Testing of self-(in)compatibility in apricot cultivars from European breeding programmes

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Abstract

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Self-(in)compatibility was tested in 40 new apricot cultivars from European breeding programmes. Pollen-tube growth in pistils from laboratory pollinations was analysed using the fluorescence microscopy. Cultivars were considered self-compatible if at least one pollen tube reached the ovary in the majority of pistils. Cultivars were considered self-incompatible if the growth of pollen tubes in the style stopped along with formation of characteristic swellings. Of the examined cultivars, 18 were self-compatible and 22 were self-incompatible. Fluorescence microscopy provides a relatively rapid and reliable method to determine self-incompatibility in apricot cultivars.

Keywords: *Prunus armeniaca*; pollen tube growth; pollination; fluorescence microscopy

Apricot (*Prunus armeniaca* L.), like other fruit species of the *Rosaceae* family, exhibits a gametophytic self-incompatibility system, which is controlled by a single polymorphic locus with multiple alleles (termed *S*-alleles). Presently, 21 *S*-alleles are known in apricot, 20 of which (S_1–S_{20}) code for self-incompatibility, and one (S_c) allows self-compatibility (Burgos et al. 1998; Alburquerque et al. 2002; Halász et al. 2005, 2010).

Self-incompatibility is common in apricot cultivars of Central Asian and Iranian-Caucasian ecogeographical groups. In contrast, cultivars of European group are traditionally considered self-compatible (Kostina 1970; Layne et al. 1996). Until recently, only a few cases of self-incompatibility were registered in apricot cultivars of this group (Schultz 1948; Nyújtó et al. 1985; Egea et al. 1991). The number of known self-incompatible apricot cultivars of the European group has increased rapidly over the last two decades. Thus, Szabó and Nyéki (1991) reported self-incompatibility in nine cultivars, Burgos et al. (1997) in 42 cultivars, Paydas et al. (2006) in 37 cultivars and hybrids, and Milatović and Nikolić (2007) in 14 cultivars.

Traditionally, self-incompatibility is determined by monitoring fruit set in controlled pollination under field conditions. The disadvantage of this method is that fruit set varies from year to year, depending on weather conditions. The second method used is the observation of pollen tube growth in the pistil using fluorescence microscopy. It enables more reliable conclusions compared to the field tests (Viti et al. 1997). In addition to these biological methods, two molecular methods have recently been used to determine self-incompatibility in apricot: detection of stylar ribonucleases (*S*-RNases) (Burgos et al. 1998; Alburquerque

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et al. 2002; Halász et al. 2005) and DNA amplification and identification by PCR (polymerase chain reaction) analysis (Badenes et al. 2000; Halász et al. 2005; Jie et al. 2005; Vilanova et al. 2005).

The objective of this paper was to examine self-incompatibility using fluorescence microscopy in apricot cultivars released from the European breeding programmes. Knowledge of self-incompatibility trait of new cultivars is of great importance for both apricot breeders and producers. In apricot breeding, it is important to select the appropriate cultivars that will be used as parents in controlled crosses. Apricot producers should know the self-compatibility status of new cultivars to choose the adequate pollinators in new orchard plantings.

MATERIAL AND METHODS

Plant material was taken from the apricot cultivar collection of the Faculty of Agriculture in Belgrade. Total number of analyzed cultivars was 40. The largest number of cultivars originates from Czech Republic (16), then from France (6), Italy (5), and Slovak Republic (5). Three cultivars originate from Romania, two from Ukraine, and one cultivar from Serbia, Bulgaria and Moldova each (Table 1).

The apricot collection orchard was established in 2007. The rootstock was Myrobalan (*Prunus cerasifera* Ehrh.) seedling, and tree spacing was 4.5 × 3 m. Studies were carried out over a two-year period (2010–2011).

Shoots with flower buds at the "balloon" stage were collected in the orchard and transported to the laboratory. They were placed in jars with 5% (w/v) sucrose solution and kept at room temperature (20 ± 2°C). Emasculation of flowers was done immediately, and the extracted anthers were placed in open 10 cm-diameter Petri dishes to desiccate. Pistils were hand-pollinated 24 h after emasculation.

Fixation of pistils was done four days (96 h) after pollination. Fixation was carried out in a 5:5:90 (v/v/v) mix of 40% (v/v) formaldehyde, glacial acetic acid, and 70% (v/v) ethanol (Burgos et al. 1997). Fixed material was kept at +4°C (in the refrigerator) until staining.

Before staining, pistils were rinsed in running water for 15 minutes. Thereafter, they were immersed overnight in 8M NaOH to soften the tissues. Then they were rinsed again in running water for 2 hours. Staining was done with 0.1% (w/v) aniline blue dissolved in 0.1M K_3PO_4 for approximately 24 hours. To prepare pistils for microscopic examination, the style was separated from the ovary. The style was squashed, while the ovary was cut longitudinally with a razor blade.

Examination of pistils was carried out by fluorescence microscopy using a Leica DM LS, (Leica Microsystems, Wetzlar, Germany), equipped with an A filter (wavelength 340–380 nm). Only pistils with at least 20 pollen grains on the stigma were analysed.

The numbers of pollen tubes that reached the middle of the style, the base of the style, and the ovule were recorded, and standard errors were calculated for these three parameters. Statistical data analysis was performed by using analysis of variance, and Duncan's multiple range test at $P \leq 0.05$ to determine a significance of the differences between the mean values. The percentages of pistils with at least one pollen tube reaching the middle of the style, the base of the style, and the ovule were also calculated.

RESULTS AND DISCUSSION

Pollen tube growth in the apricot cultivars studied is presented in Table 2. Cultivars were considered self-compatible when at least one pollen tube reached the ovary within 96 h following pollination in majority of pistils. Of the 40 apricot cultivars studied, 18 were self-compatible. They were: Forum, Gergana, Helena du Roussillon, Ivonne Liverani, Kioto, Leala, Litoral, Marlen, Minaret, Ninfa, NS-6, Palummella, Pisana, Sylvercot, Veharda, Veselka, Vestar, and Vitillo. In these cultivars, pollen tubes reached the ovary in the majority (85–100%) of pistils (Fig. 1). Also, in these cultivars the pollen tube reached the ovule in the range of 40–85% (Fig. 2). The number of pollen tubes in the middle of the style ranged from 10.2 to 25.9, at the base of the style it ranged from 3.7 to 17.0, and at the ovule ranged from 0.7 to 2.9.

Cultivars were considered self-incompatible if the pollen tubes stopped their growth in the style, with plugs forming at the tips due to the deposition of callose (Figs 3 and 4). Self-incompatibility was found in 22 of the apricot cultivars studied: Bergarouge, Dacia, LE-3276 (Betinka), LE-8311, Lebela, Lebona, Legolda, Lejuna, Lemeda, Lemira, Lenova, Lerosa, Leskora, Moldavskyi olimpic, Neptun, Palava, Pinkcot, Radka, Strepet, Sylred, Velita,

Table 1. Origin of studied cultivars

Cultivar	Country of origin	Pedigree
Bergarouge (Avirine)	FR	Bergeron × Orangered
Dacia	RO	Marculeşti 19 (Luiset × Re Umberto cl. 305) × CR 5-180
Forum	UA	Mulla Sadik × Udarnik
Gergana	BG	not known
Helena du Roussillon (Aviera)	FR	Bergeron × Rouge de Rivesaltes
Ivonne Liverani	IT	Local selection (Emilia Romagna)
Kioto	FR	not known
LE-3276 (Betinka)	CZ	Vestar × Stark Early Orange
LE-8311	CZ	Lejuna × Goldrich
Leala	CZ	Růžová raná o.p.
Lebela	CZ	D1R16T9 × NJA 34
Lebona	CZ	multiple crossing
Legolda	CZ	RR-2054 × D1R70T84
Lejuna	CZ	C4R8T143 × NJA 11
Lemeda	CZ	multiple crossing
Lemira	CZ	multiple crossing
Lenova	CZ	seedling from open pollination
Lerosa	CZ	D1R16T9 × NJA 32
Leskora	CZ	C4R8T143 × C4R9T26
Litoral	RO	Marculeşti 23-52-50 × Marculeşti 37/1 (Ananas × Ananas)
Marlen (Lednická)	CZ	clone of Hungarian Best
Minaret	CZ	Velkopavlovická × Stark Early Orange
Moldavskyi olimpic	MD	2-25-22 × (Badem-Erik + Nahichevanskyi krasnyi)
Neptun	RO	Hungarian Best × Pionier (Silistra × Ananas)
Ninfa	IT	Ouardy × Tyrinthos
NS-6	RS	local selection
Pálava	CZ	Stark Early Orange × Velkopavlovická LE-19/2
Palummella	IT	local selection (Vesuvian area)
Pinkcot (Cotpy)	FR	not known
Pisana	IT	ICAPI 26/5 o.p.
Radka	CZ	V 66 052 × KŠ 5-17-103
Strepet	UA	Vinoslivij × Erevani
Sylred	FR	not known
Sylvercot (Versyl, Cotsy)	FR	not known
Veharda	SK	Julskij × Hungarian Best
Velita	SK	Hungarian Best × (Achrori, Arzami, and Zard)
Veselka	SK	Vesna × Vegama
Vesprima	SK	Hungarian Best × (Achrori, Arzami, and Zard)
Vestar	SK	Hungarian Best × mixture of pollen from Chinese cultivars
Vitillo	SK	local selection (Campania)

o.p. – open pollination

Table 2. Pollen tube growth in pistils of apricot cultivars 96 h after self-pollination (means ± standard errors)

Cultivar	Number of pistils examined	Number of pistils with at least one pollen tube			Mean number of pollen tubes		
		in the middle of the style	at the base of the style	reached the ovule	in the middle of the style	at the base of the style	reached the ovule
Self-compatible cultivars							
Forum	20	100.0	95.0	40.0	11.80 ± 0.97^{efg}	4.60 ± 0.71^{gh}	1.29 ± 0.33^{de}
Gergana	20	100.0	100.0	70.0	14.95 ± 1.50^{cde}	8.25 ± 0.95^{def}	2.13 ± 0.28^{b}
Helena du Roussillon	20	100.0	85.0	80.0	18.00 ± 2.16^{bcd}	11.50 ± 1.84^{bc}	1.79 ± 0.26^{bc}
Ivonne Liverani	20	100.0	100.0	75.0	13.05 ± 0.72^{de}	8.45 ± 0.75^{def}	2.87 ± 0.28^{a}
Kioto	20	100.0	95.0	55.0	10.15 ± 0.89^{fgh}	4.35 ± 0.49^{h}	1.18 ± 0.28^{def}
Leala	18	100.0	100.0	61.1	15.14 ± 1.74^{cde}	9.29 ± 1.34^{cde}	1.91 ± 0.40^{bc}
Litoral	20	100.0	90.0	60.0	12.85 ± 1.23^{ef}	6.30 ± 0.82^{fgh}	1.29 ± 0.18^{cde}
Marlen	16	100.0	93.8	50.0	10.56 ± 0.81^{fgh}	3.69 ± 0.49^{h}	0.69 ± 0.22^{f}
Minaret	20	100.0	100.0	70.0	20.80 ± 1.75^{b}	11.20 ± 1.19^{bc}	2.76 ± 0.55^{a}
Ninfa	20	100.0	100.0	75.0	25.90 ± 1.53^{a}	13.60 ± 1.22^{b}	1.15 ± 0.22^{def}
NS-6	20	100.0	100.0	60.0	11.10 ± 1.04^{efg}	7.05 ± 1.08^{efg}	1.50 ± 0.18^{cd}
Palumella	21	100.0	100.0	76.2	11.05 ± 1.01^{fg}	5.52 ± 0.68^{gh}	1.11 ± 0.13^{def}
Pisana	20	100.0	100.0	60.0	13.56 ± 1.02^{de}	9.95 ± 1.05^{cd}	1.58 ± 0.35^{bcd}
Sylvercot	20	100.0	100.0	65.0	22.45 ± 1.81^{ab}	16.25 ± 1.82^{a}	1.73 ± 0.24^{bc}
Veharda	20	100.0	100.0	85.0	21.40 ± 1.64^{ab}	17.05 ± 1.51^{a}	2.79 ± 0.33^{a}
Veselka	20	100.0	100.0	60.0	11.15 ± 0.98^{efg}	8.20 ± 0.82^{def}	1.00 ± 0.18^{ef}
Vestar	15	100.0	86.7	53.3	19.23 ± 1.22^{bc}	8.31 ± 1.40^{def}	0.82 ± 0.21^{ef}
Vitillo	20	100.0	90.0	40.0	10.50 ± 0.75^{fgh}	3.75 ± 0.54^{h}	1.00 ± 0.24^{ef}
Self-incompatible cultivars							
Bergarouge	20	100.0	10.0	0.0	8.10 ± 1.02^{ghi}	0.00 ± 0.00^{i}	0.00 ± 0.00^{g}
Dacia	20	90.0	0.0	0.0	2.35 ± 0.42^{jk}	0.00 ± 0.00^{i}	0.00 ± 0.00^{g}
LE-3276 (Betinka)	22	36.4	0.0	0.0	0.77 ± 0.31^{k}	0.00 ± 0.00^{i}	0.00 ± 0.00^{g}
LE-8311	22	63.6	0.0	0.0	1.55 ± 0.36^{jk}	0.00 ± 0.00^{i}	0.00 ± 0.00^{g}
Lebela	20	35.0	0.0	0.0	0.50 ± 0.15^{k}	0.00 ± 0.00^{i}	0.00 ± 0.00^{g}
Lebona	18	100.0	5.6	0.0	2.79 ± 0.46^{jk}	0.07 ± 0.07^{i}	0.00 ± 0.00^{g}
Legolda	20	42.9	0.0	0.0	0.71 ± 0.30^{k}	0.00 ± 0.00^{i}	0.00 ± 0.00^{g}
Lejuna	16	86.7	25.0	6.3	8.53 ± 1.81^{fgh}	0.60 ± 0.32i	0.07 ± 0.07^{g}
Lemeda	20	75.0	0.0	0.0	1.50 ± 0.40^{jk}	0.00 ± 0.00^{i}	0.00 ± 0.00^{g}
Lemira	20	70.0	0.0	0.0	1.40 ± 0.27^{jk}	0.00 ± 0.00^{i}	0.00 ± 0.00^{g}
Lenova	20	100.0	30.0	0.0	9.25 ± 1.21^{fgh}	0.70 ± 0.29^{i}	0.00 ± 0.00^{g}
Lerosa	20	100.0	15.0	5.0	6.00 ± 0.83^{hij}	0.15 ± 0.08^{i}	0.05 ± 0.05^{g}
Leskora	20	100.0	10.0	0.0	3.50 ± 0.75^{ijk}	0.20 ± 0.20^{i}	0.00 ± 0.00^{g}
Moldavskyi olimpic	20	85.0	0.0	0.0	5.55 ± 1.13^{ij}	0.00 ± 0.00^{i}	0.00 ± 0.00^{g}
Neptun	20	80.0	5.0	0.0	2.05 ± 0.43^{jk}	0.05 ± 0.05^{i}	0.00 ± 0.00^{g}
Palava	20	85.0	5.0	0.0	3.10 ± 0.81^{jk}	0.05 ± 0.05^{i}	0.00 ± 0.00^{g}
Pinkcot	20	90.0	0.0	0.0	3.45 ± 0.65^{ijk}	0.00 ± 0.00^{i}	0.00 ± 0.00^{g}
Radka	20	45.0	0.0	0.0	0.45 ± 0.11^{k}	0.00 ± 0.00^{i}	0.00 ± 0.00^{g}
Strepet	20	25.0	0.0	0.0	0.40 ± 0.18^{k}	0.00 ± 0.00^{i}	0.00 ± 0.00^{g}
Sylred	20	80.0	0.0	0.0	2.45 ± 0.47^{jk}	0.00 ± 0.00^{i}	0.00 ± 0.00^{g}
Velita	20	95.0	10.0	0.0	8.05 ± 1.54^{ghi}	0.25 ± 0.18^{i}	0.00 ± 0.00^{g}
Vesprima	20	45.0	0.0	0.0	0.65 ± 0.20^{k}	0.00 ± 0.00^{i}	0.00 ± 0.00^{g}

mean values followed by different letters within a column represent significant differences at $P \leq 0.05$ according to Duncan's multiple range test

Fig. 1. The base of the style with many pollen tubes in the self-compatible apricot cv. Silvercot

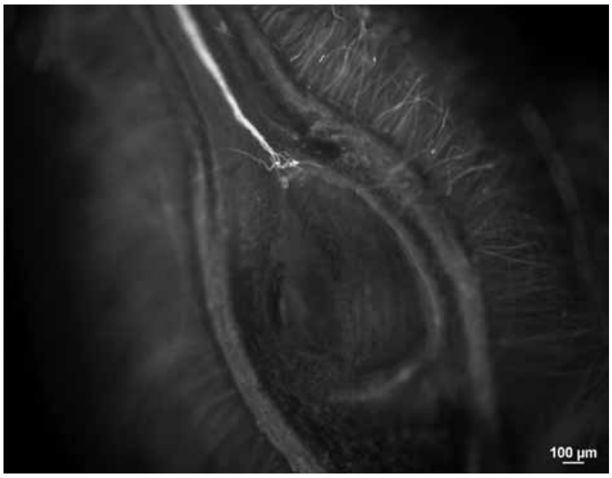

Fig. 2. Pollen tubes reaching the ovule in the self-compatible apricot cv. Gergana

Fig. 3. Incompatible pollen tubes with swellings at the tips in the middle part of the style in the self-incompatible apricot cv. LE-8311

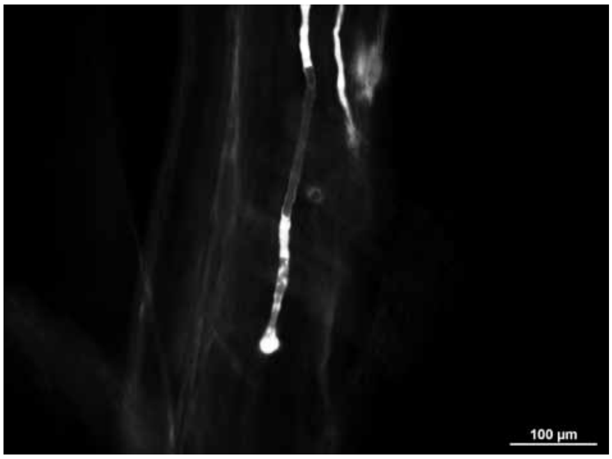

Fig. 4. Incompatible pollen tube with a broadened tip in the lower half of the style in the self-incompatible apricot cv. Lejuna

and Vesprima. In these cultivars pollen tubes often (25–100%) reached the middle of the style, but rarely (0–30%) reached the base of the style. Pollen tubes reached the ovule only in two of these cultivars (Lejuna and Lerosa). The number of pollen tubes in the middle of the style ranged from 0.4 to 9.3, and at the base of the style it ranged from 0.0 to 0.7.

Published data on self-compatibility in apricot cultivars were obtained mostly by studying fruit set following controlled pollination under field conditions (Kostina 1970; Nyújtó et al. 1985; Szabó, Nyéki 1991). Burgos et al. (1997), studying fruit set, found that Italian apricot cvs Palummella and Pisana are self-compatible. We confirm their results in laboratory tests.

Data vary on the number of hours required for pollen tubes to reach the ovary. Thus, Egea et al. (1991) reported that pollen tubes reached the ovary in 48 h, while Guerriero and Bartolini (1995) concluded that, under ideal conditions, they reach the ovary in 48 h, but most often in 72 hours. However, according to Milatović and Nikolić (2007), 72 h was insufficient for most cultivars, so they extended this period to 120 hours. VITI et al. (1997) point out that in apricot it takes pollen tubes at least 96 h to reach the ovary. Also, Audergon et al. (1999) obtained better results when fixation of pistils was done 96 h rather than 72 h after pollination. In this study, 96 h proved to be enough time to allow compatible pollen tubes to reach the ovary and ovule.

The results obtained in this study lead to the conclusion that self-incompatibility is frequent among new apricot cultivars from European breeding programmes. This phenomenon was found in 22 of 40 studied cultivars. Our results are in accordance with previous findings that many new apricot cultivars are self-incompatible (Burgos et al. 1997; Milatović, Nikolić 2007).

The increasing number of self-incompatible cultivars in the last years can be explained by using Asian or North American self-incompatible cultivars in breeding programmes that aim to create new genotypes with the traits such as: *Plum pox virus* resistance (Badenes, Llácer 2006; Karayiannis 2006; Krška et al. 2011), frost tolerance (Benediková 2006; Krška et al. 2006), increase of the sugar content (Ledbetter et al. 2006), or extending the harvest time (Pedryc, Kerek 1999; Topor et al. 2010). Some of the self-incompatible cultivars are frequently used in apricot breeding programmes. Thus, the American cvs Perfection and Goldrich, for example, are used in breeding for their large fruits (Layne et al. 1996), and cvs Stark Early Orange and Harlayne for resistance to *Plum pox virus* (Karayannis et al. 2008). Use of these cultivars in apricot breeding can lead to the development of new, undesirable, self-incompatible selections. Some of the studied cultivars also have in their pedigree self-incompatibble cultivars either of North American origin (Bergarouge, LE-3276, LE-8311, Palava) or of Central Asian origin (Velita, Vesprima).

Self-incompatibility is an undesirable trait in fruit crop production, because self-incompatible cultivars cannot be grown in single-cultivar orchards, and it is necessary to provide additional pollinators. These cultivars produce a lower yield if appropriate pollinator schemes were not planted (McLaren et al. 1996) Apricot flowering takes place in early spring and often proceeds in unfavourable weather conditions, such as low temperatures, rainfall, and wind. Such conditions limit bee flight and cross-pollination. Hence, when growing self-incompatible cultivars adequate pollinators should be selected. They need to be cross-compatible, because cross-incompatibility was found between some apricot cultivars (Szabó, Nyéki 1991; Egea, Burgos 1996; Jie et al. 2005; Hajilou et al. 2006; Zhang et al. 2008; Halász et al. 2010; Milatović et al. 2010).

Considering that self-incompatibility occurs frequently among newly bred European apricot cultivars, care should be taken in deciding the cultivar composition of new orchard plantings. Self-compatibility should be one of the most important objectives in apricot breeding programmes, because self-compatible cultivars can ensure more successful pollination, and thereby higher and more regular yield.

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