Vol. 42, 2015 (2): 70–82 Hort. Sci. (Prague)

doi: 10.17221/273/2014-HORTSCI

Studies on the interspecific crossing compatibility among three *Prunus* species and their hybrids

M. Szymajda, B. Napiórkowska, M. Korbin, E. Żurawicz

Department of Breeding of Horticultural Crops, Research Institute of Horticulture, Skierniewice, Poland

Abstract

Szymajda M., Napiórkowska B., Korbin M., Żurawicz E. (2015): **Studies on the interspecific crossing compatibility among three *Prunus* species and their hybrids.** Hort. Sci. (Prague), 42: 70–82.

In 2011–2013, a study on the crossing compatibility and the setting of fruit in distant hybridization within the genus *Prunus*, among the species *P. armeniaca* L. (apricot), *P. salicina* Lindl. (Japanese plum), *P. cerasifera* Ehrh. (myrobalan plum) and the hybrids *P. salicina* × *P. cerasifera* was conducted at the Institute of Horticulture in Skierniewice, Poland. The percentage of fruit set depended on the direction of pollination of the crossed species. Most fruits were obtained by crossing *P. salicina* × *P. armeniaca* and *P. salicina* × *P. cerasifera*. The largest number of fruitlets in relation to the number of pollinated flowers was obtained when two genotypes of *P. salicina* – Czernuszka and D 17-73, were the maternal parents. The results show that the crossing compatibility and effectiveness in the cross-breeding of the studied species of *Prunus* are influenced to a greater extent by the genotype of the maternal form of *P. salicina* than by the genotype of the paternal form of *P. armeniaca* and *P. cerasifera*.

Keywords: Prunus; distant hybridization; breeding; pollen germination; fruit set

Distant hybridization allows the transfer of genes between genetically distant species of plants. It is used especially when there are no sources of genes coding for desirable traits within a certain plant species (Layne, Sherman 1986). Distant hybridization makes it possible to obtain hybrid genotypes characterized by new traits such as increased resistance/tolerance to biotic and abiotic stresses, reduced growth vigour, improved fruit quality (attractiveness, shelf-life, flavour), or higher levels of bioactive compounds in the fruit. Interspecific crossing usually results in a very small number of fruits in relation to the number of pollinated flowers (Yosida et al. 1975; Jun, Chung 2007). The reason for the low effectiveness of distant hybridization is the existence of numerous morphological, anatomical, and physiological and biochemical barriers (Zenkteler 1990). These barriers prevent fertilization and the formation of embryo (pre-zygotic barriers) or impair its growth (post-zygotic barriers) (Perez, More 1985; Rubio-Cabates, Socias 1996; Liu et al. 2007). A way to increase the effectiveness of distant hybridization is to introduce into the cross-breeding programme species that are genetically closely related, that is, belonging to the same genus, or even subgenus (Layne, Sherman 1986), and with the same ploidy level and the same number of chromosomes (Okie, Weinberger 1996).

Distant hybridization is also used in the breeding of fruit plants, including the breeding of fruit trees of the genus *Prunus*, and in particular of the subgenus *Prunophora* (plums) (Layne, Sherman 1986;

Supported by the Ministry of Agriculture and Rural Development in 2011–2013, the task No. 110.

doi: 10.17221/273/2014-HORTSCI

Duval et al. 1994; Hakoda et al. 1998). However, obtaining interspecific hybrids within the genus *Prunus* is difficult (Perez, More 1985). The literature on deriving interspecific hybrids in the genus *Prunus* is not very extensive, but there are reports on hybrids of the Japanese plum × apricot, which have been named plumcots (Okie 2005). As a result of backcrossing plumcots and the Japanese plum, hybrids have been obtained and given the name pluots. Examples of such hybrids include the cvs Flavor Fall and Flavorich, derived and cultivated in the warm climate of California (Top et al. 2012).

The lack of apricot and Japanese plum varieties well adapted for growing in the conditions of colder parts of the 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. Trees of these species do not have sufficient resistance to low sub-zero temperatures during winter, and consequently their flower buds often freeze (Szabó, Nyeki 1994; Szabó 2003; Yao 2011; Szymajda et al. 2013). Distant hybridization can enable the transfer of the genes coding for higher winter hardiness, e.g. from the species *P. spinossa* or *P. cerasifera* to the species *P. armeniaca* and *P. salicina* (Layne, Sherman 1986; Duval et al. 1994; Neumüller 2011).

The aim of this study is a preliminary assessment of the possibility and effectiveness of distant hybridization of selected genotypes within the genus *Prunus*, belonging to the species *P. armeniaca* L., *P. salicina* Lindl. and *P. cerasifera* Ehrh.

MATERIAL AND METHODS

Study location and plant material. The study was conducted in 2011–2013 in the Fruit Breeding Department of the Research Institute of Horticulture in Skierniewice, Poland. Interspecific pollination was performed on trees growing in a field in the Experimental Orchard of the Institute in Dąbrowice (central Poland). The cross-breeding programme included 13 genotypes of *P. armeniaca* – Early Orange, Harcot, Somo, Sirena, Kijewskij Krasen, Poleskij Krupnopłodnyj, Pietropawłowskij, Czerniewyj, M I-7, M I-33, M I-69, M II-19, M II-42; 11 genotypes of *P. salicina* – Santa Rosa, Trumlar, Czuk, Czernuszka, D17-73, OSL 57, OSL 58, OSL 59, OSL 60, OSL 65, OSL 69; three genotypes of *P. cerasifera* – Anna, Agata, Amelia and two hybrids of *P. salicina* × *P. cerasifera* – Kometa and Najdiena.

Assessment of pollen viability. Before performing pollination, the viability of the pollen of the paternal forms selected for pollination was assessed. The analyses were conducted for 17 paternal genotypes, i.e. *P. armeniaca* – 12 genotypes, *P. cerasifera* – three genotypes, *P. salicina* × *P. cerasifera* – two genotypes (Table 1). The source of pollen were anthers from heavily swollen flower buds (just before they opened), which were dried on paper trays at room temperature for several hours. Pollen viability was assessed by two methods:

(1) assessing the staining of pollen grains with 2% aceto-orcein (Małuszyńska, Olszewska 1999). Pollen grains were considered viable if the red-stained cytoplasm represented at least 75% of the volume of the grain (Chrząstek et al. 2009),

(2) assessing the growth of pollen tubes on artificial growth media. In this method, two culture media were used:

- sucrose (10%), H_3BO_3 (100 mg/l), $Ca(NO_3)\cdot 4H_2O$ (300 mg/l), $MgSO_4\cdot 7H_2O$ (200 mg/l), KNO_3 (100 mg/l), Bactoagar (0.8%) (Brewbaker, Kwack 1964),
- sucrose (15%), H_3BO_3 (5 mg/l) and Bactoagar (1%) (Sharafi 2011a). Pollen tube growth was assessed after pollen grains had been incubated for 20 h in the dark, at a temperature of 24°C. Grains were considered viable if the length of the pollen tube growing out of them was at least twice the diameter of the grain. Burst grains were regarded as non-viable (Khan, Perveen 2008).

In either method, pollen viability of each genotype was assessed with at least 100 pollen grains taken randomly from a larger sample.

Pollination programme. Emasculation of flowers was performed during the closed white bud stage, using a scalpel. Open and poorly developed flower buds were removed. Immediately after emasculation and again the following day, pollen of known viability was deposited on the stigmata of the flowers with a brush. After the pollination, the branches with pollinated flowers were isolated to prevent uncontrolled cross-pollination by insects. The programme of pollination between the selected parental forms of the studied species was conducted according to the following design: *P. armeniaca* × *P. cerasifera*, *P. armeniaca* × (*P. salicina* × *P. cerasifera*), *P. salicina* × *P. armeniaca*, *P. salicina* × *P. cerasifera*, (*P. salicina*

Table 1. Pollen viability of different genotypes of *P. armeniaca*, *P. cerasifera*, and *P. salicina* × *P. cerasifera*, depending on the assessment method (Skierniewice, 2011–2013)

Genotype	Assessment method								
	staining with aceto-orcein (%)			pollen tube growth					
				Brewbaker and Kwack culture medium (%)			Sharafi culture medium (%)		
	2011	2012	2013	2011	2012	2013	2011	2012	2013
P. armeniaca									
Early Orange	67.0	100.0	96.0	74.0	48.0	60.0	–	49.0	52.0
Harkot	–	100.0	100.0	90.0	52.0	72.0	–	47.0	66.0
Somo	100.0	97.0	99.0	8.0	80.0	88.0	–	73.0	88.0
Sirena	100.0	100.0	98.0	60.0	12.0	68.0	–	24.0	74.0
Kijewskij Krasen	–	98.0	100.0	–	17.0	77.0	–	–	71.0
Poleskij Krupnopłodnyj	–	100.0	100.0	–	58.0	30.0	–	57.0	40.0
Pietropawłowskij	–	100.0	96.0	–	22.0	28.0	–	22.0	24.0
Czerniewyj	–	92.6	99.0	–	20.0	71.0	–	23.0	73.0
M I 7	–	100.0	100.0	–	26.0	79.0	–	33.0	77.0
M I 33	–	100.0	–	–	73.0	–	–	74.0	–
M II 19	–	100.0	100.0	–	73.0	60.0	–	77.0	36.0
M II 42	–	100.0	100.0	–	64.0	26.0	–	62.0	37.0
P. cerasifera									
Anna		100.0	98.0		–	9.0	–	–	5.0
Agata		75.0	97.0		–	1.0	–	18.0	3.0
Amelia		100.0	100.0		16.0	13.0	–	31.0	16.0
P. salicina × *P. cerasifera*									
Kometa	64.0	88.0	–	1.0	0	–	–	0	–
Najdiena	65.0	100.0	–	2.0	0	–	–	0	–

× *P. cerasifera*) × *P. armeniaca*, (*P. salicina* × *P. cerasifera*) × *P. cerasifera, P. cerasifera* × *P. armeniaca*, *P. cerasifera* × (*P. salicina* × *P. cerasifera*). In a few crossing combinations, pollination was carried out with a mixture of pollen from different genotypes of *P. armeniaca*, *P. cerasifera*, and *P. salicina* × *P. cerasifera*. In that way, by performing one pollination combination, the compatibility of one maternal genotype with several paternal genotypes could be assessed. Pollination with pollen mixtures was carried out according to the following design: *P. salicina* × A, B and C, *P. salicina* × D, *P. cerasifera* × A and E, (*P. salicina* × *P. cerasifera*) × D, where A is a mixture of pollen from four genotypes of *P. armeniaca* (Early Orange, Harcot, Somo, Sirena); B – a mixture of pollen from four genotypes of *P. armeniaca* (Kijewskij Krasen, Poleskij Krupnopłodnyj, Pietropawłowskij, Czerniewyj); C – a mixture of pollen from four genotypes of *P. armeniaca* (M I-7, M I-33, M II-19, M II-42); D – a mixture of pollen from three genotypes of *P. cerasifera* (Anna, Agata, Amelia); E – a mixture of pollen from two interspecific hybrids of *P. salicina* × *P. cerasifera* (Kometa and Najdiena). The genotypes that had set fruit after pollination with a mixture of pollen were pollinated the following year with the pollen of each paternal form separately.

Weather history. For the first 12 days of the pollination programme each year, the daily average, min. and max. air temperatures were recorded at a height of approx. 1.8 m above the ground (Fig. 1).

doi: 10.17221/273/2014-HORTSCI

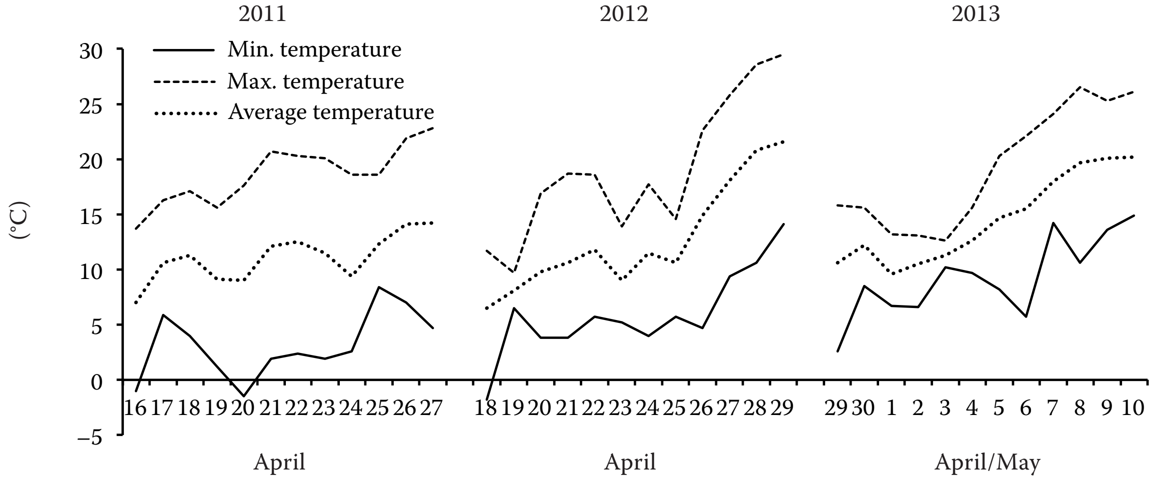

Fig. 1. Daily temperatures (°C) during hand pollination recorded at 1.8 meters above the ground level (pollination programme implementation dates: April 16–20, 2011; April 18–22, 2012; April 29–May 4, 2013)

RESULTS AND DISCUSSION

Pollen viability

The viability of pollen grains of paternal forms varied depending on the assessment method and the genotype being assessed (Table 1). The results of the analysis of pollen tube growth on the Brewbacker and Kwack medium (Fig. 2a) indicate lower pollen viability than the results of the staining of pollen grains with aceto-orcein (Fig. 2b). However, the introduction of the Sharafi (2011a) medium into the analysis in 2012 confirmed that the allegedly lower pollen viability, manifesting itself, for example, in the germination of only 8% of the grains of the genotype *P. armeniaca* Somo (2011 season) compared with 100% viability demonstrated by staining with aceto-orcein, was not caused by an improperly formulated composition of the medium. The results of the pollen germination tests, carried out on the two growth media of different compositions, showed a similar percentage range of germinating grains. On the other hand, it is known that aceto-orcein has the ability to stain the cytoplasm of both mature and immature pollen (Dafni, Firmage 2000). This may lead to an overestimation of the results of pollen viability assessment by this method relative to other methods.

Different numbers of germinating pollen grains depending on the medium used were recorded only in the 2012 season for the genotypes *P. armeniaca* Sirena and *P. cerasifera* Amelia, and in the 2013 season for the genotypes *P. armeniaca* M II-19 and *P. cerasifera* Anna and Agata. Differences in the number

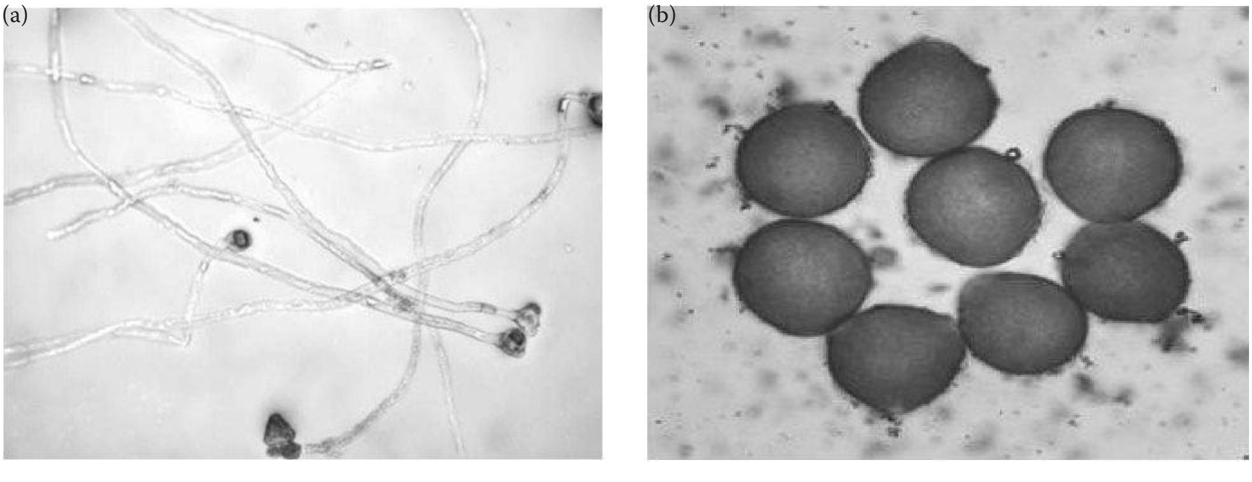

Fig. 2. Pollen grains of *P. armeniaca* Somo: (a) with pollen tubes growing out on the Brewbaker and Kwack medium (Brewbaker, Kwack 1964), (b) stained with aceto-orcein

doi: 10.17221/273/2014-HORTSCI

of germinating pollen grains, depending on the species/variety, were also observed both in the tests carried out in a given year and when comparing the results from successive years. These findings confirm the observations by Sharafi (2011a,b) who, when assessing pollen viability within the genus *Prunus*, revealed variation not only between the species but also between the varieties within a species.

Assessment of fruit set

In the distant crossing combinations, the largest number of fruits was obtained when *P. salicina* served as the maternal parent (Table 2). On average for the three years of the study, the number of fruitlets in relation to the number of pollinated flowers in the crossing combinations *P. salicina* × *P. armeniaca* was 7.2%, and in the combinations *P. salicina* × *P. cerasifera* – 3.5%. When *P. armeniaca* or *P. cera-si-fera* served as the maternal parent, the percentage of fruit set was much lower. After two years of cross-fertilizations in the direction *P. armeniaca* × (*P. salicina* × *P. cerasifera*), the number of fruitlets in relation to the number of pollinated flowers was only 0.3%, while the crossings *P. cerasifera* × (*P. salicina* × *P. cerasifera*) produced no fruit. More fruits in the crossing *P. salicina* × *P. armeniaca* than in the reciprocal crossing were also obtained by Yosida et al. (1975) and Jun and Chung (2007). This confirms the greater usefulness of *P. salicina* compared with *P. armeniaca* as a maternal parent in programmes of distant hybridization of these species.

The reason for the better setting of fruit in the combinations of *P. salicina* × *P. armeniaca* compared with those of *P. armeniaca* × (*P. salicina* × *P. cerasifera*) could have been the ability of pollen tubes of *P. armeniaca* to grow faster than the tubes of *P. salicina* and *P. cerasifera* (Perez, Moore 1985). Moreover, the flowers of *P. salicina* have a shorter pistil than those of *P. armeniaca*, and therefore the pollen tube of *P. armeniaca* has a shorter path to travel to the ovary than the pollen tube of *P. salicina* when crossing these species (Perez, Moore 1985; Jun, Chung 2007). These factors, under the low temperature conditions during pollination, could have had a significant impact on the setting of fruit.

The poor setting of fruit in the crossing combinations of *P. armeniaca* × (*P. salicina* × *P. cerasifera*) and *P. cerasifera* × (*P. salicina* × *P. cerasifera*) could have also been caused by the low germination capacity of the pollen grains of the genotypes Kometa and Najdiena, being the hybrids of *P. salicina* × *P. cerasifera* (Table 1). In addition, the temperature at which pollen tubes germinated under field conditions was lower (Fig. 1) than during the laboratory assessment, which could have been an additional factor inhibiting pollen germination and pollen tube growth. Because the effective pollination period (EPP) in stone fruit trees is 2 to 5 days (Bubán 1996), and pollen tube growth is very slow at temperatures below 10°C (Layne 1983; Bubán 2003; Hedhly et al. 2005), the pollen tubes might not have been able to reach the embryo sacs before their degeneration (Cheung 1996; Sharafi, Bahmani 2011). Hence, low temperatures during pollination could have been the reason for poor setting of fruit.

In the crossing of the genotypes of *P. armeniaca* × *P. cerasifera*, no fruits were obtained. In the reverse crossing combination (*P. cerasifera* × *P. armeniaca*), the number of fruitlets in relation to the number of pollinated flowers was very small – 0.2%. Arbeola et al. (2006) obtained much more fruitlets in the crossing of these species. Depending on the year and the genotypes being crossed, the percentage of fruit set in their trials ranged from 0.9% to 18.7%. The small number of fruits obtained in our study could have resulted from the genetic incompatibility between the genotypes of *P. cerasifera* and *P. armeniaca*. It is possible that by using other genotypes the setting of fruit would be better.

Incompatibility was also observed in the crossing combinations of *P. salicina* × *P. armeniaca* and (*P. salicina* × *P. cerasifera*) × *P. armeniaca*. In 2011, after pollinating the hybrids Najdiena (*P. salicina* × *P. cerasifera*) and *P. salicina* OSL 57 with the pollen of the genotypes of *P. armeniaca* (Early Orange, Harcot, Somo, and Sirena), and *P. salicina* (OSL 59 and OSL 60) with the pollen of the genotypes of *P. armeniaca* (Early Orange and Harcot), no fruits were obtained. However, when two new maternal genotypes of *P. salicina* (Czernuszka and D 17-73) were introduced into the pollination programme, they proved to be compatible with the four genotypes of *P. armeniaca* (Early Orange, Harcot, Somo, and Sirena) and set fruit after being pollinated with the pollen of these genotypes (Table 3). These results confirm that the effectiveness of distant hybridization depends on the compatibility of the genotypes being crossed. The two genotypes of *P. salicina* (Czernuszka and D 17-73) also set fruit after being pollinated with a mixture of pollen from

Table 2. Effectiveness of interspecific hybridization depending on the direction of pollination (Dąbrowice, 2011–2013)

Cross-species	Year	No. of crossing combinations	No. of pollinated flowers	No. of fruits obtained	Fruits from the No. of flowers pollinated (%)	No. of seeds obtained	Seeds from the No. of flowers pollinated (%)	Malformed seeds (%)
P. armeniaca × *P. cerasifera*	2012	6	770	0	0.0	0	0.0	0.0
	2013	6	660	0	0.0	0	0.0	0.0
Total		12	1,430	0	0.0	0	0.0	0.0
P. armeniaca × (*P. salicina* × *P. cerasifera*)	2011	5	525	2	0.4	2	0.8	0.0
	2012	5	257	0	0.0	0	0.0	0.0
Total		10	782	2	0.3	2	0.3	0.0
P. salicina × *P. armeniaca*	2011	13	1,402	17	1.2	8	0.6	52.9
	2012	21	3,545	99	2.8	84	2.4	15.2
	2013	36	7,349	767	10.4	654	8.9	14.7
Total		70	12,296	883	7.2	746	6.1	15.5
P. salicina × *P. cerasifera*	2012	8	1,193	13	1.1	13	1.1	0.0
	2013	8	964	63	6.5	52	5.4	17.5
Total		16	2,157	76	3.5	65	3.0	14.5
(*P. salicina* × *P. cerasifera*) × *P. armeniaca*	2011	4	776	0	0.0	0	0.0	0.0
	2012	4	450	0	0.0	0	0.0	0.0
	2013	4	520	0	0.0	0	0.0	0.0
Total		12	1,746	0	0.0	0	0.0	0.0
(*P. salicina* × *P. cerasifera*) × *P. cerasifera*	2012	1	40	0	0.0	0	0.0	0.0
	2013	1	160	5	3.1	4	2.5	20.0
Total		2	200	5	2.5	4	2.0	20.0
P. cerasifera × *P. armeniaca*	2011	3	777	0	0.0	0	0.0	0.0
	2012	3	535	2	0.4	2	0.4	0.0
	2013	3	490	1	0.2	0	0.0	100.0
Total		9	1,802	3	0.2	2	0.1	33.3

Table 2. to be continued

Cross-species	Year	No. of crossing combinations	No. of pollinated flowers	No. of fruits obtained	Fruits from the No. of flowers pollinated (%)	No. of seeds obtained	Seeds from the No. of flowers pollinated (%)	Malformed seeds (%)
P. cerasifera × (*P. salicina* × *P. cerasifera*)	2011	3	764	0	0.0	0	0.0	0.0
	2012	3	500	0	0.0	0	0.0	0.0
Total		6	1,264	0	0.0	0	0.0	0.0
Total for 2011		28	4,244	19	0.4	10	0.2	47.0
Total for 2012		51	7,290	114	1.6	99	1.4	13.2
Total for 2013		58	10,143	836	8.2	710	7.0	15.1
Total for 2010–2013		137	21,677	969	4.5	819	3.8	15.5

the three genotypes of *P. cerasifera* (Anna, Agata, Amelia). By contrast, the genotypes Najdiena (*P. salicina* × *P. cerasifera*) and *P. salicina* OSL 60 set no fruit after being pollinated with either the pollen of *P. armeniaca* or *P. cerasifera*. This indicates that the influence of the genotype of maternal from of *P. salicina* on the compatibility and effectiveness of distant hybridization of these species is greater than the influence of the paternal form of *P. armeniaca* and *P. cerasifera*.

Fruit and seed development

In many crossing combinations, a large number of fruitlets were shed in the first 2–3 weeks after pollination. The growth of the fruitlets remaining on the trees varied. Those whose growth was very slow usually changed colour from green to yellow-green and were shed between the 4th and 6th week after pollination. The fruitlets that fell off during this period contained browned ovules with no visible embryos, surrounded by a soft endocarp. From the 7th week after pollination, only in some crossing combinations there were fruitlets still remaining on the trees. Some of the fruitlets changed colour from green to yellow-green, ceased to increase in size, and also fell to the ground, but the shedding was not as intense as in the previous weeks. The fruitlets that were shed between the 7th and 10th week after pollination contained browned ovules with small degenerated embryos (Fig. 3), which indicated that they were unviable. From the 7–8th week after pollination, the process of endocarp hardening began in the fruitlets.

Fig. 3. A browned ovule with undeveloped embryo of *P. salicina* D 17-73 in the 9th week after pollination

doi: 10.17221/273/2014-HORTSCI

Table 3. Results of the interspecific crossing combinations (Dąbrowice, 2011–2013)

Cross-combinations	No. of flowers pollinated			No. of fruits obtained			Fruits from the No. of flowers pollinated (%)			No. of seeds obtained			Seeds from the No. of flowers pollinated (%)			Malformed seeds (%)		
	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013
P. armeniaca × *P. cerasifera*																		
M I-7 × Anna	–	150	120	–	0	0	–	0.0	0.0	–	0	0	–	0.0	0.0	–	0.0	0.0
M I-7 × Agata	–	110	140	–	0	0	–	0.0	0.0	–	0	0	–	0.0	0.0	–	0.0	0.0
M I-7 × Amelia	–	230	50	–	0	0	–	0.0	0.0	–	0	0	–	0.0	0.0	–	0.0	0.0
M II-42 × Anna	–	100	140	–	0	0	–	0.0	0.0	–	0	0	–	0.0	0.0	–	0.0	0.0
M II-42 × Agata	–	120	150	–	0	0	–	0.0	0.0	–	0	0	–	0.0	0.0	–	0.0	0.0
M II-42 × Amelia	–	60	60	–	0	0	–	0.0	0.0	–	0	0	–	0.0	0.0	–	0.0	0.0
P. armeniaca × (*P. salicina* × *P. cerasifera*)																		
Early Orange × Kometa	90	40	–	0	0	–	0.0	0.0	–	0	0	–	0.0	0.0	–	0.0	0.0	–
Early Orange × Najdiena	90	40	–	1	0	–	1.1	0.0	–	1	0	–	1.1	0.0	–	0.0	0.0	–
M I-33 × Kometa	163	25	–	0	0	–	0.0	0.0	–	0	0	–	0.0	0.0	–	0.0	0.0	–
M I-33 × Najdiena	70	52	–	0	0	–	0.0	0.0	–	0	0	–	0.0	0.0	–	0.0	0.0	–
M I-69 × Najdiena	112	100	–	1	0	–	0.9	0.0	–	1	0	–	0.9	0.0	–	0.0	0.0	–
P. salicina × *P. armeniaca*																		
Santa Rosa × Early Orange	302	670	1,270	15	7	46	5.0	1.0	3.6	7	6	27	2.3	0.9	2.1	53.3	14.3	41.3
Santa Rosa × Sirena	298	340	750	2	0	25	0.7	0.0	3.3	1	0	18	0.3	0.0	2.4	50.0	0.0	28.0
OSL 57 × Early Orange	105	110	57	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
OSL 57 × Harkot	46	150	100	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
OSL 57 × Somo	57	100	110	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
OSL 57 × Sirena	48	90	120	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
OSL 58 × Early Orange	53	120	160	0	0	11	0.0	0.0	6.9	0	0	11	0.0	0.0	6.9	0.0	0.0	0.0
OSL 59 × Early Orange	72	120	140	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
OSL 59 × Harkot	78	160	50	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0

doi: 10.17221/273/2014-HORTSCI

Table 3. to be continued

Cross-combinations	No. of flowers pollinated			No. of fruits obtained			Fruits from the No. of flowers pollinated (%)			No. of seeds obtained			Seeds from the No. of flowers pollinated (%)			Malformed seeds (%)		
	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013
OSL 60 × Early Orange	75	89	120	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
OSL 60 × Harkot	117	130	100	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
OSL 60 × Somo	86	10	120	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
OSL 60 × Sirena	65	90	140	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
OSL 65 × Early Orange	–	90	417	–	3	48	–	3.3	11.5	–	3	32	–	3.3	7.7	–	0.0	33.3
OSL 69 × Early Orange	–	91	115	–	0	4	–	0.0	3.5	–	0	0	–	0.0	0.0	–	0.0	100.0
Czernuszka × A^z	–	125	–	–	5	–	–	4.0	–	–	5	–	–	4.0	–	–	0.0	–
Czernuszka × Early Orange	–	–	210	–	–	3	–	–	1.4	–	–	3	–	–	1.4	–	–	0.0
Czernuszka × Harkot	–	–	130	–	–	3	–	–	2.3	–	–	3	–	–	2.3	–	–	0.0
Czernuszka × Somo	–	–	110	–	–	14	–	–	12.7	–	–	14	–	–	12.7	–	–	0.0
Czernuszka × Sirena	–	–	90	–	–	2	–	–	2.2	–	–	2	–	–	2.2	–	–	0.0
Czernuszka × B^y	–	150	–	–	10	–	–	6.7	–	–	10	–	–	6.7	–	–	0.0	–
Czernuszka × Kijewskij Krasen	–	–	290	–	–	14	–	–	4.8	–	–	14	–	–	4.8	–	–	0.0
Czernuszka × Poleskij Krupnopłodnyj	–	–	220	–	–	6	–	–	2.7	–	–	6	–	–	2.7	–	–	0.0
Czernuszka ×Pietropawłowskij	–	–	100	–	–	1	–	–	1.0	–	–	1	–	–	1.0	–	–	0.0
Czernuszka × Czerniewyj	–	–	100	–	–	9	–	–	9.0	–	–	8	–	–	8.0	–	–	11.1
Czernuszka × C^x	–	180	–	–	7	–	–	3.9	–	–	7	–	–	–	–	–	0.0	–
Czernuszka × M I-7	–	–	140	–	–	16	–	–	11.4	–	–	16	–	–	11.4	–	–	0.0
Czernuszka × M I-33	–	–	80	–	–	7	–	–	8.8	–	–	6	–	–	7.5	–	–	14.3
Czernuszka × M II-19	–	–	160	–	–	1	–	–	0.6	–	–	1	–	–	0.6	–	–	0.0
Czernuszka × M II-42	–	–	120	–	–	5	–	–	4.2		–	5	–	–	4.2	–	–	0.0
D 17-73 × A	–	330	–	–	34	–	–	10.3	–	–	32	–	–	–	–	–	5.9	–
D 17-73 × Early Orange	–	–	318	–	–	129	–	–	40.6	–	–	125	–	–	39.3	–	–	3.1

doi: 10.17221/273/2014-HORTSCI

Table 3. to be continued

Cross-combinations	No. of flowers pollinated			No. of fruits obtained			Fruits from the No. of flowers pollinated (%)			No. of seeds obtained			Seeds from the No. of flowers pollinated (%)			Malformed seeds (%)		
	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013
D 17-73 × Harkot	–	–	204	–	–	98	–	–	48.0	–	–	88	–	–	43.1	–	–	10.2
D 17-73 × Somo	–	–	350	–	–	33	–	–	9.4	–	–	29	–	–	8.3	–	–	12.2
D 17-73 × Sirena	–	–	70	–	–	4	–	–	5.7	–	–	2	–	–	2.9	–	–	50.0
D 17-73 × B	–	170	78	–	3	29	–	1.8	37.2	–	2	22	–	1.2	28.2	–	33.3	24.1
D 17-73 × C	–	230	–	–	30	–	–	13.0	–	–	29	–	–	12.6	–	–	3.3	–
D 17-73 × M I-7	–	–	50	–	–	10	–	–	20.0	–	–	10	–	–	20.0	–	–	0.0
D 17-73 × M I-33	–	–	420	–	–	169	–	–	40.2	–	–	145	–	–	34.5	–	–	14.2
D 17-73 × M II-19	–	–	120	–	–	25	–	–	20.8	–	–	20	–	–	16.7	–	–	20.0
D 17-73 × M II-42	–	–	220	–	–	55	–	–	25.0	–	–	46	–	–	20.9	–	–	16.4
P. salicina × *P. cerasifera*																		
Czernuszka × D	–	220	160	–	3	6	–	1.4	3.8	–	3	6	–	1.4	3.8	–	0.0	0.0
Czuk × D	–	76	90	–	2	21	–	2.6	23.3	–	2	18	–	2.6	20.0	–	0.0	14.3
Trumlar × D	–	250	114	–	6	2	–	2.4	1.8	–	6	2	–	2.4	1.8	–	0.0	0.0
Santa Rosa × D	–	89	220	–	0	25	–	0.0	11.4	–	0	20	–	0.0	9.1	–	0.0	20.0
D 17-73 × D	–	158	120	–	0	7	–	0.0	5.8	–	0	6	–	0.0	5.0	–	0.0	14.3
OSL 57 × D	–	80	140	–	2	0	–	2.5	0.0	–	2	0	–	2.5	0.0	–	0.0	0.0
OSL 59 × D	–	180	60	–	0	2	–	0.0	3.3	–	0	0	–	0.0	0.0	–	0.0	100.0
OSL 60 × D	–	140	60	–	0	0	–	0.0	0.0	–	0	0	–	0.0	0.0	–	0.0	0.0
(*P. salicina* × *P. cerasifera*) × *P. armeniaca*																		
Najdiena × Early Orange	150	150	150	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
Najdiena × Harkot	400	10	130	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
Najdiena × Somo	130	90	140	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
Najdiena × Sirena	96	110	100	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0

doi: 10.17221/273/2014-HORTSCI

Table 3. to be continued

Cross-combinations	No. of flowers pollinated			No. of fruits obtained			Fruits from the No. of flowers pollinated (%)			No. of seeds obtained			Seeds from the No. of flowers pollinated (%)			Malformed seeds (%)		
	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013
(*P. salicina* × *P. cerasifera*) × *P. cerasifera*																		
Najdiena × D[w]	–	40	160	–	0	5	–	0.0	3.1	–	0	4	–	0.0	2.5	–	0.0	20.0
P. cerasifera × *P. armeniaca*																		
Anna × A	196	150	290	0	1	0	0.0	0.7	0.0	0	1	0	0.0	0.7	0.0	0.0	0.0	0.0
Agata × A	411	175	140	0	1	0	0.0	0.6	0.0	0	1	0	0.0	0.6	0.0	0.0	0.0	0.0
Amelia × A	170	210	60	0	0	1	0.0	0.0	1.7	0	0	0	0.0	0.0	0.0	0.0	0.0	100.0
P. cerasifera × (*P. salicina* × *P. cerasifera*)																		
Anna × E[v]	281	200	–	0	0	–	0.0	0.0	–	0	0	–	0.0	0.0	–	0.0	0.0	–
Agata × E	193	115	–	0	0	–	0.0	0.0	–	0	0	–	0.0	0.0	–	0.0	0.0	–
Amelia × E	290	185	–	0	0	–	0.0	0.0	–	0	0	–	0.0	0.0	–	0.0	0.0	–

[z] mixture of pollen from four genotypes of *P. armeniaca* – Early Orange, Harcot, Somo, Sirena; [y] mixture of pollen from four genotypes of *P. armeniaca* – Kijewskij Krasen, Poleskij Krupnopłodnyj, Pietropawłowskij, Czerniewyj; [x] mixture of pollen from four genotypes of *P. armeniaca* – M I-7, M I-33, M II-19, M II-42; [w] mixture of pollen from three genotypes of *P. cerasifera* – Anna, Agata, Amelia; [v] mixture of pollen from two genotypes of *P. salicina* × *P. cerasifera* – Kometa, Najdiena

doi: 10.17221/273/2014-HORTSCI

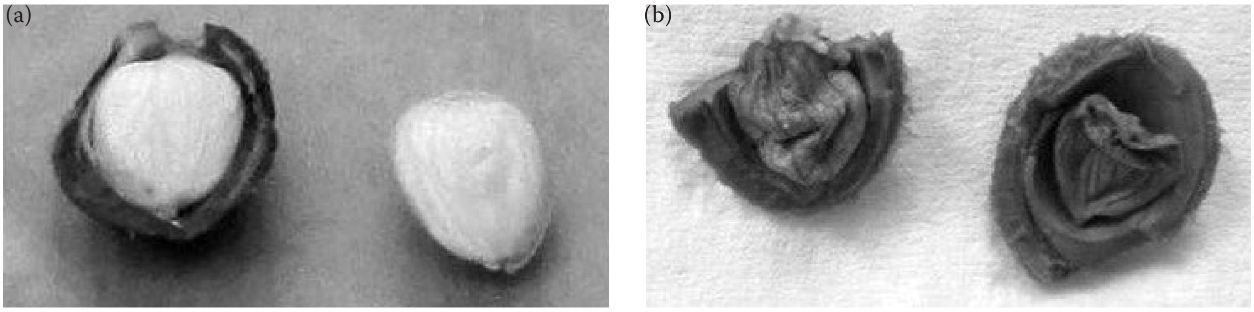

Fig. 4. Stones with seeds extracted from ripe fruits: (a) properly developed seeds of *P. armeniaca* Early Orange and (b) malformed seeds of *P. salicina* D 17-73

The mature fruits were found to contain properly developed seeds (Fig. 4a) as well as seeds that had a wrinkled seed coat and more or less malformed embryos (Fig. 4b). The proportion of fruits with underdeveloped seeds varied and depended on the genotype of the parental forms being crossed. The genotypes *P. salicina* D 17-73 and *P. salicina* Santa Rosa produced more underdeveloped seeds than *P. salicina* Czernuszka (Table 3). Embryo mortality and abnormal seed formation indicate the existence of post-zygotic barriers to crossability, which had been observed previously also by other authors (Rubio-Cabates et al. 1996; Liu et al. 2007). Overcoming post-zygotic barriers and saving at least some hybrid embryos is possible through the use of the *embryo-rescue* technique (Golis et al. 2002; Kukharchyk, Kastrickaya 2006; Liu et al. 2007). It enables the development and growth of immature embryos *in vitro*, which makes it a very useful tool in the distant hybridization of plants of the genus *Prunus* (Arbeola et al. 2003).

CONCLUSION

The effectiveness of distant hybridization between the different genotypes of *P. armeniaca*, *P. salicina*, and *P. cerasifera* is generally low, even though these species belong to the same genus and are diploids with the same basic chromosome number $2n = 2x = 16$. This effectiveness depends on the direction of the crossing. In the distant hybridization of these species, *P. salicina* is more useful as a maternal parent than *P. armeniaca* or *P. cerasifera*. The highest effectiveness of distant hybridization was obtained when the genotypes *P. salicina* Czernuszka and *P. salicina* D 17-73 served as the maternal parent. Using these genotypes, despite the existence of crossability barriers, it is possible to transfer genes between the species *P. armeniaca*, *P. salicina*, and *P. cerasifera*. It allows to increase genetic variation by introduction of this genotypes to the interspecific crossing programs and to obtain new hybrids combining the desired traits of these species in a conventional manner.

Acknowledgement

The authors thank G. Lewandowska, D. Dziubińska and K. Strączyńska for technical assistance in the studies.

References

Arbeola A., Daorden M.E., García E., Marín J.A. (2003): Successful establishment of in vitro culture of *Prunus cerasifera* hybrids by embro culture of immature fruits. Acta Horticulturae (ISHS), 616: 375–378.

Arbeola A., Daordon M.E., García E., Wünsch A., Hormaza J.I., Marín J.A. (2006): Significant effect of accidental pollinations on progeny of setting *Prunus* interspecific crosses. Euphytica, 147: 389–394.

Brewbaker J.L., Kwack B.H. (1964): The calcium ion and substance influencing pollen growth. In: Linskens H.F. (ed.): Pollen Physiology and Fertilization. Amsterdam, North-Holland Publishing: 143–151.

Bubán T. (1996): Pollination and Fertilization. In: Nyéki J., Soltész M. (eds.): Floral Biology of Temperate Zone Fruit Trees and Small Fruits. Budapest, Akadémiai Kiadó: 154–184.

Bubán T. (2003): Hormonal aspects of flower formation and fruit set. In: Kozma P., Nyéki J., Soltész M., Szabó Z. (eds): Floral Biology, Pollination and Fertilisation in Temperate Zone Fruit Species and Grape. Budapest, Akadémiai Kiadó: 3–24.

Cheung A.Y. (1996): Pollen-pistil interactions Turing pollen tube growth. Trends in Plants Science, 1: 45–51.

Chrząstek M., Kruk K., Okoń S., Wojtowicz E. (2009): Wpływ formy ojcowskiej na żywotność pyłku i niektóre cechy

doi: 10.17221/273/2014-HORTSCI

plonotwórcze mieszańców międzygatunkowych *Avena sativa* L. cv. Borowiak × *Avena sterilis* L. Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin, 252: 245–253.

Dafni A., Firmage D. (2000): Pollen viability and longevity: practical, ecological and evolutionary implications. Plant Systematics and Evolution, 222: 113–132.

Duval H., Poëssel J.L., Olivier G. (1994): Evaluation and selection of progeny of an interspecific cross between *Prunus cerasifera* and *Prunus salicina*. Acta Horticulturae (ISHS), 359: 87–92.

Golis A., Korbin M., Pluta S. (2002): The development of *Ribes* embryos by interspecific hybridisation. Acta Horticulturae (ISHS), 585: 155–158.

Hakoda N., Toyoda R., Tabuchi T., Ogiwara I., Ishikawa S., Shimura I. (1998): Morphological characteristics of the interspecific hybrids between Japanese apricot (*Prunus nume*) and Plum (*P. salicina*). Journal of the Japanese Society for Horticultural Science, 67: 708–714.

Hedhly A., Hormaza J.I., Herrero M. (2005): The effect of temperature on pollen germination, pollen tube growth, and stigmatic receptivity in peach. Plant Biology, 7: 476–483.

Jun J.H., Chung K.H. (2007): Interspecific cross compatibility among plum, apricot and plumcot. Korean Journal of Horticultural Science and Technology, 25: 217–222.

Khan S.A., Perveen A. (2008): Germination capacity of stored pollen of *Ficus carica* (Moraceae) and their maintenance. Pakistan Journal of Botany, 40: 2251–2254.

Kukharchyk N., Kastrickaya M. (2006): Embryo rescue techniques in *Prunus* L. breeding. Journal of Fruit and Ornamental Plant Research, 14: 129–135.

Layne R.E.C. (1983): Hybridization. In: Moore J.N., Janick J. (eds): Methods in Fruit Breeding. West Lafayette, John Wiley & Sons, Inc. Purdue University Press: 48–65.

Layne R.E., Sherman C.W.B. (1986): Interspecific hybridization of *Prunus*. HortScience, 21: 48–51.

Liu W., Chen X., Liu G., Liang Q., He T., Feng J. (2007): Interspecific hybridization of *Prunus persica* with *P. armeniaca* and *P. salicina* using embryo rescue. Plant Cell Tissue and Organ Culture, 88: 289–299.

Małuszyńska J., Olszewska M.J. (1999): Wybrane metody w badaniach cytogenetycznych In: Rogalska S., Małuszyńska J., Olszewska M.J. (eds): Podstawy cytogenetyki roślin. Warszaw, PWN: 210–249.

Neumüller M. (2011): Fundamental and applied aspects of plum (*Prunus domestica* L.) breeding. In: Flachowsky H., Hanke V.M. (eds): Fruit, Vegetable and Cereal Science and Biotechnology, 5(Special Issue): 139–154.

Okie W.R. (2005): 'Spring Satin' plumcot. Journal of The American Pomological Society, 59: 119–124.

Okie W.R., Weinberger J.H. (1996): Plums. In: Janick J., Moore J.N. (eds): Fruit Breeding: Tree and Tropical Fruits. New York, John Wiley & Sons, Inc.: 559–608.

Perez S., Moore J.N. (1985): Prezygotic endogenous barriers to interspecific hybridization in *Prunus*. Journal of the American Society for Horticultural Science, 110: 267–273.

Rubio-Cabates M.J., Socias i Company R. (1996): Fertilization assessment and postzygotic development in several intra- and interspecific *Prunus* hybrids. Euphytica, 90: 325–330.

Sharafi Y. (2011a): Pollen viability and longevity in some selected genotypes of peach, plum, prune and sour cherry. Journal of Medicinal Plants Research, 5: 275–279.

Sharafi Y. (2011b): An investigation on the pollen germination and tube growth in some *Prunus persica* genotypes and cultivars. African Journal of Microbiology Research, 5: 2003–2007.

Sharafi Y., Bahmani A. (2011): In vitro study of pollen traits after short storage in some almond, apricot and sweet cherry favorable genotypes. Journal of Medicinal Plants Research, 5: 266–269.

Szabó Z. (2003): Frost injuries of the reproductive organs in fruit species. In: Kozma P., Nyéki J., Soltész M., Szabó Z. (eds): Floral Biology, Pollination and Fertilisation in Temperate Zone Fruit Species and Grape. Budapest, Akadémiai Kiadó: 59–74.

Szabó Z., Nyeki J. (1994): Frost injury to European and Japanese plum flower buds in Hungary. Kerteszeti Tudomany, 26: 11–15.

Szymajda M., Pruski K., Żurawicz E., Sitarek M. (2013): Freezing injuries to flower buds and their influence on yield of apricot (*Prunus armeniaca* L.) and peach (*Prunus persica* L.). Canadian Journal of Plant Science, 93: 191–198.

Topp B.L., Russell D.M., Neumüller M., Dalbó M.A., Liu W. (2012): Plum. In: Badenes M.L. Byrne D.H. Fruit Breeding (eds): Handbook of Plant Breeding 8. New York, Springer Science+Business Media, LLC: 576–621.

Yoshida M., Kyotani H., Yasuno M. (1975): Studies on interspecific crossing in *Prunus* spp. I. Cross compatibility. Japanese Journal of Breeding, 25: 17–23.

Yao S. (2011): Winter 2011 low-temperature injury to stone fruit buds in New Mexico. HortTechnology, 21: 767–772.

Zenkteler M. (1990): *In vitro* fertilization and wide hybridization in higher plants. Critical Reviews in Plant Sciences, 9: 267–279.

Received for publication October 8, 2014
Accepted after correction January 12, 2015

Corresponding author:

MSc. Marek Szymajda, Research Institute of Horticulture, Department of Breeding
of Horticultural Crops, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland
phone: + 48 46 834 5406, e-mail: marek.szymajda@inhort.pl