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USE OF GENETIC DIVERSITY OF THE GENUS *PRUNUS* L. IN SELECTION OF CLONAL ROOTSTOCKS FOR STONE FRUIT CROPS AND FEATURES OF THEIR REPRODUCTION

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On the basis of diversity of wild types of the genus Prunus *L. (*P. cerasifera, P. armeniaca, P. persica, P. fruticosa, P. lannesiana, P. maackii, P. tomentosa, P. pumila, *and* P. incana*) in the collection of the Krymsk Experiment Breeding Station, highly adaptive, medium or weak vigour clonal rootstocks for stone fruit crops with good compatibility with grafted cultivars were selected: for plum, apricot and peach — Kuban 86, VVA-1, Evrika 99, Zarevo (Glow), Alab 1, Speaker, Best, VSV-1; and for sweet cherries and sour cherries — L-2, LC-52, VSL-1, VSL-2, and RVL-9. Part of the rootstocks were tested and successfully used in different parts of Russia and some near and far countries. The applied integration of tissue culture in vitro in the selection process at the station considerably promoted the rapid introduction of new rootstocks into world production. Technologies were developed for microclonal reproduction, and green and woody cuttings. The revealed light rooting of woody cuttings of stocks Kuban 86, Evrika 99, Zarevo, Best, VSL-1, RVL-9 (50–80%) allowed to develop technology for growing of young plants on these rootstocks directly in the first field of the nursery.*

Key words: *gene pool, clonal rootstock, cultivar, tissue culture, reproduction, inter-species hybridisation, cutting.*

INTRODUCTION

The industrial production of stone fruit crops in many countries is conducted by intensive technologies, where the use of weak vigour clonal rootstocks is the most important element. Such rootstocks have been created in the world, however, in more severe natural-climatic conditions of Russia and states close to it, their absolute majority cannot realize be used to reach the full potential productivity, as they were created mainly in countries with more favourable conditions in warm climate where the main producers of stone fruit crops are located. This made it necessary to create a number of easily propagated and adaptive clonal rootstocks for stone fruit crops with wide use in world production, including in the climatic conditions of different regions of Russia.

A programme for selection of stone fruit crop clonal rootstocks was initiated and is constantly being improved (Eremin, 2012) at Krymsk Experiment Breeding Station in the period of 60 years. The genotypes that were sources of the most important traits were selected from the genetic diversity of the genus *Prunus* L, and amounted to more than 5000 genotypes used as the initial material in this programme.

MATERIALS AND METHODS

The study was conducted at the Krymsk Experimental Breeding Station, Branch of Federal State Budgetary Scientific Institution, Federal Research Center the N. I. Vavilov All-Russian Institute of Plant Genetic Resources.

The studied species were wild types of the genus *Prunus* L. — *P. cerasifera*, *P. armeniaca*, *P. persica*, *P. fruticosa*, *P. lannesiana*, *P. maackii*, *P. tomentosa*, *P. pumila*, and *P. incana,* rootstocks of different interspecific origin.

The study was conducted according to "Programme and methods of study of fruit, berry-like and caryocarpous crop varieties" (Sedov and Ogol'tseva, 1999), and "Programme and methods of fruit, berry-like and caryocarpous crop selection" (Sedov, 1995).

The scientific and-methodical recommendations published previously on clonal micro-propagation of stone fruit crops and clonal rootstocks (Medvedeva *et al.,* 2014) were used for the work with tissue culture

Analysis of variance was used in the mathematical processing of experimental material (Dospekhov, 1979).

RESULTS

The development of new clonal rootstocks is one of the most effective trends in selection of stone fruit crops allowing to use them already in the first generation, to obtain economically valuable forms, even involving wild forms having low-quality fruit, because there is no need for recrossing of hybrids with them to improve the quality of the fruit. Already in the F1 generation, it is possible to combine the features of high adaptability (winter resistance, drought resistance, resistance to soil pathogens, waterlogging, and excess moisture) together with agronomical characteristics like weak growth, ability of vegetation reproduction, early fruiting of grafted cultivar and others, and with the trait of ńompatibility with main cultivars of stone fruit crops and vegetative reproduction by different ways.

In the gene pool of the genus *Prunus* L. collected at Krymsk EBS, the sources of all traits valuable for selection of stone fruit clonal rootstocks were selected. Moreover, among the members of single types of this genus, there are genotypes that have combined several of these characteristics (Table 1).

However, efforts to select the genotypes presenting interest for use as clonal rootstocks among the samples of pure species allowed to select the forms for practical application (L-2), yet showed their slight insufficiency in comparison with interspecific hybridisation. The crossing of species differing by presence of characteristics valuable in selection allowed to obtain hybrids with more favourable combinations of traits. Such hybrids that combine complexes of parent type signs, which are valuable in selection in one genotype, can achieve the production level of the varieties allowed in Russia (Table 2).

It should be noted that in the process of use of different species of the genus *Prunus* L. in breeding, genotypes were selected that were donors of traits like light reproducibility by cuttings, adaptability, compatibility with grafted cultivars, early fruiting of grafted cultivar, good anchoring of trees and others. Specimens of the species *P. cerasifera* Ehrh., *P. pumila* L., *P.maackii* Rupr., and *P.lannesiana* Wils. became parents for the majority of clonal rootstocks selected at Krymsk EBS.

DISCUSSION

The great advantage of many interspecific hybrids is high adaptability allowing to reveal its positive qualities in different countries and regions of Russia with different climatic conditions (Eremin and Eremin, 2014; 2015). The clonal rootstocks VVA-1 (*P. tomentosa* × *P. cerasifera*), Kuban 86 (*P. cerasifera* × *P. persica*), VSL-2 (*P. fruticosa* × *P. lannesiana*), LC-52 (*P. cerasus* × (*P. cerasus* × *P. maakii*)), Evrika 99 ((*P. pumila* × *P. salicina*) × *P. cerasifera*), which showed good trial results in some countries, present great interest in this direction in regard to international testing.

One of the reasons restraining the spread of stone fruit clonal rootstocks in Russia is an insufficient readiness of available, economically profitable ways of their reproduction as well as the technology of obtaining of stone fruit planting material. In many countries that are producers of stone fruit crops, the main means of vegetative reproduction of stone fruit clonal rootstocks is by clonal microreproduction *in vitro*. All types of clonal rootstocks selected at Krymsk EBS successfully propagate by this means. However, their application in Russia is limited by the insufficient number of biotechnological laboratories and high-tech specialised complexes. Unlike the rootstocks of pome crops grown in industrial nurseries mostly by vertical and horizontal layering, the most simple and effective ways of reproduction of stone fruit clonal rootstocks are green and woody cuttings. All clonal rootstocks selected on Krymsk EBS are rooted well by green cuttings. Woody cuttings are rooted insufficiently for rootstocks Druzhba, VSV-1, L-2 or remain absolutely non-rooted for LC-52, VC-13 (Eremin, 2000).

It is economically reasonable to propagate all rootstocks by woody cuttings, except the above mentioned rootstocks, including Krymsk EBS rootstocks. This method is simpler, accessible and effective, not demanding the additional expenses associated with construction of cultivation facilities, and provides a high coefficient of propagation at minimal expenses including labour costs.

All plum and peach stocks propagate by woody cuttings. The easiest rooted are: Kuban 86, Evrika 99, and Best.

Table 1

SPECIES OF THE GENUS *PRUNUS* L. FROM WHICH GENOTYPES WERE SELECTED AS SOURCES OF CHARACTERISTICS THAT ARE IMPORTANT FOR SELECTION

Characteristics	Species
Weak growth	*P. pumila, P. incana, P. tomentosa. P. spinosa, P. nana, P. kurilensis, P. incisa, P. prostrata, P. canescens*
Rooting with wood cuttings	*P. cerasifera, P. pumila, P. dasycarpa, P. lannesiana, P. mahaleb, P. pseudocerasus, P. serrulata*
Winter resistance	*P. pumila, P. tomentosa, P. spinosa, P. nana, P. fruticosa, P. sachalinensis, P. kurilensis, P. ulmifolia, P. davidiana*
Drought resistance	*P. nana, P. bucharica, P. spinosissima, P. mahaleb, P. fruticosa, P. spinosa, P. incana, P. armeniaca*
Flooding resistance	*P. cerasifera, P. tomentosa, P. dasycarpa*
Resistance to soil pathogens	*P. cerasifera, P. spinosa, P. tomentosa, P. pumila, P. davidiana, P. fruticosa*

Table 2

CHARACTERISTICS OF SELECTED STONE FRUIT CLONAL ROOTSTOCKS AT KRYMSK EBS

Clonal rootstock	Origin	Vigour	Winter resistance	Drought resistance	Frost resistance	Flood resistance	Rooting with wood cuttings
Rootstocks for plum, apricot, peach							
Alab 1	*P. cerasifera* × *P. armeniaca*	4	3	4	3	3	3
Best	*P. pumila* × *P. cerasifera*	3	5	4	4	5	5
VVA-1 (Krymsk 1)*	*P. tomentosa* × *P. cerasifera*	2	5	3	4	5	4
VSV-1 (Krymsk 2)*	*P. incana* × *P. tomentosa*	3	4	5	5	3	3
Druzhba**	*P. pumila* × *P. armeniaca*	3	5	3	3	4	3
Zarevo	*(P. armeniaca* × *P. salicina)* × *P. cerasifera*	4	4	4	3	5	5
Kuban 86 (Krymsk 86)*	*P. cerasifera* × *P. persica*	5	3	5	5	3	5
Speaker	*(P. pumila* × *P. salicina)* × *P. cerasifera*	3	4	4	3	3	5
Fortuna	*P. cerasifera* × *(P. salicina* × *P. persica)*	5	4	4	3	5	5
Evrika 99 (Krymsk 99)*	*(P. pumila* × *P. salicina)* × *P. cerasifera*	4	4	4	4	5	5
Rootstocks for sweet cherries and cherries							
VSL-1	*P. fruticosa* × *P. lannesiana*	3	4	5	4	5	5
VSL-2 (Krymsk 5)*	*P. fruticosa* × *P. lannesiana*	4	4	5	4	5	5
VC-13***	*P. cerasus* × *(P. cerasus* × *P. maackii)*	5	5	5	3	4	1
L-2 (Krymsk 7)	*P. lannesiana* c. o.	4	2	5	3	3	4
LC-52*** (Krymsk 6)*	*P. cerasus* × *(P. cerasus* × *P. maackii)*	4	4	5	4	4	5
RVL-9	*(P. cerasus* × *P. maackii)* × *P. lannesiana*	4	4	5	4	4	5

* trade mark; ** selected at All-Russian Scientific Research Institute of Genetics and Selection of Fruit Trees, *** selected at All-Russian Selection and Technological Institution of Horticulture and Nursery Breeding

Degree of the characteristic's intensity:

1 – absent;
2 – weakly expressed;
3 – expressed in medium level;
4 – well expressed;
5 – expressed in maximum.

Among clonal stocks for cherries, VSL-1, VSL-2, RVL-9, and L-2 are easily rooted. Clonal rootstocks of the Krymsk EBS selection are quite suitable to obtain high-quality planting material, which is needed for a suitable cherry rootstock (Table 3).

Some clonal rootstocks propagate quite well by horizontal layers; these include the microcherries (*P. pumila* var. *besseyi*) Evrika 99, Druzhba, and Best, and the steppe cherries (*P. fruticosa*) VSL-1, VSL-2. However, this method of propagation is more labour-intensive and thus is applied to lesser extent than propagation by cuttings.

To accelerate the breeding process and the introduction of newly developed rootstocks directly into production, it is necessary to use biotechnological methods. In this regard, the potential of the biotechnological laboratory available at the Station is used for several aims: overcoming postgamous incompatibility with use of culture of embryos *in vitro*, creation of necessary number of copies of unique samples of hybrids and improvement with following rooted clonal micropropagation of the newly developed and tested

Table 3

PARAMETERS OF PLANTING MATERIAL GROWN FROM ROOTED WOOD CUTTINGS OF CHERRY CLONAL ROOTSTOCKS, 2011–2015

Rootstocks	Rooting of woody cuttings, %	Parameters of planting material from rooted cuttings			
		diameter of trunk, mm	height, cm	number of lateral shoots, units	general length of lateral shoots, cm
VSL-1	82	26.8	194.0	2.0	74.3
VSL-2	84	23.4	247.9	0.9	62.5
VC-13	10	25.1	207.3	0.3	30.7
L-2	68	27.8	223.3	0.2	20.1
LC-52	12	24.6	186.4	0.9	47.5
RVL-9	85	26.3	201.0	1.2	74.9
LSD_{05}	10.3	2.8	32.8	0.09	14.1

LSD, least significant difference

rootstocks, with the aim of mass production of improved planting material.

We use the method of hybridisation between different genera to create fundamentally new adaptive rootstocks of stone fruit crops that are suitable for applying modern technologies in their growing and propagation. However, we often observed postgamous incompatibility, which makes it impossible to obtain viable embryos. Embryo culture *in vitro* developed at our laboratory allowed to overcome the full self- and cross-incompatibility in interspecific and intergeneric crossings in rootstock selection (Medvedeva *et al*., 2014). This allowed us to obtain to 35–40% of seedlings of complex hybrids in culture from seeds that do not produce suitable shoots in common conditions (*in vivo*).

The combination of culture of embryos *in vitro* with clonal micropropagation practiced in future work allows us to replicate only a single seedling, gives the opportunity for plant breeders to obtain the required number of plants for detailed evaluation for further research. Moreover, this method ensures the preservation of a unique plant by production of identical copies (clones), and excludes the death of the initial plant in the course of biotechnological manipulations as *in vitro*, as occurs in adaptation *ex vitro* and at the following cultivation *in vivo*.

To improve efficiency of using a hybrid as a promising rootstock in production in the future, it is necessary to improve disease resistance, primarily viral. For this purpose, as the most appropriate way is a dry-air thermotherapy in combination with culture of isolated apexes, we designed an original device for dry-heat thermotherapy (Podorozhniy, 2012). During culture of apexes, in combination of accelerated clonal micropropagation, a variety of specific reaction of explants represented in complex hybrids grown in physical conditions of cultivation *in vitro* demands constant improvement of the process that is regularly replicated. Firstly, this concerns optimisation of nutritive media at different stages of cultivation, correction of their growth medium and biologically active substances, as well as other factors influencing the maximum production of micro plants of optimal quality. We developed a patented method for successful adaptation of micro plants *in vitro* (Podorozhniy and Mayorova, 2011).

As a consequence, the complex of biotechnological methods of new clonal rootstocks applied in their sampling, propagation and subsequent introduction into practice promotes the acceleration of the selection process to 2–3 years and allows us to introduce new rootstocks into practice in 5–6 years.

CONCLUSIONS

Use of genotypes from the gene pool of *Prunus* L. allowed to create some clonal rootstocks for stone fruit crops characterised by adaptability, easy vegetative propagation and weak vigour on Krymsk Experimental Breeding Station (EBS). These rootstocks are valuable for use in intensive technologies of stone fruit crop cultivation.

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2. New clonal rootstocks of Krymsk EBS selection propagate well by methods of clonal micro-reproduction and green cuttings, and the majority of them by woody cuttings. The best of them are capable to propagate by all methods: Kuban 86, Evrika 99, Best, Zarevo, VSL-1, VSL-2, and RVL-9.

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PRUNUS L. ĢINTS ĢENĒTISKĀS DAUDZVEIDĪBAS IZMANTOŠANA KAULEŅKOKU KLONU POTCELMU SELEKCIJĀ UN TO PAVAIROŠANAS ĪPATNĪBAS

Izmantojot *Prunus* L. ģints savvaļas sugu — *P. cerasifera, P. armeniaca, P. persica, P. fruticosa, P. lannesiana, P. maackii, P. tomentosa, P. pumila, P. incana* daudzveidību, Krimskas selekcijas izmēģinājumu stacijā selekcionēti labi adaptēti vidēja vai vāja auguma kauleņkoku klonu potcelmi ar labu saderību ar potētajām šķirnēm. Plūmēm, aprikozēm un persikiem tie ir Kuban 86, VVA-1, Evrika 99, Zarevo (Glow), Alab 1, Speaker, Best, VSV-1; saldajiem un skābajiem ķiršiem – L-2, LC-52, VSL-1, VSL-2 un RVL-9. Daļa potcelmu pārbaudīti un tiek veiksmīgi izmantoti dažādās Krievijas daļās kā arī dažās tuvākās un tālākās ārvalstīs. Selekcijas procesā integrētās *in vitro* audu kultūras ievērojami veicinājušas jauno potcelmu ātrāku ieviešanu globālā ražošanā. Izstrādātas tehnoloģijas mikroklonālai pavairošanai, kā arī pavairošanai, izmantojot zaļos un koksnainos spraudeņus. Potcelmiem Kuban 86, Evrika 99, Zarevo, Best, VSL-1, RVL-9 (50–80%) pētījumos atklātā vieglā apsakņošanās ļāvusi izstrādāt tehnoloģiju stādu iegūšanai uz šiem potcelmiem jau kokaudzētavas pirmajā laukā.