

# Wpływ wybranych form rodzicielskich na zawiązywanie owoców i nasion w hybrydyzacji oddalonej trzech gatunków drzew owocowych z rodzaju *Prunus* (śliwa japońska, morela, ałycza)

Influence of selected parental forms on fruit and seed set in distant hybridization of three fruit tree species of the genus *Prunus* (Japanese plum, apricot, myrobalan plum)

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Celem badań była ocena efektywności hybrydyzacji oddalonej wybranych genotypów drzew owocowych należących do trzech gatunków z rodzaju *Prunus* – śliwa japońska (*P. salicina* Lindl.), morela (*P. armeniaca* L.) i ałycza (*P. cerasifera* Ehrh.). Program hybrydyzacji wykonano w wysokim tunelu foliowym w Sadzie Pomologicznym Instytutu Ogrodnictwa w Skierniewicach, wiosną 2019 r. Do badań użyto 8 genotypów rodzicielskich, tj. morela – 4 genotypy ('Early Orange', 'Harcot', 'Sirena', 'Somo'), śliwa japońska – 3 genotypy ('Czarnuszka', 'Trumlar', 'D 17–73') oraz ałycza – 1 genotyp ('Amelia'). Program hybrydyzacji wykonano w układzie: śliwa japońska × morela – 12 kombinacji krzyżowań, śliwa japońska × ałycza – 3 kombinacje krzyżowań. Uzyskane wyniki pokazały, że wpływ genotypu śliwy japońskiej jako formy matecznej jest większy niż moreli jako formy ojcowskiej na zawiązywanie owoców w hybrydyzacji oddalonej tych gatunków. Śliwa japońska 'Czarnuszka' znacznie lepiej zawiązywała owoce niż 'Trumlar' po zapyleniu pyłkiem tych samych form ojcowskich moreli. W dojrzałych owocach znajdowały się zarówno nasiona wykształcone prawidłowo (żywotne), jak i takie, które miały pomarszczoną okrywą nasienną i w różnym stopniu zniekształcone zarodki (nieżywotne). Udział owoców z niewykształconymi nasionami był różny i zależał od krzyżowanych gatunków oraz od genotypu formy matecznej. W kombinacjach krzyżowań śliwa japońska × ałycza uzyskano więcej wykształconych nasion niż w kombinacjach śliwa japońska × morela, w stosunku do całkowitej liczby uzyskanych nasion (pestek). Obumieranie zarodków i nieprawidłowe wykształcenie otrzymanych nasion wskazuje na istnienie postzygotycznych barier krzyżowalności w krzyżowaniu śliwy japońskiej, moreli i ałyczy.

**Słowa kluczowe:** ałycza, krzyżowanie międzygatunkowe, morela, *Prunus*, śliwa japońska

**The aim of the study** was to evaluate the efficiency of distant hybridization of selected fruit tree varieties in three species of the genus *Prunus* – Japanese plum (*P. salicina* Lindl.), apricot (*P. armeniaca* L.) and myrobalan plum (*P. cerasifera* Ehrh.). The hybridization programme was carried out in a high plastic tunnel in the Pomological Orchard of the Research Institute of Horticulture in Skierniewice, in the spring of 2019. Eight parental genotypes, i.e. apricot – 4 genotypes ('Early Orange', 'Harcot', 'Sirena', 'Somo'), Japanese plum – 3 genotypes ('Czarnuszka', 'Trumlar', 'D 17–73') and myrobalan plum – 1 genotype ('Amelia') were used for the study. The hybridization programme was carried out while following the system of Japanese plum × apricot – 12 cross combinations, Japanese plum × myrobalan plum – 3 cross combinations. The obtained results showed that the influence of the Japanese plum genotype as a maternal form was greater than that of apricot as a paternal form on fruit-setting in the distant hybridization of these species. Furthermore, Japanese plum 'Czarnuszka' set fruit much better than 'Trumlar' after pollination with pollen of the same paternal forms of apricot. The ripe fruit also contained both properly developed seeds (viable), as well as those that had a wrinkled seed coat and malformed embryos (non-viable). The proportion of fruits with underdeveloped seeds varied and depended on the crossed species and on the genotype of the maternal form. In the crossing of Japanese plum × myrobalan plum, less underdeveloped seeds were obtained in relation to the total number of seeds (stones) obtained than in the crossing of Japanese plum × apricot. Embryo mortality and abnormal seed formation indicate the existence of post-zygotic barriers to crossability in the crossing of Japanese plum, apricot and myrobalan plum.

**Keywords:** apricot, interspecific crossing, Japanese plum, myrobalan plum, *Prunus*,

## Introduction

Crossing plants from genetically distant species (distant hybridization) allows for the creation of new genotypes characterized by innovative

traits, such as increased resistance/tolerance to biotic and abiotic factors, lower plant vigour or improved fruit quality. This method is used especially when a given plant species is lacking

a source of genes that determine the desired traits, or their expression is insufficient (Layne and Sherman, 1986).

Distant hybridization is also used in the breeding of fruit plants, including fruit trees from the genus *Prunus* (Layne and Sherman, 1986, Duval et al., 1994; Hakoda et al., 1998). However, very few fruit, compared to the number of all pollinated flowers, are obtained from distant hybridization within the genus *Prunus* species (Yoshida et al., 1975; Jun and Chung, 2007). The low efficiency of distant hybridization is caused by the presence of numerous morphological, anatomical and physiological-biochemical barriers (Zenktele, 1990). These barriers prevent fertilization and embryo formation (prezygotic barriers) or disturb embryo development (postzygotic barriers) (Perez and More, 1985; Rubio-Cabates, Socias and Company, 1996; Liu et al., 2007).

The literature on obtaining of interspecific hybrids of fruit trees within the genus *Prunus* is not extensive, but there are some reports on hybrids of Japanese plum (*P. salicina* Lindl.), myrobalan plum (*P. cerasifera* Ehrh.), or their crosses with apricot (*P. armeniaca* L.) which are called plumcots (Okie, 2005). Backcrossing of (Japanese plum × apricot) × Japanese plum has led to the creation of hybrids called the pluot. Examples of such hybrids are ‘Flavor Fall’ and ‘Flavorich’ cultivars, created and cultivated in a warm Californian climate (Top et al., 2012).

The lack of apricot and Japanese plum cultivars well adapted to the cooler temperate zone is one of the most important problems in the cultivation of these species in the countries of Central and Northern Europe, including Poland. The trees of these species are not sufficiently resistant to sub-zero temperatures in winter, and their flower buds are often damaged by frost (Szabó and Nyeki, 1994; Szabó, 2003; Yao, 2011; Szymajda et al., 2013). Distant hybridization may allow for the transfer of genes determining greater resistance to frost in winter from such species as *P. spinosa* (sloe) or *P. cerasifera* to *P. armeniaca* and *P. salicina* (Duval et al., 1994; Layne and Sherman, 1986; Neumüller, 2011). Good examples of such possibilities are the Ukrainian cultivars ‘Kometa’ and ‘Najdiena’, which are hybrids of Japanese plum and myrobalan plum. These cultivars are more tolerant to winter frost than traditional cultivars of Japanese plum.

The aim of the presented study was to evaluate the efficiency of distant hybridization of selected fruit tree varieties from three species of the genus

*Prunus* – Japanese plum, apricot and myrobalan plum.

## Material and Methods

### Study location and plant material

The study was carried out in 2019 at the Department of Horticultural Plant Breeding, the Research Institute of Horticulture in Skierniewice. Eight parental genotypes from the genus *Prunus* were crossed, i.e. 4 genotypes of apricot (‘Early Orange’, ‘Harcot’, ‘Sirena’, ‘Somo’), 3 genotypes of Japanese plum (‘Czarnuszka’, ‘Trumlar’, ‘D 17-73’) and one genotype of myrobalan plum (‘Amelia’).

### Pollination programme

Pollination was performed on trees growing inside a high plastic tunnel in the Orchard of the Research Institute of Horticulture in Skierniewice (Central Poland). Pollination was done on flowers castrated in the phase of white, very swollen but closed buds. Poorly developed flower buds were removed. Directly after castration, pollen of known viability was applied with a brush onto the stigmas of the castrated flowers. Pollination was repeated on the following day. Pollination was carried out in the following system: Japanese plum × apricot – 12 cross combinations, Japanese plum × myrobalan plum – 3 cross combinations.

Before pollination, the germination capacity of pollen grains from the paternal forms of apricot and myrobalan plum was analysed. Pollen was collected from anthers selected from very swollen flower buds (just before their opening). The anthers were dried at room temperature on paper trays for several hours. The dried pollen grains were placed on the artificial medium proposed by Sharafi (2011), composed of saccharose (15%),  $H_3BO_3$  (5 mg L<sup>-1</sup>) and Bactoagar (1%). The tube cell growth was assessed after 20 hours’ incubation of pollen grains in the dark at 24°C. Pollen grains were regarded as viable if the growing pollen tube was at least two-fold longer than the pollen grain diameter (Khan and Perveen, 2008). Pollen viability was assessed for at least 100 pollen grains, randomly selected from a larger sample within each genotype.

### Formation of fruits and seeds

Fruits were counted at the stage of their maturity for harvest and their formation was expressed as a percentage of all pollinated flowers. Stones removed from the ripe fruit were thoroughly cleaned of pulp residues by repeated washing under running water, and endocarps were stripped

by means of table clamps in order to extract seeds. The seeds were divided into two groups – properly developed and malformed. Further assessment (seed germination capacity test) was only done for properly formed seeds. Seed formation was expressed in percent, as the ratio of the total number of seeds (stones) obtained.

### ***Stratification of seeds***

Properly formed seeds were disinfected by soaking for 45–60 minutes in a 0.5% solution of fungicide suspension (Kaptan 50 WP, 50% kaptan) (Arysta Life Science North America Co., San Francisco, USA). Disinfected seeds were mixed with moist stratification medium (perlite), wrapped in plastic bags and stratified in a MIR-554 cool incubator (SANYO, Moriguchi, Japan) at a temperature of approx. 5°C. The seeds from individual combinations were stratified on different dates depending on the date of fruit ripening. The first two seed inspections were performed 30 and 60 days after the start of stratification, and the next ones every 10 days. During these inspections germinating seeds were removed and counted. Seeds with a visible 5–15 mm long radicle were regarded as germinated. Seed stratification was continued until day 160. Seed germination capacity was expressed in percent as the ratio of the total number of seeds subjected to stratification.

## **Results and Discussion**

### ***Evaluation of the effectiveness of distant hybridization***

In the performed 15 combinations of distant crosses, 1047 fruits were obtained, which accounted for 15.2% of all pollinated flowers. The percentage of fruit developed from Japanese plum × apricot and Japanese plum × myrobalan plum cross combinations was similar (15.6% vs 12.1%) (Table 1). Since these were interspecific crosses where different barriers to crossability exist, this result can be considered good. The selection of parental forms made in previous years of the study certainly had a positive effect on the formation of fruit (Szymajda et al. 2015). This study analysed crosses in the direction Japanese plum × apricot and Japanese plum × myrobalan plum instead the opposite direction, and compatible genotypes of Japanese plum with the parental forms of apricot and myrobalan plum. A greater number of fruit produced by the Japanese plum × apricot cross compared to the reciprocal cross was also reported by Yoshida et al. (1975) and Jun and Chung (2007). This shows that Japanese plum is more suitable as a maternal

form than the apricot in the distant hybridization of these species.

Good fruit formation in the Japanese plum × apricot cross could also be attributed to the faster growth of pollen tubes in apricot compared to Japanese plum (Perez and Moore, 1985). In addition, the flowers of Japanese plum and myrobalan plum have a shorter pistil than apricot flowers, and therefore the apricot pollen tube has a shorter distance to reach the ovary than Japanese plum tube in the crossing of these species (Jun and Chung, 2007; Perez and Moore, 1985). These factors, when pollination takes place under unfavourable weather conditions, could have a significant impact on fruit formation.

Fruit formation depended on the maternal form of Japanese plum. Two genotypes of Japanese plum, ‘Czarnuszka’ and D 17–73, formed fruits much better than Japanese plum ‘Trumlar’ considering the average for the paternal forms of apricots used. These results suggest that the genotype of Japanese plum as the maternal form has a stronger impact than that of the apricot paternal form on fruit formation in the distant hybridization of these species. The evaluation of pollen germination capacity on the artificial medium showed that pollen from each paternal form had a high germination capacity. Thus, pollen viability had no effect on the differences in fruit formation in maternal forms of the Japanese plum.

Fruit formation also depended on the crossed parental forms. Japanese plum ‘Czarnuszka’ set fruit very well after pollination with ‘Somo’ apricot pollen; D 17–73 after pollination with ‘Early Orange’ apricot pollen. This indicates different degrees of genetic compatibility of crossed parental forms of Japanese plum and apricot, and their specific combining ability in terms of this trait. Different genetic compatibility of parental forms was also demonstrated for the Japanese plum × myrobalan plum cross. The ‘Trumlar’ genotype formed fruits better than ‘Czarnuszka’ and D 17–73 after pollination with pollen from the ‘Amelia’ myrobalan plum. Arbeola et al. (2006) reported that fruit formation in the crossing of myrobalan plum and apricot also depended on the crossed parental forms and ranged from 0.9% to 18.7%.

Ripe fruits contained both properly formed seeds (viable) and those with a wrinkled seed coat and unformed embryos (non-viable). A total of 752 properly formed hybrid seeds of Japanese plum and apricot or myrobalan plum were obtained from 15 cross combinations, which accounted for 71.8% of all seeds obtained and only 10.9% of all pollinated

flowers. The proportion of fruit with undeveloped seeds was different and depended on the species crossed and on the genotype of the maternal form (Table 1). In the Japanese plum × myrobalan plum cross, the number of formed seeds was higher than that for the Japanese plum × apricot cross in relation to the total number of obtained seeds (stones). Considering maternal forms, Japanese

plum D 17–73 set fruit better than ‘Czarnuszka’ or ‘Trumlar’, regardless of the paternal form used in the cross. Embryo mortality and seed malformation indicate the existence of postzygotic barriers to crossing, as previously observed by other authors in their research (Rubio-Cabates, Socias and Company, 1996; Liu et al., 2007). Overcoming postzygotic barriers and saving at least some

Tabela 1.  
Table 1.

Efektywność hybrydyzacji oddalonych śliwy japońskiej, alyczy i moreli (Skierniewice, 2019 r.)  
Effectiveness of distant hybridization of Japanese plum, apricot and myrobalan plum (Skierniewice, 2019 r.)

Krzyżowane formy rodzicielskie Crossed parental forms ♀ × ♂	Zapylone kwiaty (szt.) Polinated flowers (No.)	Uzyskane owoce Obtained fruit		Wykształcone nasiona Developed seeds		Skiełkowane nasiona Germinated seeds	
		(szt.) (No.)	(%) <sup>z</sup>	(szt.) (No.)	(%) <sup>y</sup>	(szt.) (No.)	(%) <sup>x</sup>
śliwa japońska × morela							
Czarnuszka × Early Orange	720	162	22,5	68	42,0	60	88,2
Czarnuszka × Harkot	730	143	19,6	75	52,4	70	93,3
Czarnuszka × Sirena	200	17	8,5	15	88,2	6	40,0
Czarnuszka × Somo	790	208	26,3	173	83,2	158	91,3
Suma Total	2440	530	21,7	331	62,5	294	88,8
Trumlar × Early Orange	170	18	10,6	9	50,0	9	100,0
Trumlar × Harkot	220	8	3,6	5	62,5	5	100,0
Trumlar × Sirena	110	7	6,4	6	85,7	6	100,0
Trumlar × Somo	85	6	7,1	5	83,3	5	100,0
Suma Total	585	39	6,7	25	64,1	25	100,0
D 17–73 × Early Orange	570	158	27,7	110	69,6	108	98,2
D 17–73 × Harkot	830	82	9,9	63	76,8	60	95,2
D 17–73 × Sirena	750	23	3,1	21	91,3	21	100,0
D 17–73 × Somo	860	109	12,7	104	95,4	104	100,0
Suma Total	3010	372	12,4	298	80,1	293	98,3
Suma Total (śliwa japońska × morela)	6035	941	15,6	654	69,5	612	93,6
śliwa japońska × alycza							
Czarnuszka × Amelia	150	10	6,7	10	100,0	6	60,0
Trumlar × Amelia	133	35	26,3	34	97,1	34	100,0
D 17–73 × Amelia	590	61	10,3	54	88,5	52	96,3
Suma Total (śliwa japońska × alycza)	873	106	12,1	98	92,5	92	93,9
Suma (dla wszystkich kombinacji krzyżowań) Total (for all crosses)	6908	1047	15,2	752	71,8	704	93,6

<sup>z</sup> procent z liczby zapylonych kwiatów

<sup>z</sup> percentage of the number of pollinated flowers

<sup>y</sup> procent z liczby z całkowitej liczby uzyskanych nasion

<sup>y</sup> percentage of the total number of seeds obtained

<sup>x</sup> procent z liczby z liczby nasion zastratyfikowanych

<sup>x</sup> percentage of the total number of seeds subjected to stratification

of the hybrid embryos is possible through the use of the embryo-rescue technique (Golits et al., 2002; Kukharchyk and Kastrickaya, 2006; Liu et al., 2007). In this technique, immature embryos can develop and grow *in vitro*, which is useful in the distant hybridization of plants from the genus *Prunus* (Arbeola et al., 2003).

#### Germination capacity of obtained hybrid seeds

The seeds from *Prunus* species when removed from the fruit cannot germinate because they are in deep physiological dormancy (Suszka 1962, 1967). These seeds do not germinate even when temperature, soil moisture and oxygen supply are suitable (Baskin and Baskin, 2004). This dormancy is overcome during stratification (Szymajda and Żurawicz, 2014; Szymajda et al. 2019b) when seeds are exposed to low temperatures, which degrade chemical compounds called germination inhibitors. Of all 752 seeds stratified in our tests 704 (93.9%) germinated. Although not all seeds germinated, their germination capacity was quite good. It depended more on the genotype of the maternal form than the paternal form. Regardless of the paternal form of apricot or myrobalan plum, the seeds of the Japanese plum 'Trumlar' had a greater germination capacity than the seeds of the Japanese plum 'Czarnuszka'. The influence of the maternal genotype on the germination capacity of seeds of *Prunus* species has also been found in our other studies (Szymajda et al., 2019a; 2019b) and by other authors (Jensen and Kristiansen, 2009; Seliga and Żurawicz, 2011; Souza et al., 2017).

#### Conclusions

1. Traditional distant hybridization of Japanese plum and apricot or myrobalan plum is not very effective and provides a small number of fruit and hybrid seeds in relation to all pollinated flowers.
2. The genotype of Japanese plum used as a maternal form has a stronger influence than the paternal genotype of apricot on fruit formation in the distant hybridization of these species.
3. A large proportion of seeds obtained from distant hybridization of Japanese plum × apricot and Japanese plum × myrobalan plum are malformed (non-viable).
4. Not all properly formed hybrid seeds from the crosses of Japanese plum and apricot or myrobalan plum genotypes are able to germinate, despite long stratification. With a small number of seeds obtained, this creates

a great obstacle to obtaining a larger population of hybrid seedlings from the crossed species.

5. The germination capacity of seeds obtained by crossing Japanese plum × apricot depends on the maternal genotype of Japanese plum.

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