

The Role Of Inter-Species Hybridization In Expanding The Sortiment Of Plum

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Abstract—As a result of hybridization of species: *Prunus domestica* L., *Prunus salicina* Lindl., *Prunus rossica* Erem., *Prunus brigantina* Vill., *Prunus persica* (L.) Batsch., *Prunus armeniaca* Lin., *Prunus pumila* L., *Prunus spinosa* L. crossed between Interspecific hybrids combining genomes of 2 or more species ((*Prunus rossica* Erem. × *Prunus domestica* L.) × ((*Prunus pumila* L. × *Prunus salicina* Lindl.) × *Prunus spinosa* L.), *Prunus domestica* L. × (*Prunus brigantina* Vill. × *Prunus persica* (L.) Batsch.)). Depending on the combination groups, without using the *in vitro* method of introducing isolated hybrid embryos into the culture, the percentage of surviving seeds varies from 0% to 15.4%.

Introduction to the culture of *in vitro* isolated hybrid embryos on Murashige and Skoog medium with the inclusion of thidiazuron in the medium at a concentration of 1.0 mg / l for 20-23 days after pollination allows to save them from death and increase the frequency of development of adventitious shoots to 34.5 ± 4.2 % The application of this method allowed to obtain varieties ‘Tulitsa’ and ‘Velichavaya’.

Keywords— *Prunus*, hybridization, interspecific hybrids, isolated nuclei, cultivars

I. INTRODUCTION

The modern basis of the plum assortment is varieties of home hexaploid plum (*Prunus domestica* L.) and Japanese diploid plum (*P. salicina* Lindl) as well as their hybrids with other species of the genus *Prunus* L. [1]. To date, more than 30 species are actively involved in the breeding process. Many of which are natural spontaneous hybrids. [1-2].

The basis of selection work is the creation of industrial varieties of various types of consumption. New species should be self-fertile, stably fruiting, high-yielding and highly adaptive in relation to climatic indicators, cultivation zones resistant to pathogens, pests and viruses, and also comply with new intensive cultivation technologies [3].

To solve this problem, remote hybridization is of particular importance, since certain species involved in the crossing process have unique characteristics.

Hybridization of *P. cerasifera* Ehrh has been successfully used in the selection of diploid plums. and its hybrids F1 from crossing with *P. salicina*, isolated in a separate type of plum - Russian plum (*P. rossica* Erem.) [4]

In the selection of *P. domestica* for winter hardiness and overgrownness, *P. spinosa* L. is used [5]. In the first generation, hybrids have 40 chromosomes and low fecundity. Upon their repeated crossing with plum, the home part of F₂ hybrids have 48 chromosomes and normal fertility. The fruits of hybrids are close in size and quality to the varieties of *P. domestica* and have high winter hardiness.

The United States, in particular Zaiger'sInc Genetics, is the undisputed world leader in the achievements of interspecific plum hybridization. Over the past 4 decades, they have obtained groups of complex hybrids involving species *P. salicina*, *P. persica* var. *nucipersica* (Borkh.) C.K. Schneid., *P. persica* (L.) Batsch., *P. armeniaca* Lin., *P. cerasifera* named Aprium®, Pluot®, NectaPlum®, Peacotum®, Color-Cot™, Pluerry™. The names are patented at the federal level as trademarks, Aprium®, Pluot®, NectaPlum® - are used as industrial cultivars in the USA and Australia [1].

Despite the presence of certain achievements in the selection of plums with the participation of various species, there is a number of main problems, of which are the low survival rate of hybrid seeds as a result of impaired fertilization and embryogenesis, which leads to the death of the embryo and the optimal selection of parental pairs to obtain interspecific hybrids with unique properties.

The aim of the study is to identify successful combinations of crosses of plum species in the conditions of the Central region of Russia, to determine the optimal environment for the cultivation of isolated hybrid embryos and to obtain interspecific hybrids - cultivars.

II. EXPERIMENTAL

Studies are observed in the conditions of the Central region of Russia. The objects of study are species and interspecific hybrids: *P. domestica*, *P. salicina*, *P. rossica*, *P. spinosa*, *P. brigantina* Vill., *P. persica*, *P. armeniaca*, *P. pumila* L., Species were represented by characteristics of cultivars and selected forms: *P. domestica* – ‘Stanley’, ‘Aleksiy’, ‘Renkloda Ulana’, ‘Pamyat Satarovoy’, ‘Utro’, ‘Smolinka’, ‘Sukhanovskaya’, ‘Skorospelka krasnaya’, ‘Izyum Erik’, ‘Opal’, ‘Yakhontovaya’, ‘CV 17’, ‘Naroch’; *P. salicina* – ‘Black Gold’, ‘Skoroplodnaya’; *P. rossica* – ‘7-1-10 PP’, ‘Mara’, ‘Kubanskaya Kometa’, ‘Rannyaya

Rozovaya'; *P. spinosa* – '6/24'; *P. armeniaca* – 'Feeriya', 'Poputchik', 'Professor Smykov', 'Gamlet', 'Iskorka Tavridy', 'Yuzhneda', 'Chistenkiy'; *P. rossica* × *P. domestica* – 'Velichavaya'; *P. pumila* × *P. salicina* – 'GES 37-210'; (*P. pumila* × *P. salicina*) × *P. spinosa* – 'GPT'; *P. brigantina* × *P. persica* – variety F₁. Hybridization was carried out annually from 1992 to 2019 in late April - early May, depending on the weather conditions of the year. Pollen was isolated from flowers, in the state of a loose bud, plants grown under the conditions of the study, and also obtained from Nikitsky Botanical Gardens – National Scientific Center of the RAS ('Feeriya', 'Poputchik', 'Professor Smykov', 'Gamlet', 'Iskorka Tavridy', 'Yuzhneda', 'Chistenkiy', 'Izyum Erik', 'Renkloda Ulena', 'Black Gold').

Pollen viability was determined by the acetocarmine method [6]. An analysis of the selection of parental couples is presented for the period from 2017 to 2019.

The cultivation of isolated hybrid embryos was carried out on Murashige and Skoog culture medium [7] with the addition of preparations with cytokinin activity (6-benzylaminopurine 0.5-1.0 mg / l, kinetin 0.5-1.0 mg / l, thidiazuron - 0.05-2.0 mg / l).

Varietal study of interspecific hybrids was carried out in accordance with the "Program and methodology for selection of fruit, berry and nut crops" [8].

The results were processed statistically using the Statistica 6.0 (StatSoft, Russia) software package.

III. RESULTS AND DISCUSSION

In the period from 2017 to 2019, we conducted pollination of 14,223 flowers in 44 cross combinations shown in Table I. The pollen viability of all paternal forms involved in hybridization was not lower than 94%. Each combination was selected taking into account the dominant traits of the genotypes and the compilation of the offspring model. In all the selected combinations, one of the parental forms was represented by a variety or selected hybrid adapted to the research conditions. With the involvement in the hybridological process of 3 or more species ((*P. rossica* × *P. domestica*) × ((*P. pumila* × *P. salicina*) × *P. spinosa*), *P. domestica* × (*P. brigantina* × *P. persica*), (*P. rossica* × *P. domestica*) × *P. domestica* had 18% of their success combinations, except for the group (*P. rossica* × *P. domestica*) × *P. domestica* (11.3%) ranged from 0% to 1.3%.

The proportion of hybrid seeds obtained without using the method of introducing into the culture in vitro isolated hybrid embryos varies from 0 % (*P. salicina* × (*P. brigantina* × *P. persica*), *P. rossica* × (*P. pumila* × *P. salicina*), *P. spinosa* × *P. rossica*, (*P. rossica* × *P. domestica*) × *P. rossica*, *P. domestica* × *P. rossica*, *P. domestica* × (*P. brigantina* × *P. persica*) to 15.4 % (*P. domestica* × *P. salicina*).

The number of interspecific hybrids depends on the particular genotype involved in hybridization. In the group of *P. domestica* × *P. salicina* combinations consisting of 'CV 17' × 'Black Gold' combinations, the percentage of seeds obtained is 0%, and in the ol 'Smolinka' × 'Black Gold' combination is 35%. The only group where all combinations were successful, that is, seed sets exceeded 0.8%, was *P.*

rossica × *P. salicina*, the maternal form of which was the cultivar 'Kubanskaya kometa'.

The hybridization of 'Kubanskaya kometa' × 'Utro' cultivars with double repetition in 2017 (9.2%) and 2019 (25.3%) revealed the influence of the year conditions on seed set.

TABLE I. THE EFFECTIVENESS OF INTERSPECIFIC PLUM HYBRIDIZATION DEPENDING ON THE COMBINATION OF CROSSES

CROSS COMBINATION GROUPS	TOTAL COMBINATIONS	POLLINATED FLOWERS	NUMBER	
			OVARIES	
			PC.	%
<i>PRUNUS DOMESTICA</i> L. × <i>PRUNUS SALICINA</i> LINDL.	2	182	28	15.4
<i>PRUNUS DOMESTICA</i> L. × <i>PRUNUS ROSSICA</i> EREM.	1	131	0	0
<i>PRUNUS DOMESTICA</i> L. × <i>PRUNUS ARMENIACA</i> LIN.	3	420	16	3.8
<i>PRUNUS DOMESTICA</i> L. × (<i>PRUNUS BRIGANTINA</i> VILL. × <i>PRUNUS PERSICA</i> (L.) BATSCH.)	1	114	0	0
<i>PRUNUS SALICINA</i> LINDL. × <i>PRUNUS DOMESTICA</i> L.	2	444	62	14.0
<i>PRUNUS SALICINA</i> LINDL. × <i>PRUNUS ROSSICA</i> EREM.	2	470	38	8.1
<i>PRUNUS SALICINA</i> LINDL. × <i>PRUNUS ARMENIACA</i> LIN.	2	615	31	5.0
<i>PRUNUS SALICINA</i> LINDL. × (<i>PRUNUS BRIGANTINA</i> VILL. × <i>PRUNUS PERSICA</i> (L.) BATSCH.)	1	362	0	0
<i>PRUNUS ROSSICA</i> EREM. × <i>PRUNUS DOMESTICA</i> L.	8	4030	493	12.2
<i>PRUNUS ROSSICA</i> EREM. × <i>PRUNUS SALICINA</i> LINDL.	2	1894	14	0.74
<i>PRUNUS ROSSICA</i> EREM. × <i>PRUNUS ARMENIACA</i> LIN.	7	2195	42	1.9
<i>PRUNUS ROSSICA</i> EREM. × (<i>PRUNUS PUMILA</i> L. × <i>PRUNUS SALICINA</i> LINDL.)	1	367	0	0
(<i>PRUNUS ROSSICA</i> EREM. × <i>PRUNUS DOMESTICA</i> L.) × ((<i>PRUNUS PUMILA</i> L. × <i>PRUNUS SALICINA</i> LINDL.) × <i>PRUNUS SPINOSA</i> L.)	1	616	8	1.3
<i>PRUNUS SPINOSA</i> L. × <i>PRUNUS DOMESTICA</i> L.	4	1102	5	0.5
<i>PRUNUS SPINOSA</i> L. × <i>PRUNUS ROSSICA</i> EREM.	2	583	0	0
<i>PRUNUS SPINOSA</i> L. × (<i>PRUNUS ROSSICA</i> EREM. × <i>PRUNUS DOMESTICA</i> L.)	1	148	1	0.7
(<i>PRUNUS ROSSICA</i> EREM. × <i>PRUNUS DOMESTICA</i> L.) × <i>PRUNUS DOMESTICA</i> L.	3	407	46	11.3
(<i>PRUNUS ROSSICA</i> EREM. × <i>PRUNUS DOMESTICA</i> L.) × <i>PRUNUS ROSSICA</i> EREM.	1	143	0	0

In 64.5%, the low survival rate of hybrid seeds is caused by impaired fertilization and embryogenesis (the rest is due to damage by insects and other factors). The biotechnological method of in vitro cultivation of isolated embryos allows to increase the survival of hybrid seeds by more than 30% (Table II) depending on the composition of the cultivation medium.

A study of the effect of BAP, kinetin, and thidiazuron preparations in different concentrations on the development of adventitious shoots in cotyledons of embryos of interspecific hybrids of *P. rossica* × *P. domestica* taken from the fruit 20–23 days after pollination revealed an increase in the frequency of shoot development from $2.2 \pm 0.1\%$ (kinetin 0.5 mg / l), up to $34.5 \pm 4.2\%$ (thidiazuron 1.0 mg / l).

As a result of the cultivation of cotyledons of embryos of hybrids *P. rossica* × *P. domestica* ('Kubanskaya kometa' × 'Naroch') on a culture medium, tetraploid cultivars 'Tulitsa' and 'Velichavaya' were studied, studied in the Moscow region of the Central region of Russia. Below is a brief description of them:

'Tulitsa'. Authors of the cultivar: V.S. Simonov, S.N. Kulimekov. V.A. Vysotsky, Somo-sterile medium-ripening cultivar. The tree is medium-sized, fast-growing, with a raised, oval crown, of medium density. Fruits weighing up to 40 g, on average weight is 28 g, oval-elongated. The skin color is purple-red, the pulp is orange, dense, juicy, with a harmonious, unusual combination of the taste of the parent forms.

The stone is small, half lagging behind the pulp. The leaves are obovate in size, shape and venation have an intermediate shape between the leaves of *P. domestica* and *P. rossica*. The internodes and buds are as short and small as in *P. rossica*. Highly winter resistant. Resistance to diseases is average.

TABLE II. THE EFFECT OF VARIOUS DRUGS WITH CYTOKININ ACTIVITY ON THE DEVELOPMENT OF ADVENTITIOUS SHOOTS IN COTYLEDONS OF THE EMBRYOS OF INTERSPECIFIC HYBRIDS *PRUNUS ROSSICA* EREM. × *PRUNUS DOMESTICA* L.

Drug, concentration mg / l	The frequency of development of adventitious shoots, %
BAP* 0.5	6.5±0.4
BAP 1.0	9.2±0.8
KINETIN 0.5	2.2±0.1
KINETIN 1.0	2.5±0.2
TDZ** 0.01	5.5±0.3
TDZ 0.05	10.4±0.9
TDZ 0.1	15.0±3.0
TDZ 0.2	22.2±2.8
TDZ 0.5	25.6±3.2
TDZ 1.0	34.5±4.2
TDZ 2.0	28.6±3.8

BAP* - 6-Benzylaminopurine, TDZ** - thidiazuron

'Velichavaya'. Authors of the cultivar: V.S. Simonov, S.N. Kulimekov. V.A. Vysotsky. Somo-sterile cultivar of late-ripening. The tree is tall, fast-growing, with an oval crown, of medium density. The fruits are large, weighing up to 55 g, an average weight is 37 g with excellent taste. The skin color is dark purple, almost black. Subcutaneous points are hardly noticeable. Peel of medium thickness, glabrous, with a medium waxy coating. The pulp is orange, medium density, juicy, fibrous. The stone is medium, The stone is medium, half lagging behind the pulp. Winter hardiness is high, disease resistance is average. The features of *P. domestica* in this form prevail over the features of *P. rossica*.

The hybrid origin of the 'Tulitsa' and 'Velichavaya' cultivars was exposed by DNA analysis using primers (Paw S 5, Paw S 6, Paw S 11, Paw S 16, Paw S 17) flanking moderately repeating R 173 family of retrotransposons [9].

IV. CONCLUSION

Thus, it was found that the success of interspecific plum hybridization depends on the specific genotype involved in hybridization and the conditions of the year. The most successful crossbreeding group was *P. domestica* × *P. salicina* (15.4%). When 3 or more species are involved in hybridization, its success is from 0 % to 1,3%, except group (*P. rossica* × *P. domestica*) × *P. domestica* (11.3 %). The group where all combinations were successful was the group *P. rossica* × *P. salicina*.

The most successful interspecific combination was a cross combination 'Smolinka' × 'Black Gold'.

The hybridization of 'Kubanskaya kometa' × 'Utro' varieties with double repetition in 2017 and 2019 revealed the influence of the year conditions on seed set.

A study of the effect of drugs with cytokinin activity on the development of adventitious shoots in cotyledons of embryos of interspecific hybrids in various concentrations showed that thidiazuron 1.0 mg / l is most successful when added to the medium.

The use of the in vitro cultivation method of isolated embryos made it possible to preserve hybrids from which cultivars 'Tulitsa' и 'Velichavaya' were obtained.

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