DEVELOPMENT OF METHODS FOR CRYOCONSERVATION OF VEGETATIVE SHOOTS (CUTTINGS) EMPLOYING VARIETIES OF RED CURRANT (*R. RUBRUM* L.) FROM THE GENE POOL PRESERVED AT THE POLAR EXPERIMENT STATION OF THE VAVILOV INSTITUTE

V.G. Verzhuk, A.V. Pavlov, L.V. Sukhareva, I.I. Gracheva, T.D. Kuvaeva

The N.I. Vavilov Institute of Plant Genetic Resources (VIR), St. Petersburg, Russia, *vverzhuk@mail.ru, lyubov.suxareva@yandex.ru*

Abstract. The work on cryoconservation of vegetative shoots and buds of red currant was based on the technique developed by P. Forsline for dormant apple-tree buds, which we modified to make it applicable to our plant. Cuttings were selected from the collection of small fruit plants held by the Polar Experiment Station of VIR. They were cut into sections 7–8 cm long with 2–3 buds, and dried in an incubator at –4–5°C down to 28–32% moisture content. After that they underwent two-step freezing in Sanyo Medical Freezer MDF-U442(T), and were placed for long-term storage into liquid nitrogen vapor. Assessment of the cuttings' viability prior to their placement into nitrogen (initial), and after their storage and planting in the field showed their differences and variety-specific dependence. The minimum initial value for this parameter was 56.6±5.0% demonstrated by var. Natali (No. K-202), while the maximum of 90.0±3.0% was shown by var. Laplandia (No. K-315). The same correlation between these varieties was recorded after their cryopreservation and taking root in the field: 46.6±5.0% for var. Natali, and 76.6±4.2% for var. Laplandia. The remaining varieties demonstrated intermediate values, but had a rather good percentage of rooting after cryostorage.

Keywords: *cryoconservation, vegetative shoots, gene pool, berry plants, red currant, liquid nitrogen vapor*

DOI: 10.31255/978-5-94797-319-8-858-860

At present, the importance of crop genetic diversity conservation is constantly growing. The problems of collecting, conservation, study and sustainable utilization of plant genetic resources are economically of national significance and great strategic import, and directly connected with the provision of both national and global food and environmental security [Verzhuk et al., 2007; Dzyubenko et al., 2016].

Today, when climatic, environmental and economic conditions are unstable both within and outside the country, there is a threat of irretrievable losses of valuable collection material representing vegetatively propagated fruit and berry plants. A practical solution to the problem is long-term storage of the endangered accessions under controlled conditions at low and ultralow temperatures, and establishment of plant genetic collections with improved health by means of *in vitro* culture [Butenko, 1964; Kalinin et al., 1980].

A promising method of fruit plant preservation is cryoconservation of vegetating plant parts (annual shoots, buds or meristems), seeds and pollen in liquid nitrogen or its vapor at the temperatures of –183–185°C [Verzhuk, Filipenko et al., 2012; Pavlov et al., 2016; Verzhuk, Pavlov et al., 2017]. Plant cells, tissues and organs are kept in a deep freeze environment in anabiotic state, and the material preserved at ultralow temperatures remains genetically stable, which prevents genetic changes typical of the organisms preserved under ordinary conditions [James, 1987; Kozaki et al., 1988; Benson, 2008].

The work on cryoconservation of vegetative shoots and buds of fruit and berry plants was based on the technique developed by P. Forsline [Forsline et al., 1998] for dormant apple-tree buds, which we modified, improved and applied to such cultivated plants as apricot, quince, cherry, pear, black currant, honeysuckle, bird cherry, etc.

While working out the technique for cryoconservation of red currant shoots, prior to their placement for storage in nitrogen vapor, the yearly gain of cuttings from different varieties underwent selection during the period of plant dormancy (November to December)

in the collection orchards at the Polar Experiment Station of the Vavilov Institute (VIR). Then, they were cut in the CryoLab into smaller sections: 7–8 cm long, with 2–3 buds per section, and predried in the INCUBATOR–818 device at –4°– 5°C down to the residual moisture of 28–32%. Prior to predrying (before freezing and placement for storage into nitrogen vapor), a reference batch of cuttings from the selected set of varieties underwent initial viability assessment in %. After predrying, the experimental plant material was gradually frozen in two steps. A decrease of negative temperatures was initially accomplished within a speed of –1°–2°C every half hour. After reaching –30°–32°C, freezing was accelerated by –3°–4°C per hour, and the cuttings were frozen to –48°–50°C using Sanyo Medical Freezer MDF–U442(T). Afterwards, laminated packages with the frozen accessions were placed into cryotanks (HB-0.5 m^3) for long-term preservation in liquid nitrogen vapor at –183°–185°C. After 5–6 months of storage, the cuttings of the studied varieties were removed from the cryotanks, rapidly unfrozen in cold water, and planted in spring in the field to evaluate their viability, growth and development during the entire growing season under the conditions of the field. Cryostorage research was performed for 7 red currant varieties: 1. Natali (K-202), 2. Rolan (K-309), 3. Zarya Zapolyarya (K-200), 4. Svetlana (K-201), 5. Tatyana (K-313), 6. Laplandia (K-315), 7. Det Van (K-204). Cultivated red currant has certain advantages if compared with black currant: it is more productive, as the average yield of black currant is 5 kg per bush against 8 kg with red currant. Valuable properties of red currant are earliness and longevity of fruiting: under favorable conditions its plants start to bear fruit in their third year after planting, and continue fructification for 20 years. An important trait of red currant is the ability of its berries to stay on the branches for a very long time after ripening — they do not fall, and often not only retain their flavor but even improve it due to accumulation of sugars. One more useful quality is the existence of early-ripening varieties whose berries reach maturity almost simultaneously with strawberry, and late-ripening ones which make it possible to consume fresh berries sometimes until late autumn.

Table.

Viability of red currant cuttings: a) initial (cuttings were grown in water inside a thermostatic room), b) planted in the field after cryopreservation in liquid nitrogen vapor.

NNo	Variety/Catalog No. of the Polar Experiment Station, VIR	a) Initial viability of cuttings and buds before cryopreservation when grown under light in water, %	b) Viability of cuttings and buds planted in the soil after cryostorage in liquid nitrogen vapor, %
Red currant (*R. rubrum* L.), Polar Experiment Station of VIR (Apatity), Pushkin/Pavlovsk Science & Production Association, 2015–2017			
1	Natali/K-202	56.6±5.0	46.6±5.0
2	Rolan/K-309	76.6±4.2	63.3±4.8
3	Zarya Zapolyarya/ K-200	83.3±3.7	70.0±4.6
4	Svetlana/K-201	73.3±4.4	66.6±4.7
5	Tatyana/K-313	86.7±3.4	73.3±4.4
6	Laplandia/K-315	90.0±3.0	76.6±4.2
7	Det Van/K-204	83.3±3.7	73.3±4.4

Table presents the results of initial viability assessment for red currant cuttings after their segmentation at the collection site of the Polar Experiment Station of VIR (Apatity Town), and their viability rates after storage in liquid nitrogen vapor and transplanting in spring onto the field of the Pushkin/Pavlovsk Science & Production Association where they have successfully taken root and yielded new young shoots from their buds.

The analysis of viability data for the studied red currant varieties has disclosed variety-specific differences both in the initial version (prior to cryopreservation) and after long-term

storage of the cuttings in liquid nitrogen vapor, when they were transplanted into a field. Initial viability of the varieties was within the scope from 56.6±5.0% to 90.0±3.0%, while after they were transplanted into the field and took root, their viability decreased by a dozen or dozen and a half points and varied from 46.6±5.0% to 76.6±4.2%. Minimum values of this parameter were shown by var. Natali (K-202): 56.6±5.0% in the initial assessment, and 46.6±5.0% after storage in nitrogen vapor and establishment in the field. Maximum viability values were recorded for the cuttings of var. Laplandia (K-315): 90.0±3.0% (initial planting) and 76.6±4.2% (after cryostorage and planting in the field). The remaining varieties demonstrated intermediate values, but had a rather good percentage of rooting after cryostorage. During the spring/summer growing season the plants developed from their buds 2–3 young shoots which survived the winter well, and next spring produced new shoots. It should also be mentioned that the plants grown after cryostorage in nitrogen were less susceptible to various diseases and pests than the reference ones (which did not undergo cryopreservation in nitrogen vapor).

List of references

Arsenyeva T.V. Handbook of a horticulturist. St. Petersburg. – 2000. – P. 316–336. [available in Russian]

Benson E.E. Cryopreservation theory // In: Reed, B.M. Plant cryopreservation, A Practical Guide. – Springer, New York, 2008. – P. 15–32.

Butenko R.G. Culture of isolated tissues and plant physiology. – Moscow, 1964. – 18 p. [available in Russian]

Dzyubenko N.I., Goncharova E.A., Verzhuk V.G., Murashev S.V. Innovative trends and methods of prognosticating environmental safety and conservation of plant resources // Proceedings of a scientific and practical conference, Sept. 4–6, 2015, "Increasing the efficiency of modern horticulture to improve the structure of nutrition for the population of Russia". Michurinsk – Naukograd RF, 2016. – P. 53–57. [available in Russian]

Forsline P.I., Towill L.E., Waddel J.W. et al. Recovery and longevity of cryopreserved dormant apple buds // J. Amer. Soc. Hort. Sci. – 1998. – V. 123, No. 3. – P. 365–370.

James E. Preservation of cells under low temperature conditions // In: Biotechnology of Crop Plants. – Moscow: Agropromizdat, 1987. – 301 pp. [available in Russian]

Kalinin F.L., Sarnatskaya V.V., Polishchuk V.E. Tissue culture methods in plant physiology and biochemistry. – Kiev, 1980. – 142 p. [available in Russian]

Kozaki I., Omura M., Matsuta N., Moriguchi T. Germplasm preservation of fruit trees // Preservation of Plant Genetic Resources. Japan International Operation Agency. – 1988. – P. 65–74.

Pavlov A.V., Porotnikov I.V., Verzhuk V.G., Vorobeykov G.A. Preservation of fruit and berry crop breeding material at ultralow temperatures // Scientific Journal of ITMO University. Series "Processes and Devices of Food Industries", St. Petersburg, 2016. – No. 1. – P. 55–60. [available in Russian]

Verzhuk V.G., Pavlov A.V., Dzyubenko N.I., Novikova L.Y., Murashev S.V., Eremina O.V. Cryoconservation in liquid nitrogen: a promising method of preserving the biodiversity of stone and pome fruit crop plants // Fruit and Berry Plant Growing in Russia. – Moscow, 2017, vol. XXXXVIII, No. 1. – P. 33–36. [available in Russian]

Verzhuk V.G., Filipenko G.I., Safina G.F., Pavlov A.V., Zhestkov A.S. Cryoconservation as an efficient method of fruit and berry plant genetic resources preservation // Proceedings on Applied Botany, Genetics and Breeding, V. 169. – St. Petersburg, 2012. – P. 270–279. [available in Russian]

Verzhuk V.G., Tikhonova N.G., Tikhonova O.A. Cryoconservation of the germplasm of black currant (*Ribes nigrum* L.) at ultralow temperatures // Intern. Conf. "Modern physiology: from molecules to ecosystems". – Syktyvkar, 2007. – P. 152–153/ [available in Russian]