The determination and counting of germinated pollen have been done in 10-fold repetition under a Motic BA 300 TYPE 102M microscope. Pollen with a pollen tube length exceeding the pollen grain diameter was considered germinated [10].

3 Results and discussions

There was a gap in the studies over the years due to the covid-19 pandemic and the impossibility of traveling to the experimental station for grafting cuttings directly in the garden. Therefore, all work on material preparation, freezing, storage and post-cryogenic evaluation in 2018 have been done in the laboratory for long-term storage of plant genetic resources. According to the results obtained, it was revealed (Table 2) that the initial viability of quince cuttings after cutting in the garden is almost the same and averages 93.5±3.74%. After drying, before laying the cuttings for storage in nitrogen vapor, their viability decreased by 10-20% and was in the range of 73.0±3.3% - 83.0±3.3%. After 6 months of storage in a refrigerator at -5°C and germination in a light room, the viability of the control cuttings decreased by approximately 30% compared to the initial one and averaged 63.8±3.3%. With such storage, the varieties Nivushka, Novogodnaya, From the Fergana Range No. 2 turned out to be more resistant (66.0 ± 3.3%). The results after cryopreservation showed that the viability of the cuttings decreased by about 10% compared to the control, and, depending on the variety, its values ranged from 43.0±3.3% to 53.0±3.3%. Among the varieties in this storage option, such varieties as Rajabli, Novogodnaya and From the Ferghana Range No. 2 stood out (53.0±3.3% -53.0±6.7%). Comparing the data on the viability of varieties with the characteristics of winter hardiness (Table 1), our values coincided with the variety Nivushka, with other varieties there was little or no coincidence.

Table 2. Assessment of the viability of quince cuttings obtained under laboratory conditions after cold storage at -5°C (control) and cryostorage in liquid nitrogen vapor (experiment), 2018

№	Variety	VIR catalog number	Viability of cuttings, %			
			Initial (after cutting cuttings in the garden)	After drying in an incubator before laying for cryostorage	Germination in the light room	
					After cold storage (control)	After cryosto-rage (experi-ment)
1	Rajabli No. 2	k-18539	93.0±3.3	83.0±3.3	63.0±3.3	53.0±3.3
2	Nivushka	k-48860	93.0±3.3	73.0±3.3	66.0±3.3	50.0±5.7
3	Novogodnaya	k-48868	96.0±3.3	83.0±3.3	66.0±3.3	53.0±6.6
4	Kubanochka	k-48864	90.0±5.8	73.0±3.3	56.0±3.3	43.0±3.3

5	From the Ferghana Range No. 1	k-48886	93.0±3.3	83.0±3.3	63.0±3.3	46.0±3.3
6	From the Ferghana Range No. 2	k-48887	96.0±3.3	73.0±3.3	66.0±3.3	53.0±3.3
	Average		93.5±3.7	78.0±3.3	63.8±3.3	49.7±4.2

Table 3 shows the results of research in 2021-2022. and assessing the viability of cuttings grafted in the garden after cold storage and cryopreservation in nitrogen vapor.

Table 3. Viability assessment of quince cuttings after cold storage at -5°C (control) and cryopreservation in liquid nitrogen vapor (-183°…-185°C), 2021-2022

№	Variety	VIR catalog number	Viability of cuttings, %			
			Initial (after cutting cuttings in the garden)	After drying in an incubator before laying for cryostorage	Прививка на дерево в саду	
					After cold storage (control)	After cryosto-rage (experi-ment)
1	Rajabli No. 2	k-18539	96.6±0.1	84.8±1.8	63.3±0.0	50.0±3.3
2	Nivushka	k-48860	90.0±0.0	75.0±5.0	65.0±1.7	51.6±1.6
3	Novogodnaya	k-48868	96.6±0.1	79.6±3.6	66.7±0.0	55.0±1.7
4	Kubanochka	k-48864	91.6±1.6	73.3±3.3	58.1±5.1	51.7±5.0
5	From the Ferghana Range No. 1	k-48886	94.6±1.4	81.6±1.6	61.6±1.6	48.1±1.8
6	From the Ferghana Range No. 2	k-48887	93.3±0.0	76.1±0.1	63.3±0.0	49.8±3.5
	Average		93.8±0.5	78.4±2.6	63.0±1.4	51.0±2.8

Spring grafting of cuttings on the shoots of skeletal branches of trees showed a decrease in viability by 30-40% from the inital, after cutting in the garden: in the control variant, it was at the level of 66.7±0.0% - 58.1±5.1%, after cryostorage – from 55.0±1.7% to 48.1±1.8%. Among the quince varieties taken for the study, the varieties Novogodnyaya (66.7±0.0) and Nivushka (65.0±1.7%) were more resistant in the control variant, the remaining varieties showed slight differences - 1-3%, but all of them met the accepted international standards, allowing for a 40% level of post-cryogenic viability of the cuttings, which is necessary for recovery in the event of the loss of one or another variety.

Statistical analysis of the viability of cuttings in terms of storage conditions and years of research did not reveal significant differences. Cryopreservation significantly ($p=0.002$) reduced the viability of cuttings from 93.5% to 49.7% when determined in a light room (2018). The viability of cuttings grafted in the garden after cryopreservation (51.0%) is significantly ($p=0.012$) lower than the viability of control cuttings grown after cold storage and also grafted in the garden (63.0%) (2021-2022). The results of statistical data processing showed the absence of significant differences in the viability of cuttings between the original and control variants. The decrease in the viability of quince cuttings after cryopreservation in laboratory and field conditions can be explained by the stress effect of exposure to ultralow temperatures on the content of cells and the plants themselves with a preliminary decrease in their moisture, but does not affect the process of their survival after cryopreservation.

The results of assessing the initial viability of the pollen of the studied pear varieties and after cryogenic storage are shown in Table. 4.

Table 4. Pear varieties pollen viability before (initial) and after storage in liquid nitrogen vapor, branch of the Krymsk EBS, 2022

№	Variety	VIR catalog number	Pollen viability, %	
			Initial, after collecting pollen in the garden	After cryostorage
1	Chinese 7	к-6268	3.11	30.47
2	Suxen early ripening	к-6318	4.37	47.5
3	Elite 12-69-37	к-6165	0	25.75
4	Elite 17-62-26	к-6168	3.36	12.57
5	Georgievskaya Early	к-6194	6.95	52.81

The analysis of the results showed that the initial values of viability of pollen of pear varieties were small, and in variety 12-69-37, they were generally equal to zero. With this definition, increased values were observed in varieties Georgievskaya early (6.95%) and Suksen early ripening (4.37%). After a year of cryopreservation of pollen in liquid nitrogen, its viability in varieties increased by 4–10 or more times. By varieties, the same sequence is observed: the variety Georgievskaya Early viability of pollen is 52.81%, Suksen early ripening - 47.5%, Chinese 7 - 30.47%, and Elite 12-69-37, which has a zero value in the original variant, after cryostorage was - 25.75%. This increase can be explained by the fact that after a change in storage conditions and sudden temperature changes, the upper shell of pollen grains bursts, releasing a large amount of pollen. For example, it would be appropriate to draw such an analogy with the process of scarification, when the top layer of poorly germinating seeds (recalcitrant ones) is violated by physical impact, then after that they begin to germinate.

4 Conclusions

Post-cryogenic assessment showed that the viability of quince cuttings averaged 51.0±2.8%, which exceeds the international requirements for samples placed in cryostorage. Cold storage of cuttings at -5°C can be used for a short period of time, up to 1-1.5 years.

An assessment of the viability of pear pollen revealed small initial values after collection in the garden (from 0 to 6.95%), but after cryopreservation in nitrogen vapor in varieties, it increased by 4-10 times. According to the initial values, the variety Georgievskaya Early stood out - 6.95%, after cryopreservation - 52.81% and Suksen early ripening (4.37% initial value and 47.5% after cryopreservation), in the elite 12-69-37 with zero initial value after cryo pollen viability was 25.75%.

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