

DOI: 10.31676/0235-2591-2020-3-11-17

Признаки и свойства гибридов мандарина, полученных от отдаленных скрещиваний (*Citrus* var. *unshiu* × *Poncirus trifoliata*)

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Резюме. Среди цитрусовых культур в субтропической зоне Черноморского побережья Краснодарского края можно выращивать только мандарины (*Citrus reticulata* Blanco var. *unshiu* Tan.). Расширению насаждений препятствует их слабая зимостойкость, поэтому перед селекционерами стоит задача создания новых форм мандарина, максимально приспособленных к экстремальным климатическим условиям региона. Создание таких форм возможно с использованием отдаленной гибридизации. Целенаправленная отдаленная гибридизация позволяет передавать свойства одного рода или вида другому, что приводит к фенотипическому и генотипическому изменению в гибридном потомстве. В статье подчеркивается важность отдаленных скрещиваний. Указано, что в 1995-2019 гг. для создания нового гибридного фонда проведены скрещивания отдаленные, насыщающие и межгибридные. Представлены результаты от 19 комбинаций скрещивания с использованием *Poncirus trifoliata* Raf. и гибридов, которые получены с участием данного рода, а также сородичей цитрусовых: *C. × insitum*, *C. × citrangequat* и рода *Fortunella* Sw. Из данных комбинаций скрещиваний отобраны перспективные формы с ранним сроком созревания плодов (I декада октября): ЮМР-97; ЮКР-22; ККР-27; ККР-22; МРКР-97-25; МРКР-97-25-1; КРМР-25-97, с более поздним созреванием (II декада октября): КВИ-1, КВИ-3, ЮИ-12. Эти формы получены с участием *C. insitum*. Также выделены гибриды с поздним сроком созревания: КРФ-25; КРФ-22; МРФ-97; ЦКР-25; ЮЦ-8 – от комбинаций с участием *Fortunella margarita* и *C. × citrangequat* в качестве опылителей. От насыщающих и межгибридных скрещиваний выделены перспективные формы мандарина с положительными признаками, такими как раннеспелость, средняя масса плодов, высокая дегустационная оценка. Наибольшую селекционную ценность представляют гибриды, полученные с участием самого морозостойкого сородича цитрусовых *Poncirus trifoliata*, однако в этих семьях отобрано от 5,0 % до 6,25 % перспективных образцов. Все формы имеют селекционную ценность для дальнейшей работы по созданию зимостойких сортов мандарина.

Ключевые слова: цитрусовые, мандарин, селекция, отдаленные скрещивания, гибриды, признаки, свойства, зимостойкость

Features and properties of tangerine hybrids derived from distant hybridization (*Citrus* var. *unshiu* × *Poncirus trifoliata*)

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Abstract. Among citrus crops in the subtropical zone of the of the Russian Federation Black Sea coast of Krasnodar Territory, only tangerines can be grown (*Citrus reticulata* Blanco var. *unshiu* Tan.). The expansion of planting is hindered by poor winter hardiness, that's why breeders are faced with a task of creating new forms of mandarin that are maximally adapted to the extreme climatic conditions of the region. By using of a distant hybridisation it is possible to create such forms. Purposeful distant hybridization allows transferring the properties of one genus to another, which is leading to phenotypic and genotypic changes in hybrid generation. The article emphasizes the importance of distant hybridization. For the period 1995-2019, distant, recurrent and inter-hybrid hybridizations were carried out to create a new hybrid fund. The results of 19 combinations of crossing by using *Poncirus trifoliata* Raf., and hybrids which were obtained with the participation of this genus, as well

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Образец цитирования:

Кулян Р.В. Признаки и свойства гибридов мандарина, полученных от отдаленных скрещиваний (*Citrus* var. *unshiu* × *Poncirus trifoliata*). Садоводство и виноградарство. 2020;3:11-17
doi: 10.31676/0235-2591-2020-3-11-17
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For citation:

Kulyan R. V. Features and properties of tangerine hybrids derived from distant hybridization (*Citrus* var. *unshiu* × *Poncirus trifoliata*). Sadovodstvo i vinogradarstvo. 2020;3:11-17
doi: 10.31676/0235-2591-2020-3-11-17

as hybrid species: *C. × insitorum*, *C. × citrangeqwa* and the genus *Fortunella* Sw. Expanded forms were selected for further breeding with an early term of fruit ripening (I decade of October): YuMR-97; YuKR-22; KKR-27; KKR-22; MRKR-97-25; MRKR-97-25-1; KRMR-25-97, with later ripening (II decade of October: KVI-1, KVI-3, YuI-12 received with *C. insitorum*. Also with late maturity hybrids were revealed: KPF-25; KPF-22; MPF-97; TsKR-25; YuTs-8 with the immixture of *Fortunella margarita* and *C. citrangeqwa*. From recurrent and inter-hybrid hybridization were marked out promising forms of mandarin with positive features such as early ripening, average fruit mass and a high tasting assessment. The greatest breeding value is represented by hybrids derived with the immixture of the most frost-resistant congeners of the citrus *Poncirus trifoliata*, however, from 5.0 % to 6.25 % of promising samples were selected in these families. All forms have breeding value for further work on creating winter hardiness varieties of mandarin.

Keywords: citrus fruits, mandarin, breeding, distant hybridization, hybrids, features, properties, winter hardiness

Introduction

The humid subtropical zone of the Krasnodar Territory is favorable for the cultivation of heat-loving subtropical crops, including citrus plants. However, Russia's subtropics represent the northernmost limit of the citrus growing range. Consequently, cultivating them in this zone entails specific characteristics that must be taken into account during breeding work.

A limiting factor for citrus cultivation on Russia's Black Sea coast is the plants' low tolerance for freezing temperatures. Plantings suffer significant damage from recurring winters characterized by prolonged periods of low temperatures. On average, a severe winter occurs every 8–10 years, during which freezing temperatures cause plant mortality [1]. Consequently, the primary focus of citrus breeding—particularly for mandarins—is the development of varieties and forms resistant to extreme cold during dormancy, as well as low-growing plants that mature early and produce fruit of high commercial and gustatory quality.

This can be achieved through distant hybridization. Incorporating wild and semi-wild relatives—such as *P. trifoliata* Raf., *C. × citrangequat*, *C. × insitorum*, as well as *C. ichangensis* Sw. and *Fortunella* Sw.—into breeding programs makes it possible to develop forms that are more winter-hardy and resistant to biotic and abiotic environmental factors [2–5]. These relatives possess unique traits such as high winter hardiness, a deciduous habit, and a deep dormancy period.

The ability to utilize the gene pool of wild relatives and distant hybrids held in the collections of research institutions and botanical gardens is of great importance for addressing complex breeding challenges [6–8]. Distant hybridization enables the transfer of beneficial traits and resistance genes to hybrid forms [9]. Thus, the aim of this study is to develop winter-hardy, low-growing, and early-maturing mandarin cultivars with enhanced adaptability to the extreme conditions of the Black Sea coast subtropics.

Research Materials and Methods

Mandarin seedlings derived from 19 intergeneric crosses, as well as selected promising hybrids, were used as research material. The studies were conducted...

Results and Discussion

Given the low winter hardiness of citrus crops and the climatic conditions of the subtropical zone of the Krasnodar Territory, a priority task for breeders is to find ways and develop methods to enhance this trait.

Addressing this issue involves navigating the complex biological characteristics of citrus and other evergreen plants. They lack a clearly defined dormancy period and exhibit multiple growth flushes. The continuity of physiological and biochemical processes is incompatible with frost resistance [13, 14]. During winter, when temperatures exceed +10°C, citrus plants—particularly those in the orange group—begin to show signs of vegetative activity, which can lead to serious damage. Consequently, intergeneric hybridization is of great interest, as related species possess a pronounced winter dormancy period and limited growth flushes (one or two), with shoot maturation concluding by October. In distant hybridization, gene recombination between genera or species has induced significant genotypic changes. This has created opportunities to develop new forms that combine traits and properties from various species—such as enhanced winter hardiness, immunity to diseases and pests, high yield, early maturity, and compact growth habits. Thus, the challenge of winter hardiness can be addressed through distant hybridization—specifically by incorporating *Poncirus trifoliata* Raf. and previously developed *Poncirus*-derived intergeneric hybrids into the breeding program. Such crosses offer the potential to produce new mandarin cultivar types with superior adaptability compared to existing varieties, a crucial factor for the continued development of citrus cultivation in the humid subtropical zone of the Krasnodar Territory.

Between 1995 and 2019, 53 crossing combinations were performed; 19 of these involved *P. trifoliata* Raf. and hybrids derived from that genus. Cultivars of *Citrus var. unshiu* were used as the maternal parent. Crosses involving *Fortunella* Sw. were also carried out.

As part of the mandarin breeding program to develop new, high-yielding, early-maturing forms resistant to environmental stressors—and, above all, possessing enhanced winter hardiness—the following types of crosses were performed using (*Citrus var. unshiu*): distant hybridization, backcrossing, and inter-hybrid crossing. The use of the wild relative *Poncirus trifoliata* Raf. in hybridization makes it possible to produce hybrids with high winter hardiness. Backcrossing and inter-hybrid crossing lead to reduced winter hardiness but improved fruit quality. A number of complex hybrids have been obtained between-

-*Fortunella* Sw., *C. × citrangequat*, *C. × insitorum*, and previously developed distantly related forms. These hybrids are characterized by deep winter dormancy and fruits without bitter essential oil.

The juvenile period of citrus crops is significantly longer than that of other fruit plants, lasting 15-20 years, which creates certain difficulties in breeding.

The influence of the studied factor "crossing combination" on the variability of the "number of seedlings" trait was determined (Table 1).

Таблица 1. Количество отборов, полученных в результате целенаправленных скрещиваний (1995-2019 гг.)

Table 1. The number of selections obtained as a result of targeted crossing (1995-2019)

Cross-breeding combination	Quantity of 2–5-year-old seedlings	Number of samples	Selected forms, %
Distant crosses			
Kowano-Wase × <i>P. trifoliata</i>	32	2	6,25
Miyagawa Wase × <i>P. trifoliata</i>	31	2	6,4
Пионер-80 × <i>P. trifoliata</i>	30	2	6,6
Юбилейный × <i>P. trifoliata</i>	20	1	5,0
$\eta^2=96,39$	96,34 %* ($F_{\phi}=46,82>F_{\Gamma}=18,10$)		94,93 %* ($F_{\phi}=32,78>F_{\Gamma}=18,10$)
Back-crosses			
Kowano-Wase × KP-22 Kowano-	34	4	11,8
Wase × KP-27 Kowano-Wase ×	18	4	23,1
MP-97 Miyagawa Wase × KP-27	22	3	13,6
Miyagawa Wase × <i>C. × insitorum</i>	18	2	11,1
Юбилейный × KP-22	38	4	10,7
Юбилейный × MP-97	9	2	22,2
Юбилейный × <i>C. × citrangequat</i>	11	2	18,2
Юбилейный × <i>C. × insitorum</i>	22	3	15,8
KP-25 × <i>C. × citrangequat</i>	19	3	13,6
$\eta^2=98,61\%$	15	4	26,6
	98,61 %* ($F_{\phi}=157,53<F_{\Gamma}=439,26$)		99,9 %* ($F_{\phi}=2180,4>F_{\Gamma}=439,26$)
Interhybrid crosses			
MP-97× KP-25	19	3	15,8
KP-25 × MP-97	27	3	11,1
KP-25 × <i>Fortunella margarita</i>	27	2	7,4
KP-22 × <i>Fortunella margarita</i>	28	2	7,4
MP-97× <i>Fortunella margarita</i>	27	2	7,4
$\eta^2=98,67\%$	98,66 %* ($F_{\phi}=184,67<F_{\Gamma}=329,22$)		98,81 %* ($F_{\phi}=1310,5>F_{\Gamma}=329,22$)

Примечание: η^2 – вклад в общую дисперсию, или сила влияния фактора, %; F – критерий Фишера; *значения p-критерия, превышающие стандартные для 1 %-ного уровня значимости (**p<01**)

Remark: η^2 is the contribution in the general dispersion or the power of factor influence, F is the Fisher criterion; *p-test values exceeding standard for one percent significance level (**p<01**)

Примечание. KP-22; KP-27; KP-25 ('Kowano-Wase' × *P. trifoliata*); MP-97 ('Miyagawa Wase' × *P. trifoliata*); *C. × citrangequat* (*P. trifoliata* × *C. sinensis*) × *Fortunella*; *C. × insitorum* (*P. trifoliata* × *C. sinensis*)

Remark. KP-22; KP-27; KP-25 ('Kowano-Wase' × *P. trifoliata*); MP-97 ('Miyagawa Wase' × *P. trifoliata*); *C. × citrangequat* (*P. trifoliata* × *C. sinensis*) × *Fortunella*; *C. × insitorum* (*P. trifoliata* × *C. sinensis*)

At the same time, the share of influence for each of the three types of the selected combinations amounted to 96.34% (distant crosses), 98.61% (backcrosses), and 98.66% (inter-hybrid crosses), respectively. A similar pattern was observed regarding the influence of this factor on the variability of the "selected forms" trait; the contribution to variance was 94.93%, 99.9%, and 98.81%, respectively. It should be noted that the "combination" factor had a significant effect on the variability of the "number of seedlings" and "percentage of selected forms" traits (at $P < 0.01$). Overall, the factor under study exerted a significant effect—though in some instances a non-significant one (specifically, on the "number of seedlings" in distant crosses and backcrosses)—on the variability of all traits analyzed via one-way analysis of variance.

Hybrids derived from *Poncirus trifoliata*—the most frost-hardy species in the entire Rutaceae family—hold the greatest breeding value. However, only 5.0% to 6.25% of the specimens in these progenies were selected as promising. For crosses involving *Fortunella margarita*, the figure was around 7.4%. The highest number of forms were identified from backcross and inter-hybrid combinations.

For the selection of cold-hardy mandarin seedlings observations were conducted on the growth and development of hybrids derived from distant crosses. The sexual progeny are fully viable and exhibit high genetic and phenotypic variability, with the traits and characteristics of wild relatives predominating; consequently, traits associated with domestication are recessive in first-generation hybrids. Hybrids obtained using *P. trifoliata* possess enhanced winter hardiness (-15 to -16°C), a trait dominant in *P. trifoliata* [5, 15].

The fruit quality of the resulting hybrids is poor due to the accumulation of a bitter essential oil (ponciridin) in the juice vesicles. However, some hybrids show traits leaning towards cultivated varieties; they are semi-deciduous and produce fully edible fruit. Morphological characteristics of the leaf blade show segregation, resulting in the formation of three-, two-, and one-foliolate leaves (Fig. 1)—a trait characteristic of the mandarin. Such hybrids are few in number but are of great importance for future breeding aimed at cold hardiness.



Рис. 1. Морфологические изменения листовой пластинки при гибридизации с участием *Poncirus trifoliata*

Fig. 1. Morphological changes in the leaf blade during hybridization with *Poncirus trifoliata*

As a result of the study of hybrid plants were selected forms promising for further breeding. Their characteristics are presented in Table 2. The following forms are characterized by early fruit ripening (first ten days of October): YUMR-97, YUKR-22, KKR-27, KKR-22, MRKR-97-25, MRKR-97-25-1, and KRM-25-97. They possess a standard average fruit weight (60–80 g) and a high tasting score. Selections derived from inter-hybrid crosses inherit the traits of the cultivated species, yet morphological characteristics of the wild relative (numerous spines, bifoliate leaves) sometimes appear.

Hybrids with later ripening (second ten-day period of October)—KVI-1, KVI-3, and YuI-12—have been identified; these were obtained-

-using *C. insitorum* as the pollinator. Plants derived from combinations using *C. citrangequat* and *Fortunella margarita* as the pollen parents exhibited the latest ripening times (third ten-day period of November). These same hybrids are remountant.

In the humid subtropics of the Krasnodar Territory, mandarin plants suffer from low winter temperatures in some years. However, the hybrids selected from intergeneric crosses are semi-deciduous and have a single growth wave, making them better prepared for winter. The fruits are multi-seeded and of low quality. All selected forms are recommended for inclusion in the saturation.

Таблица 2. Биологические особенности развития гибридных растений

Table 2. Biological peculiarities of the development of hybrid plants

Form Number	Maternal Form ♀	Paternal Form ♂	Fruit Ripening	Fruit Mass	Deciduousness	Tasting Score Points
KBP-4	Kowano-Wase	<i>P. trifoliata</i>	<i>P</i>	<i>C</i>	<i>Л</i>	2
KBP-3	Kowano-Wase	<i>P. trifoliata</i>	<i>C</i>	<i>K</i>	<i>П</i>	2
MBP-1	Miyagawa Wase	<i>P. trifoliata</i>	<i>P</i>	<i>C</i>	<i>Л</i>	2
MBP-2	Miyagawa Wase	<i>P. trifoliata</i>	<i>C</i>	<i>K</i>	<i>П</i>	2
КВИ-1	Kowano-Wase	<i>C. insitorum</i>	<i>C</i>	<i>K</i>	<i>П</i>	2
КВИ-3	Kowano-Wase	<i>C. insitorum</i>	<i>C</i>	<i>K</i>	<i>П</i>	2
ПР-1	Пионер-80	<i>P. trifoliata</i>	<i>C</i>	<i>C</i>	<i>П</i>	2
ЮР-6	Юбилейный	<i>P. trifoliata</i>	<i>P</i>	<i>K</i>	<i>Л</i>	2
ЮЦ-8	Юбилейный	<i>C. citrangequat</i>	<i>П</i>	<i>М</i>	<i>П</i>	4
ЮИ-12	Юбилейный	<i>C. insitorum</i>	<i>C</i>	<i>K</i>	<i>П</i>	2
KKP-22	Kowano-Wase	KP-22	<i>P</i>	<i>C</i>	<i>В</i>	5
KKP-27	Kowano-Wase	KP-27	<i>C</i>	<i>C</i>	<i>В</i>	5
KMP-97	Kowano-Wase	MP-97	<i>C</i>	<i>C</i>	<i>В</i>	5
KMP-97-1	Kowano-Wase	MP-97	<i>C</i>	<i>C</i>	<i>В</i>	5
MKP-27-1	Miyagawa Wase	KP-27	<i>C</i>	<i>C</i>	<i>В</i>	5
ЮМР-97	Юбилейный	MP-97	<i>P</i>	<i>C</i>	<i>В</i>	5
ЮКР-22	Юбилейный	KP-22	<i>P</i>	<i>C</i>	<i>В</i>	5
ЦКР-25	KP-25	<i>C. citrangequat</i>	<i>П</i>	<i>М</i>	<i>П</i>	4
МРКР-97-25	MP-97	KP-25	<i>P</i>	<i>C</i>	<i>В</i>	5
МРКР-97-25-1	MP-97	KP-25	<i>P</i>	<i>C</i>	<i>В</i>	5
КРМР-25-97	KP-25	MP-97	<i>P</i>	<i>C</i>	<i>В</i>	5
КРМР-25-97-1	KP-25	MP-97	<i>C</i>	<i>C</i>	<i>В</i>	5
KPF-25	KP-25	<i>Fortunella margarita</i>	<i>П</i>	<i>М</i>	<i>В</i>	3
KPF-22	KP-22	<i>Fortunella margarita</i>	<i>П</i>	<i>М</i>	<i>В</i>	3
МРФ-97	MP-97	<i>Fortunella margarita</i>	<i>П</i>	<i>М</i>	<i>В</i>	3

Примечание: П – полулистопадные; В – вечнозелёные; Л – листопадные

Срок созревания: Р – ранний (I декада октября); С – средний (II декада октября); П – поздний (III декада ноября); К – крупные плоды (80-90 г); С – средние (60-80 г); М – мелкие (15-55 г)

Remark: П – semi-deciduous, В – evergreen, Л – deciduous

Ripening period: Р – early (the first decade of October), С – medium (the second decade of October), П – late (the third decade of November); К – large fruits (80-90 g), С – medium (60-80 g), М – small (15-55 g)

Hybrids obtained with the participation of forms of *Fortunella margarita* possess enhanced winter hardiness—though this is reduced due to their everbearing nature—and produce small fruits (weighing 15–40 g) with a tasting score of 3 points; the fruit flavor lacks the bitterness associated with ponciridin, a compound characteristic of *P. trifoliata*. Hybrids with edible fruit—KRF-25, KRF-22, and MRF-97—were obtained from the cross combinations (KR-25 × *Fortunella margarita*), (KR-22 × *Fortunella margarita*), and (MR-97 × *Fortunella margarita*).



Рис. 2. Гибрид ЮЦ-8
Fig. 2. Hybrid YuTs-8

Hybrids derived from *C. citrangequat* produce high-quality fruit; a promising form, Yuts-8 (Fig. 3), was identified with a tasting score of 4 points. However, the fruit lack uniformity in weight and size and contain a large number of seeds. Hybrids resulting from this combination represent the best material for developing new mandarin cultivars resistant to extreme growing conditions.

Conclusions

1. From crossing combinations using the wild species *P. trifoliata*, the hybrid species-

This demonstrates that the *Fortunella* genome suppresses the genes of *P. trifoliata* and inhibits the formation of ponciridin. Similar studies confirm these findings [16–18].

The semi-deciduous nature of the leaves indicates that it was possible to modify the assimilation apparatus. The leaf carries out physiological and biochemical processes that induce the plants to enter a state of dormancy. This trait is observed in the following forms: KVR-3, MVR-2, KVI-1, KVI-3, PR-1, and YuI-12 (Fig. 2). However, it should be noted that the fruits of these forms are of poor quality, receiving a tasting score of 2 points.



Рис. 3. Гибрид МВР-2
Fig. 3. Hybrid MBP-2

-*C. × insitorum*, and *C. × citrangequat* as pollinators, forms were selected that exhibit semi-deciduousness and early maturity, though they produce fruit of low quality. These hybrids are of interest for further breeding aimed at cold hardiness.

2. The majority of mandarin-type hybrids—characterized by early maturity and high fruit quality—were selected from inter-hybrid and backcrosses. These forms constitute valuable breeding material for the development of new mandarin cultivars that are better adapted to stressful growing conditions.

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Поступила: 20.03.2020

Отправлена на доработку: 17.04.2020

Принята к печати: 16.06.2020

Received: 20.03.2020

Revision received: 17.04.2020

Accepted: 16.06.2020

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