Increasing resistance to coccomycosis in sweet cherry by applying distant hybridization with cherry species

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Abstract. To reduce the pesticide load in industrial plantings, it is necessary to use pathogen-resistant varieties when establishing orchards. The problem of obtaining stable, large-fruited, adaptive varieties is acute, since there are practically no genotypes in the existing assortment that combine a complex of economically valuable traits with resistance to diseases. The isolation of new resistance genes in wild forms and the creation of genotypes resistant to fungal diseases on their basis is possible through the use of remote hybridization. Interspecific hybrids with sweet cherry were obtained at the Krymsk EBS, VIR Branch, which combine resistance to coccomycosis and increased fruit size. These genotypes are complex sources of traits for further breeding work in obtaining adaptive, large-fruited genotypes. The work carried out proves that the use of interspecific hybridization makes it possible to obtain genomes highly resistant to coccomycosis.

1 Introduction

At the present stage of society's development, the problem of agricultural production ecologization is acute. Reducing the pesticide load in industrial plantings is the most important direction in crop production. As consequence, there is a need to introduce varieties highly resistant to coccomycosis into production. To create them, it is necessary to isolate new resistance genes in wild forms and include such genotypes in the breeding process. For cost-effective, modern intensive gardening, varieties are needed that combine high productivity with sufficient adaptability to environmental conditions, have high taste and commercial qualities of fruits, in the existing assortment of almost similar genotypes. On the basis of remote hybridization using Far Eastern cherries due to the variety of stable forms available among them, a series of new economically valuable adaptive varieties of cherry and sweet cherry in the middle zone of Russia was obtained by I.V. Michurin, A.N. Venyaminov, H.K Enikeev, A.F. Kolesnikova, et al. [1]. Nevertheless, their use in the south of Russia is not effective due to low adaptability to growing conditions caused by the insufficiently high quality of fruits.

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Coccomycosis (*Coccomyces* hiemalis Higg.) is one of the most dangerous fungal pathogens in cherry and sweet cherry. Despite the increasing number of cherry varieties in the world, its assortment does not meet the production requirements [2]. It is impossible to create such varieties without using the entire genetic potential of stone fruit plants as a source material [3].

A huge work on the formation and study of the gene pool of stone plants was carried out at the All-Russian Scientific Research Institute of Plant Growing n.a. N.I. Vavilov, and in the plantings of the Krymsk EBS, VIR Branch, more than 5,000 samples are preserved and studied, of which 800 are cherry and sweet cherry phenotypes [4, 5, 6, 2].

Interspecific hybrids were created by remote hybridization, which plays a significant role in the origin of most fruit crops and the emergence of their varietal variation [7]. Genotypes with a complex of valuable traits, one of which is resistance to disease, which is of decisive importance in their spread in industrial plantations, are created with remote hybridization [8].

In the south of Russia, a new secondary center of formation in fruit crops has emerged in environmental conditions unusual for them. Hybridogenic forms and especially varieties obtained under such conditions from interspecific hybridization are easier to adapt to new conditions that contribute to the emergence of new positive transgressions for the most important traits [5].

One of the significant reasons for the constant reduction in the production of sweet cherry fruits, and especially cherry, is the widespread increase in the harmfulness of fungal diseases, the main of which is coccomycosis. In some years, the progression of the disease can contribute to great damage in the production of sweet cherry fruits [9, 7].

2 Materials and methods of research

The tests were carried out in 2014-2021 at the Krymsk EBS, VIR Branch (city of Krymsk). The objects of research were 12 combinations of crossing of 1927 hybrid forms obtained as a result of interspecific hybridization. The test area is located in a temperate continental climate, the soils are gray forest. During the research period, weather conditions were not the same, there were years with both low and high precipitation – 2014, 2016, 2018 and 2021, which contributed to the development of fungal diseases. When assessing the resistance of forms to fungal diseases, a 6-point accounting scale was used [10, 11]. Statistical processing of experimental data was carried out by methods of deviation from the mean in the variant, analysis of variance using SNEDERCOR application programs [12].

3 Results of the study and their discussion

A plant affected by fungal diseases impairs growth, loses its production value [13,14]. Disease-resistant varieties are an important part of an integrated protection system. For this reason, our attention was drawn to the East Asian cherry species of the genus *Prunus* L., to forms that are poorly susceptible to the disease. Wild forms of cherry count 18 species in the genetic fund of the Krymsk EBS, including 192 genotypes, which are mostly collected during expeditions to the Far East, mainly in the coastal areas of Primorsky Region, on the islands of Sakhalin, Kunashir, Iturup, etc. are characterized by high resistance to fungal diseases [7,14]. Many researchers (Eremin G.V., Tsarenko V.P., et al.) speak about highly resistant to coccomycosis specimens of the species *P. maximowiczii* Pupr., *P.lannesiana* Carr., *P.incisa* Thunb. Studies conducted at the Krymsk EBS, VIR Branch in the 80s in the immunity laboratory prove that with artificial infection, all samples are affected by this disease to a small extent [6,15].

To increase the effectiveness of the breeding program for the creation of new genotypes of the studied crops, an in-depth study of the sweet cherry gene pool, various types of cherry, and the isolation of gene carriers from it, both donors and sources of economically valuable traits, plays an important role. The most significant new cherry species for the breeding process are actively involved in the breeding process.

In the gene pool concentrated at the station, genotypes have been established that provide adaptability to extreme weather anomalies and large-fruited progeny (Table 1).

The gene pool of fruit plants, collected and concentrated at the Crimean experimental breeding station of the VIR branch, passed the primary study of biology, phenology, morphology, processability. As a result, the best carriers of selectively significant traits of their determinancy and features of transmission to progeny were identified.

The breeding programs use not randomly taken genotypes, but sources studied over many years and distinguished by the selected trait or verified donors.

High resistance to fungal diseases was noted in the following species – *Prunus kurilensis* Miyabe *(VIR catalog No.:39689* - 7-10, *39673* – Goryachiy Klyuch 3, *39662* – Vetrovoye 4)*, Prunus maackii* Rupr. (*42820 – V*ladivostok 4, *42844* – Lazovoe 8, *42842* – DVOS No. 1), *Prunus maximowiczii* Rupr. (*39710* – Ricorda No. 4, *39721* – Sakhalin 2/84, *39716* – Khmelnitsky *3), Prunus sachalinensis* Kom. (*39736* – BG 30, *39771* – No. 5, *39734* – Edwin Muler No. 3), *Prunus incisa* Thunb. (*39648* – №3), *Prunus serrulata* Lindl. (*39786* – №21, *39788* – № 26, *39793* – Kanzan, *39789* – Asagi)*, Prunus lannesiana* Carr. (*39696* – No.1 and *39700* – No.2).

Table 1. Cherry species in which genotypes have been identified as sources of positive traits for breeding use (Krymsk EBS, VIR Branch, 1990-2000)

Cherry species	Early flowering	Late flowering	Weak strength of growth	Resistance to coccomycosis	Resistance to moniliosis	Lack of bitterness in fruits	Short dormant period	Long dormant period
P.canescens			+			+		
P.fruticosa		+	+			+		+
P.incisa			+	+		+		
P.kurilensis			+					
P.lannesiana				+		+		
P.maackii		+			+			+
P.mahaleb		+						+
P.maximowiczii		+						+
P.nipponica	+						+	
P.pensylvanica		+			+	+		+
P.pseudocerasus	+					+	+	
P.sachalinensis	+			+	+	+		
P.serrulata	+			+	+	+	+	

Genotypes – sources of the absence of bitterness in fruits combining resistance to coccomycosis were identified: *P. sachalinensis (*BG 30, 1/75, 1-207), *P. incisa* (from the GDR), *P. canescens* (18/18), *P. fruticosa* (most samples), *P. lannesiana (No. 1 and No. 2), P. pensylvanica* L. (1-24-4), *P. pseudocerasus* Lindl., *P. serrulata* (No. 26, No. 12).

The sources used in practical breeding pass into the category of donors of breeding-valuable traits with established inheritance of the desired, they are involved in intergenerational and interspecific hybridization [8]. In the breeding plantings of the station, hybrids of sweet cherry varieties with the participation of these species are grown.

Some species have a very short dormancy period and overwintering weather conditions are not always favorable for breeding work. The years 2012 and 2013 were successful for the formation of female gametophytes during the flowering period.

Inheritance of the trait is mostly on the maternal side, therefore, cherry species showing resistance were taken as such forms, the paternal form was the sweet cherry varieties established as donors of the large-fruited trait – *26087* - Valery Chkalov and *24795* – Krupnoplodnaya (Table 2).

Table 2. Compatibility of sweet cherry varieties (2n = 2x = 16) with distant cherry species [1]

Hybrid combination	Number of pollinated flowers, pcs	Ovary formed			Seeds received			Seedlings grown			Crossing productivity index
		number	% of pollinated flowers	arcsin √%	number	% of pollinated flowers	arcsin √%	number	% of pollinated flowers	arcsin √%	
Valery Chkalov											
P.kurilensis ×	1758	704	40,05	39,20	459	26,11	30,74	87	18,95	25,82	4,94
P.sachalinensis ×	937	307	32,76	34,81	257	27,42	31,56	119	46,30	42,45	12,7
P.pensylvanica ×	10000	350	3,50	10,66	312	3,12	10,3	18	5,76	13,80	0,18
P.incisa ×	2300	783	34,04	35,07	680	29,56	32,94	312	45,88	42,65	13,56
LSD_{05}				6,38			1,15			12,94	
Krupnoplodnaya											
P.lannesiana ×	5000	900	18,00	25,15	870	17,40	24,64	54	6,20	14,11	1,08
P.serrulata ×	12500	2700	21,60	27,62	2650	21,20	28,91	876	33,05	35,07	7,00
(*P.incisa* x **V. Chkalov**) ×	2200	1200	54,54	47,58	1097	49,86	44,36	361	32,90	35,03	16,40
(*P.lannesiana* × **Krupnoplodnaya**) ×	340	115	33,82	35,77	113	33,23	35,23	24	21,23	24,93	7,05
(*P.serrulata* × **Krupnoplodnaya**) ×	970	231	23,81	29,20	210	21,64	27,65	76	36,19	36,95	7,83
LSD_{05}				4,37			8,17			9,52	
All combinations	∑ 36005	∑ 7290	$\bar{x}$ 29,12	$\bar{x}$ 32,60	∑6648	$\bar{x}$ 25,50	$\bar{x}$ 30,54	∑1927	$\bar{x}$ 27,38	$\bar{x}$ 31,59	$\bar{x}$ 7,86
All hybrid combinations (partial differences)		LSD_{05} = 5,38 h^2 = 22,33%			LSD_{05} = 4,66 h^2 = 53,59%			LSD_{05} = 7.48 h^2 = 75,03%			
Depending on paternal form		LSD_{05} = 2,69 h^2 = 58,07%			LSD_{05} = 2,33 h^2 = 32,62%			LSD_{05} = 3,74 h^2 = 13,59%			

Different types have different amounts of fruit setting. The species: *P. incisa, P. kurilensis, P. sachalinensis*, and *P. serrulata are easily crossed and give a high yield of hybrid pit.* After a month, the average number of ovaries was below 35%, which corresponds to the level of intervariety crossings in sweet cherry, but during the development part of the ovary fell off, and the number of mature hybrid fruits averaged up to 25%.

The production of hybrids in the species *P. pensylvanica* with sweet cherry of the Valery Chkalov variety, even in favorable weather conditions, is very weak, less than 4%.

About 2000 viable seedlings were grown in all combinations, the average productivity of the selected crossings was 7.86, the highest crossbreeding rates in the species *P. incisa* × Valery Chkalov – 13.56 and with (saturating) backcrossing of this species with sweet cherry (*P. incisa* × V. Chkalov) × Krupnoplodnaya – 16.40.

It is noted that with backcrossings, the percentage of obtaining breeding fruits increases in the species *P. lannesiana* to 49%.

The weather conditions of the growing season in some years contributed to the development of fungal diseases. Studying the resistance of genotypes to coccomycosis, records of the incidence in a natural provocative background were carried out. The records were carried out in two periods: in July, when the development of spotting is strong, and necrotic damage and chlorosis of the leaves are still insignificant, and in September – at the time of the manifestation of the full harmfulness of these diseases, accompanied by mass defoliation (Table 3).

Analyzing a hybrid combination in which the maternal form was genotype *39810* – 1-24-4, characterized by a sweet taste of fruits and resistance to coccomycosis of the species *P. pensylvanica*, and as a paternal one, the sweet cherry variety *26087* was taken – Valery Chkalov – a donor of large-fruitness, out of 10,000 pollinated flowers, in the fourth week after flowering, the useful ovary was up to 3.5%. Only 18 hybrid forms were preserved to the productive state, of which four samples were affected by coccomycosis by 1-2 points (on a 5-point scale). Also, 20% of hybrids turned out to be infertile, and another 20% were not productive having single fruits.

The resulting sweet cherry hybrids with the species *P. serrulata* in 75% of the breeding material inherit high disease resistance in the first generation. In terms of taste, seedlings mostly have a slight bitterness, but when saturating crossings with large-fruited sweet cherry varieties in the second generation, 90% of the tested hybrids acquire an increased fruit size - up to 4-4.5 g, nevertheless, disease resistance is lost in more than 50% of seedlings.

Table 3. Interspecific hybrids, their resistance to coccomycosis, %

Hybrid combination	Number of seedlings, pcs	Seedling resistance to coccomycosis, %					
		1-2 points	arcsin √ %	3 points	arcsin √ %	4-5 points	arcsin √ %
P.kurilensis × *P.avium*	87	78	62.0	8	16,4	14	22.1
P.sachalinensis × *P.avium*	119	97	80,0	2	8,1	1	5.7
P.pensylvanica × *P.avium*	18	75	60.0	12	20,3	13	21,1
P.incisa × *P.avium* × (*P.fruticosa* × *P.lannesiana*)	61	100	90.0	-	-	-	-
P.incisa × *P.avium*	312	98	81.9	2	8.1	-	-
P.lannesiana × *P.avium*	54	75	60.0	19	25,8	6	14,2
P.serrulata × *P. avium*	876	88	69.7	10	18,4	2	8.1
(*P.lannesiana* × *P.avium*) × Krupnoplodnaya	24	56	48.4	24	29,3	20	26.6
(*P.incisa* × *P.avium*) × Krupnoplodnaya	361	78	62,0	13	21,1	9	17,5
(*P.serrulata* × *P. avium*) × Krupnoplodnaya	76	50	45,0	34	35,7	16	23,6

P.fruticosa × *P.canescens*	54	99	84,3	1	5,7	-	-
P.fruticosa × *P.serrulata*	38	87	68.9	12	20,3	1	5,7
LSD 05			2.99		1,61		2.05

Among the studied samples of various hybrid families, high resistance to disease is shown by *P. incisa* obtained from crossing as the maternal form. Nevertheless, most hybrids had bitter fruits, with saturating crossings with sweet cherry, the taste qualities of fruits and their size improve, but resistance to disease drops sharply, less than 50% of seedlings show partial resistance up to 1-2 points in epiphytotic years.

It is noted that in the first generation, most combinations (sweet cherry with different species) inherit many disease-resistant interspecific hybrid seedlings.

Cherry species *P. sachalinensis, P. kurilensis, P. pensylvanica*, and *P. incisa* transmit small fruiting to progeny in the first generation. Forms with very small fruits are marked from 70 to 100% of phenotypes.

Table 4. The degree of fruiting and the weight of fruits in distant hybrids

Hybrid combination	Number of seedlings, pcs	% of seedlings with a score fruiting						Quantity seedlings, %					
								Fruit weight, g			Fruit taste		
		0	1	2	3	4	5	0,5-1,0	1,1-2,8	2,9-4,5	Sweet	Bitter	Light bitter
P.kurilensis × *P.avium* (Valery Chkalov)	87	2,2	20,7	34,5	42,5	-	-	100	0	0	4	96	0
P.sachalinensis × *P.avium* (Valery Chkalov)	119	5,9	10,1	6,8	71,4	2,5	3,4	78	22	0	7	87	6
P.pensylvanica × *P.avium* (Valery Chkalov)	18	22,2	-	22,2	55,6	-	-	72	22	6	0	100	0
P.incisa × *P.avium* × (*P.fruticosa* × *P.lannesiana)*	61	27,9	-	4,9	11,8	11,8	44,2	70	25	5	4	67	29
P.incisa × *P.avium*	312	-	-	23,7	38,1	16,9	21,2	78	22	0	87	2	11
P.lannesiana × *P.avium*	54	-	-	-	22,2	25,9	51,9	54	46	0	50	48	2
P.serrulata × *P. avium*	876	-	8,9	25,9	35,3	7,6	22,3	0	86	14	53	17	30
(P.lannesiana × *P.avium)* × Krupnoplodnaya	24	-	-	-	33,3	16,7	50,0	0	0	100	98	0	2
(P.incisa × *P.avium)* × Krupnoplodnaya	361	-	-	8,6	51,8	26,0	13,6	0	51	49	100	0	0
(P.serrrulata × *P. avium)* × Krupnoplodnaya	76	-	-	15,8	76,3	7,9	-	0	0	100	98	0	2
P.fruticosa × *P.canescens*	54	12,9	5,6	14,8	53,7	9,3	3,7	12	80	8	100	0	0
P.fruticosa × *P.serrulata*	38	7,9	18,4	10,5	39,5	21,0	2,6	14	78	8	100	0	0
LSD 05							2,54			4,31			3,18

There is an increase in the size of the fetus during backcrossings, an important role is played using large-fruited donors as a paternal form.

Hybrid samples obtained by crossings of *P.kurilensis P.pensylvanica*× *P.avium* – 2 forms*, P.sachalinensis* × *P.avium* – 7*, P.pensylvanica* × *P.avium* – 4*, P.incisa* × *P.avium)* × *(P.fruticosa* × *P.lannesiana)* – 17*, P.fruticosa* × *P.canescens* – 7*,* and *P.fruticosa* × *P.serrulata* – 3 with abundant flowering, do not set fruits, and if they are set, they fall off after three weeks, being not fertile.

4 Conclusions

The practical application of interspecific hybridization has shown that this method makes it possible to obtain genotypes highly resistant to coccomycosis. Large-fruited donors involved in breeding in the second generation contribute to this trait transmission. Thus, the genotypes obtained from crossing of the species *P.kurilensis, P.sachalinensis, P.canescens, P.incisa*, and *P.serrullata inherit resistance to coccomycosis well.* Nevertheless, *P.kurilensis* and *P.sachalinensis* species have bitter fruits in 90% of progeny. 3 forms of *P.kurilensis* × *P.avium* and 8 genotypes of *P.sachalinensis* × *P.avium were isolated without bitterness.* The sweet taste of fruits is inherited by genotypes from the crossing of *P.canescens, P.incisa*, and *P.fruticosa* species in 80-100% of cases. At the same time, resistance to coccomycosis remains.

In case of saturating crossings with large-fruited genotypes, there is a tendency to enlargement, while bitterness is not inherited, but stability decreases.

The selected genotypes will be used in further backcrossings as complex sources of the desired traits.

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