

Review. Flower biology in apricot and its implications for breeding

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Abstract

Different aspects related to flower biology have a close link to fruit set failures in apricot and other fruit trees. In this work, studies on pollen viability and germinability, stigma receptivity, ovule development and longevity, the different factors affecting the effective pollination period (EPP), are reviewed. The concept of EPP is based on biological parameters that are the successive steps that take place during the reproductive process and it is the frame within which the factors limiting an appropriate fruit set can be studied. The definition of this concept and its detailed study have allowed determination of the different limiting factors and the design of specific treatments to improve it. Knowledge of the incompatibility phenotype for many apricot cultivars has allowed advising about the planting of single-cultivar orchards. The study of the inheritance of this and other traits in apricot and other fruit trees has allowed planning of hybridisations to minimise or eliminate the production of undesirable seedlings, increasing the efficiency of the breeding programme. Studies on the flower biology of apricot have provided valuable information to help select the appropriate parent cultivars for breeding programmes, also this information is transferred to farmers to avoid losses produced by an inadequate cultivar selection. In this review we intend to give an updated overview of the state of the art in the research and the achievements thus far, as well as considering the implications of these studies for fruit breeding in general, with special attention to apricot breeding.

Key words: effective pollination period, inheritance, male sterility, pistil, pollen, self-(in)compatibility, S-RNases.

Resumen

Revisión. Biología floral en albaricoquero y sus implicaciones en la mejora

Diferentes aspectos de la biología de la flor tienen una estrecha relación con los problemas productivos del albaricoquero y otros frutales. En este trabajo se revisan estudios de viabilidad y germinabilidad del polen, receptividad del estigma, desarrollo y longevidad de los óvulos y en definitiva los diferentes aspectos que condicionan el periodo de polinización efectiva (PPE). El concepto de PPE está basado en parámetros biológicos que constituyen los pasos sucesivos que ocurren durante el proceso reproductor y supone el marco dentro del cual se pueden estudiar los factores limitantes para una adecuada fructificación. El establecimiento de este concepto y su estudio detallado han permitido determinar diferentes factores que lo limitan y diseñar tratamientos específicos para mejorarlo. El conocimiento del fenotipo para la incompatibilidad de muchas variedades de albaricoquero ha permitido desaconsejar su establecimiento en plantaciones monovarietales. El estudio del modo de herencia de éste y otros caracteres en albaricoquero y otros frutales, ha permitido la planificación de los cruzamientos para minimizar o eliminar la producción de descendientes indeseables, aumentando así la eficiencia de los programas de mejora. En conjunto, el estudio de la biología floral del albaricoquero ha producido información aplicable tanto en la elección de genitores en el programa de mejora, como transferible directamente al sector productivo para evitar las pérdidas causadas por una incorrecta elección varietal. En esta revisión se ha pretendido dar una visión actualizada del estado de la investigación y las metas alcanzadas, así como de la incidencia de estos estudios en la mejora genética de los frutales en general y del albaricoquero en particular.

Palabras clave: periodo de polinización efectiva, herencia, androesterilidad, pistilo, polen, auto(in)compatibilidad, S-ARNases.

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Introduction

Different aspects related to flower biology have a close link to fruit set failures in apricot and other fruit trees. Studies on the flower biology of apricot have provided valuable information to help select the appropriate parent cultivars for breeding programmes and transfer to farmers to avoid losses produced by an inadequate cultivar selection. In this review we intend to give an updated overview of the state of the art in the research and the achievements thus far, as well as considering the implications of these studies for apricot breeding.

The review covers the importance of an enough production of flower buds as well as their permanence on the branches until they develop to flowers, are pollinated and become fruits, for the suitability of apricot cultivars to give adequate yields.

Different aspects related to the pollen, the stigma and the ovules are considered that influence greatly the possibilities of the flowers to set fruits. The effective pollination period (EPP) is considered to be an expression of the likelihood that the flowers set fruit and therefore it links female fertility and pollination by considering in a global form all parameters related to reproductive biology.

The need of pollinators, with overlapping blooming times, as well as pollinating insects to transfer the pollen, make self-incompatible cultivars unsuitable to modern horticultural practises. Knowledge on the inheritance of this trait and methodologies to determine the genotypes of different cultivars as soon as possible, have allowed the planification of hybridisations so that the number of self-incompatible seedlings is minimised in the progenies from controlled crosses. A review of the main aspects of this trait in fruit trees and future prospect of the current research are outlined in this work.

Flower bud density and drop

Depending on the intensity, flower bud drop may negatively influence final yield. Several factors are considered common causes of flower bud drops (water stress, lack of chilling, high temperatures during autumn or winter, etc.).

Important losses of flower buds have been associated with deficit irrigation treatments in apricot (Hendrickson and Veihmeyer, 1950; Brown, 1952; Brown,

1953; Uriu, 1964). However, other authors did not find an influence of different irrigation treatments on flower bud drop in apricot (Albuquerque *et al.*, 2003).

Warm temperatures during autumn and winter have been considered responsible for incorrect flower development and, therefore, large flower bud drops in peach (Brown, 1958; Monet and Bastard, 1971). Unsatisfied chilling requirements have also been related to flower bud drop in apricot (Legave, 1978). However, other authors did not observe an influence of chilling on flower bud drop in apricot cultivars (Viti and Monteleone, 1991; Albuquerque *et al.*, 2003).

The different results found could be explained if flower bud density (number of flower buds per branch section) and flower bud drop were genetically conditioned traits. A strong influence of the cultivar on flower bud drop in apricot has been found (Legave, 1975; Legave *et al.*, 1982). Also in apricot, when nine different cultivars were studied during three consecutive years, flower bud density and flower bud drop were not affected by the climatic conditions of the different years but there were large differences between cultivars. Flower bud densities ranged from 63 to 180 buds cm⁻² and percentages of flower bud drops were over 50% in many cultivars and ranged from 13% to 72%, expressed as averages from the three years (Albuquerque *et al.*, unpublished results). It has been found also, in peach and nectarine, that flower bud density (Bellini and Gianelli, 1975; Okie and Werner, 1996) is highly dependent on the cultivar studied.

A scarce flower bud production and/or high flower bud drop is indicative of poor productivity. Since these characters seem to be cultivar-dependent, they may be inherited and therefore the use of such cultivars as parents within a breeding programme will not be advisable.

The pollen

When studying pollen from apricot cultivars it was found that, with the exception of some male sterile cultivars like 'Colorao' or 'Arrogante', most of the apricot cultivars produce pollen in quantities that range from 2,000 to 4,000 grains per anther, which is more than 90,000 pollen grains per flower (Egea and Burgos, 1993). Furthermore, this pollen has a high percentage of viability and germinates, emitting a pollen tube, in a wide range of temperatures (Vachun, 1981; Egea *et al.*, 1992).

Male sterility

Male sterility is defined as the deviant condition in normally bisexual plants when no viable pollen is formed (Frankel and Galun, 1977). Male sterility has been exploited as an effective tool to aid hybrid seed production in many crops. However, male sterility is an undesirable characteristic in scion cultivars of *Prunus* to be used for fruit production, because this trait would restrict yield in large monoculture production blocks. Male-sterile cultivars need cross-pollination and production would depend on an adequate pollen transfer from other cultivars.

A review of apricot pollen fertility (Burgos, 1991) indicated that only three male sterile cultivars, 'Arrogante', 'Colorao de Moxó' and 'Colorao', have been described. Male-sterile anthers can be distinguished visually from normal fertile anthers during the bloom period. Shrunken, discoloured anthers are indicative of male sterility and provide a sharp contrast to the swollen, yellow appearance of normal, pollen-fertile anthers (Burgos and Ledbetter, 1994). A relatively high number of male-sterile trees were observed by these authors in progenies from controlled hybridisations among fertile cultivars in apricot, and they proposed a preliminary model for the inheritance of the trait. Later, it was confirmed (Burgos and Egea, 2001) that the trait is controlled by one recessive gene (Table 1). Five cultivars or selections included in this study were heterozygous for this trait and, since all hybridisations among them were performed to combine fruit quality attributes and the heterozygous status was unknown, this trait can be of economic importance in the efficiency of an apricot breeding programme, since hybridisation among heterozygous cultivars would produce 25% of male-sterile progeny.

Table 1. Number of male-fertile and male-sterile seedlings obtained from crosses among cultivars with different male-sterile genotypes*

Type of hybridisation	Male-fertile seedlings	Male-sterile seedlings	Test ratio	χ^2/P
<i>Msms</i> x <i>Msms</i>	449	154	3:1	0.09/P < 0.9
<i>MsMs</i> x <i>Msms</i>	830	3	1:0	—
<i>msms</i> x <i>MsMs</i>	35	0	1:0	—
<i>msms</i> x <i>Msms</i>	14	7	1:1	2.3/P < 0.25

* Data elaborated from results published by Burgos and Ledbetter (1994) and Burgos and Egea (2001).

Recent research in peach has described a different type of male sterility, that has been proposed to be due to cytoplasmic inheritance (Werner and Creller, 1997). These authors found that all crosses between the male-sterile parent and normal cultivars resulted in a completely male-sterile offspring. Furthermore, when these F₁ seedlings were open-pollinated or backcrossed with the fertile parent all progenies were male-sterile.

The knowledge on the inheritance of this trait will help to plan hybridisations, so that production of male-sterile progeny is avoided through selection of homozygous fertile parents. Also, this information and the progenies generated to obtain it, have helped the search for molecular markers for this trait, that will allow detection and elimination of male-sterile plants at the seedling stage (Badenes *et al.*, 2000).

The pistil

It has been demonstrated that fruit set is determined by numerous factors that affect different processes occurring in the pistil during pollination, pollen tubes germination and growth through the stiles and ovule fertilisation. For instance, it has been found that high temperatures during the pre-blossom weeks produce abnormal flowers and diminish fruit set in apricot (Rodrigo and Herrero, 2002) as well as the ovule viability in almond (Egea and Burgos, 1995a). Stigma receptivity (Egea *et al.*, 1991a; Egea and Burgos, 1992; González *et al.*, 1995), the role of the pistil in controlling pollen tubes growth (Herrero, 1992; Herrero and Hormaza, 1996), ovule maturity at anthesis (Egea and Burgos, 1994; Egea and Burgos, 1998; Albuquerque *et al.*, 2000 and 2002a) and its subsequent evolution (Burgos and Egea, 1993; Burgos *et al.*, 1995) have been studied widely in apricot and other fruit trees.

Macro styles

The length of some pistils places the stigmas above the anthers when their natural position should be at the same or a lower height. Macro styles are a cultivar characteristic that is inherited, although climatic conditions, especially temperatures before or after anthesis, play an important role in regulating the manifestation of the trait. In apricot cultivars with the stigma 2 to 3 mm above the anthers, at anthesis, a much lower number of pollen grains has been found on the stigmas

than in flowers of cultivars with the anthers at the same height as or above the stigmas, when those flowers were within bagged branches and cross-pollination was absent (Egea and Burgos, 1993).

Macro styles may produce important crop failures when there are few bees or when climatic conditions do not allow the activity of these insects. Self-compatible cultivars with long styles may behave as incompatible in these conditions. Also, since stigmas project out of the flower, the risk of quick desiccation and subsequent loss of receptivity is high.

The stigma

Stigma receptivity is fundamental, in many instances, for explanation of phenomena observed during fruit setting. In some cases, the stigma has been considered responsible for the success of some cultivars like the pear 'Decana del Comizio' (Bini and Bellini, 1971; Bini, 1972). Other papers have reported immature stigmas at anthesis in the pear 'Agua de Aranjuez' (Herrero, 1983; Sanzol *et al.*, 2003) or the apple 'Cox's Orange Pippin' (Williams *et al.*, 1984). In apricot, immature stigmas at anthesis have been found also in some apricot cultivars, reaching an optimum receptivity two to four days after anthesis and losing it very quickly thereafter (Burgos *et al.*, 1991; Egea *et al.*, 1991a). In the Southeast of Spain, many apricot cultivars have an extremely short period in which stigmas are receptive (Egea and Burgos, 1992).

The ovary and the ovule

When studying the development of the megagametophyte in relation to fruit set, the occurrence of malformed ovules with degenerated embryo sacs has been observed at different stages of flower development in avocado (Tomer *et al.*, 1976), olive (Rallo *et al.*, 1981; Bini and Lensi, 1981; Bini, 1984), apple (Marro, 1976; Forino *et al.*, 1987), pear (Jaumien, 1968; Bini, 1972), cherry (Eaton, 1959; Stösser and Anvari, 1982; Furukawa and Bukovac, 1989), almond (Pimienta and Polito, 1982; Pimienta and Polito, 1983), peach (Arbeloa and Herrero, 1985) and apricot (Eaton and Jamont, 1964; Burgos and Egea, 1993; Egea and Burgos, 1994; Burgos *et al.*, 1995).

Frequently, more than two ovules have been found in apricot. However, extra ovules are generally mal-

formed or they degenerate quickly (Burgos and Egea, 1993; Egea and Burgos, 1995b).

Figure 1 shows the different embryo sac developmental stages in apricot. At anthesis, apricot ovules are not mature and frequently they are in a very immature stage (Egea and Burgos, 1994; Albuquerque *et al.*, 2000 and 2002a). Most ovules examined were within the first three stages of development in our classification (i.e. from ovules without embryo sac to four-nuclei embryo sacs), with high percentages of ovules without a differentiated embryo sac (Table 2).

Lillecrap *et al.* (1999) found small and delayed embryo sacs at anthesis in an apricot cultivar with frequent low yields, whereas most embryo sacs had eight nuclei in two other cultivars which produced good yields generally.

In South-Eastern Spain, apricot cultivars with immature ovules at anthesis (embryo sacs with four nuclei) produced normal crops (Egea and Burgos, 1998). Therefore, those ovules with, at least, a four-nuclei embryo sac at anthesis have been considered as functional (Albuquerque *et al.*, 2002a). In Table 3, the percentages of functional ovules and fructification of nine apricot cultivars are reported. Cultivars with more than 50% fruit set had also high percentages of functional ovules, suggesting that a certain degree of megagametophyte development at anthesis is necessary for fertilisation to be successful, although it may not be enough to ensure a good crop since some cultivars with high percentages of functional ovules had low fruit set.

Both the ovary and the ovule provide signals that orient and direct pollen tube growth to the right course (Herrero, 2001). In peach, particular secretions from ovary cells along the pollen tube pathway are required for the pollen tube to proceed towards the embryo sac (Arbeloa and Herrero, 1987; Herrero, 2000).

The effective pollination period

Williams (1966) introduced the concept of «effective pollination period» (EPP) as the period during which pollination is effective to produce a fruit, and described in detail the approach used to estimate the EPP in orchard conditions, which basically consists of hand-pollinating flowers at time intervals from anthesis and later recording the initial and final fruit set in these flowers (Williams, 1970a). Microscopic examination of pollen tube kinetics and ovule viability can be useful as an indirect estimation of the EPP. Since

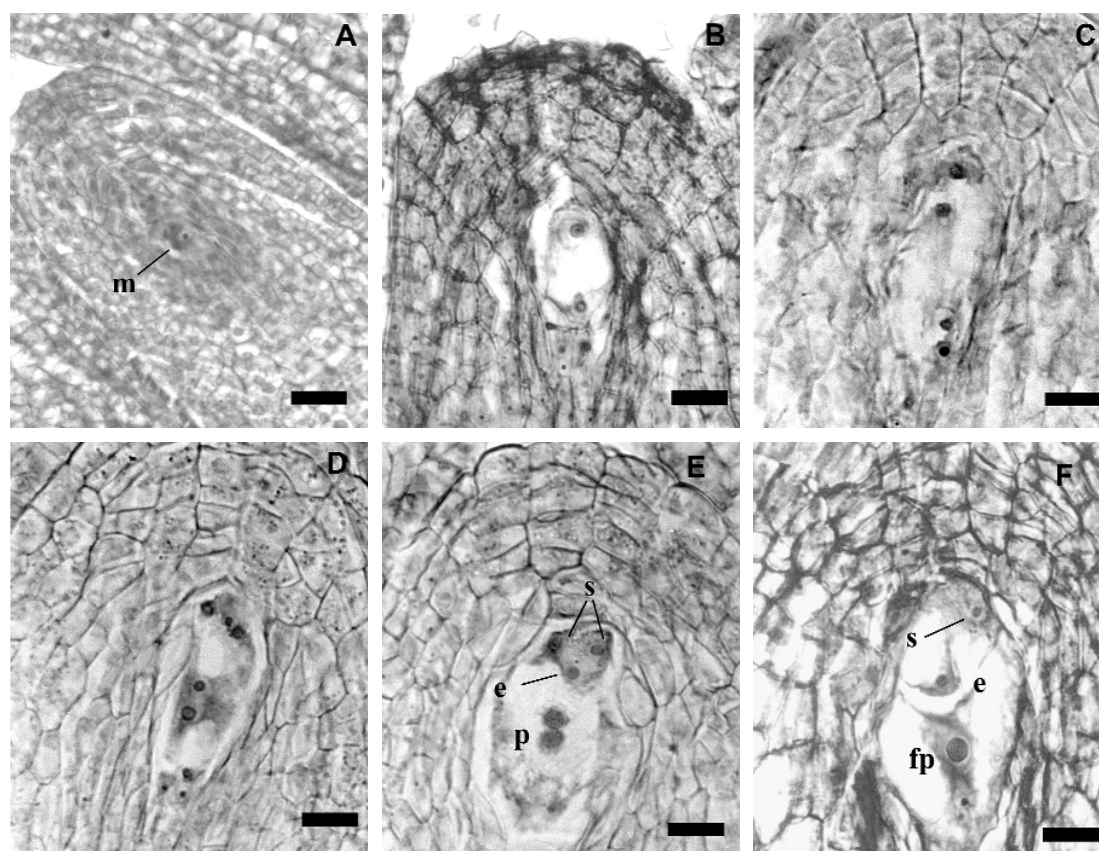


Figure 1. Developmental stages of the embryo sac. (A) No embryo sac. Mother cell of the embryo sac (m). Immature embryo sacs with two (B), four (C) and eight (D) nuclei. (E) Mature embryo sac with the egg cell (e), synergids (s) and unfused polar nuclei (p). (F) Mature embryo sac with fused polar nuclei (fp). Bars represent 20 μm in A and 50 μm in B, C, D, E, and F.

Table 2. Percentage of ovules in different developmental stages at anthesis, in eighteen apricot cultivars

Apricot cultivars	NES	2 N	4 N	8 N	DEC	FPN	M	No. of ovules
Moniquí	37.5	20	21.3	20	1.2	0	0	80
Pepito del Cura	72.5	10	17.5	0	0	0	0	40
Pepito del Rubio	50.8	25.9	19.7	2.1	0	0	1.5	193
Velázquez Fino	50	15.1	27.3	1.9	0	0	5.7	106
Velázquez Tardío	60	10	20	10	0	0	0	40
Gitano	45.8	24.3	19.6	4.7	0	0	5.6	107
Carrascal	40	18.8	31.2	10	0	0	0	80
Candelo	55	27.5	16.3	0	0	0	1.2	80
Bergeron	69	15.9	13.7	0.7	0.7	0	0	145
Modesto	45	17.5	15	3.7	11.3	0	7.5	80
Hargrand	36.3	16.2	28.7	8.8	3.8	0	6.2	80
Palstein	1.9	13.2	43.4	28.3	13.2	0	0	53
Priana	2.5	22.5	32.5	7.5	30	0	5	40
Beliana	4.5	4.5	20.5	22.8	43.2	4.5	0	44
Bebeco	45.4	36.4	18.2	0	0	0	0	33
Colorao	17.5	7.5	22.5	32.5	15	2.5	2.5	40
Guillermo	32.4	35.3	29.4	2.9	0	0	0	34
Goldrich	2.6	12.8	41	28.2	15.4	0	0	39

NES: no embryo sac; N: nuclei. DEC: differentiated egg cell with unfused polar nuclei. FPN: fused polar nuclei. M: malformed. Elaborated from results published in Alburquerque *et al.* (2002a), Burgos *et al.* (1995), Egea and Burgos (1994 and 1998).

Table 3. Percentages of functional ovules and fruit set in different apricot cultivars

Cultivar	Functional ovules	Fruit set
Palstein	84.9 ± 4.92	53.8 ± 1.90
Priana	73.7 ± 7.14	56.4 ± 4.55
Beliana	90.9 ± 4.33	63.5 ± 0.40
Colorao	74.3 ± 7.00	7.5 ± 1.67
Guillermo	32.3 ± 8.02	23.5 ± 0.42
Goldrich	84.6 ± 5.78	16.6 ± 6.66
Pepito	22.1 ± 3.01	12.0 ± 3.02
Bergeron	15.2 ± 2.98	12.8 ± 3.25
Modesto	12.5 ± 5.23	11.0 ± 3.40

Elaborated from results published in Alburquerque *et al.* (2002a).

the EPP is determined by the longevity of the ovule minus the time required by the pollen tube to reach the ovule, this indirect estimation will be valid whenever the EPP values do not exceed the stigmatic receptivity period (Williams, 1966). The microscopic approach provides additional information on the parameters that limit the EPP that is not obtained with the estimation in the orchard.

The EPP was defined as a function of pollen tube speed and ovule longevity. Therefore, it links female fertility and pollination and is an expression of the likelihood that the flowers set fruit. Flower fertility is the capability to produce fruits when flowers are pollinated, at the right time, with compatible pollen. Theoretically, each normally-developed flower is able to set a fruit if pollinated with the appropriate pollen just after anthesis. Its failure to do so is indicative of female sterility. However, under normal conditions, flowers are not always pollinated at anthesis and stigmas remain receptive for several days (Williams, 1970b; Williams *et al.*, 1984).

Stigma receptivity, the speed of pollen tube growth and ovule longevity are three factors commonly-studied in the literature about EPP. Different studies report their relative importances, depending on the species and climatic conditions. There must be a good synchronisation between them, although genetic and environmental factors may unbalance the process and, therefore, decrease fruit setting (Thompson and Liu, 1973).

In fruit trees, including apricot, EPP duration has been estimated to be very variable, depending on the species, cultivar and environmental conditions, ranging from two days to more than a week (Sanzol and Herrero, 2001). When the limiting factor of EPP was determined, in the reviewed papers, a good correlation was found between the two period lengths. In kiwi, the

short EPP found was attributed to a fast loss of pollen germinability due to high temperatures (Galimberti *et al.*, 1987) or to lack of support of pollen germination by the stigma (González *et al.*, 1995). Delays in stigma maturation (Martínez-Tellez and Crossa-Raynaud, 1982; Herrero, 1983) or a short receptivity period (Williams, 1965; Guerrero-Prieto *et al.*, 1985; Burgos *et al.*, 1991; Egea *et al.*, 1991a) may limit the EPP.

Williams (1970c) found that ovule development is affected by high temperatures, but with temperatures between 7 and 15°C ovule development is normal while there is an increase in the speed of pollen tube growth. In these conditions, the EPP is improved. In the climatic conditions of South-Eastern Spain, the limited period of stigma receptivity has been found to be responsible for a short EPP in apricot. For many cultivars examined, high temperatures at bloom limit the stigma receptivity to only one to three days after anthesis, in the most extreme cases (Egea *et al.*, 1991a; Egea and Burgos, 1992). Pollen tubes grow fast in these conditions but at least three days are necessary to reach the ovary.

The longevity of the ovule is related to its stage of development at anthesis. Ovules mature at anthesis will remain viable only a short time, limiting the EPP. On the other hand, if ovules are very immature at anthesis, there may be asynchronies between pollen tube arrival and the maturity of the ovules, which will affect fruit set. The most favourable condition for fructification would be when ovules are at intermediate stages of development (embryo sacs with four to eight nuclei) at anthesis (Alburquerque *et al.*, 2002a).

Self-(in)compatibility

Incompatibility is the inability of a fertile seeded-plant to produce zygotes after self- or cross-pollination (self- or cross-incompatibility) (Heslop-Harrison, 1975). This reaction is an active, regulated constraint of pollen tube growth where, depending on the species and the system operating, the process may be blocked at the initial steps of pollen hydration and germination on the stigma (Dickinson, 1995), during pollen tube growth in the style (Matton *et al.*, 1994) or further down in the ovary (Sage *et al.*, 1994).

Recognising and rejecting their own pollen before fertilisation allows self-incompatible plants to promote outcrossing and improve genetic variability, which is considered to play an important role in the evolutionary

success of the angiosperms. Outcrossing establishes a regulated degree of heterozygosity in the population. Incompatibility occurs in more than 3,000 species of 250 genera, that belong to about 70 families (Van Gastel, 1976).

Although, traditionally, the European group of apricot (within which the apricots grown in Europe, North

America, South Africa and Australia are included) has been described as self-compatible (Mehlenbacher *et al.*, 1991), in the last two decades many widely-cultivated apricot cultivars have been described as self-incompatible (Tables 4 and 5). In fruit trees, incompatibility complicates horticultural practices because self-incompatible clones require the addition of polli-

Table 4. Main self-compatible apricot cultivars

Cultivar	Reference	Cultivar	Reference
Acqua del Serino	Cappellini and Limongelli, 1981	Morocco	Burgos <i>et al.</i> , 1997a
Antonio Errani	Burgos <i>et al.</i> , 1997a	Muscat	Burgos <i>et al.</i> , 1997a
Amabile Vecchioni	Burgos <i>et al.</i> , 1997a	Ninfa	Bassi <i>et al.</i> , 1995
Baracca	Cappellini and Limongelli, 1981	Palummella	Cappellini and Limongelli, 1981
Bebeco	Burgos <i>et al.</i> , 1997a	Palstein	Burgos <i>et al.</i> , 1997a
Bellidieu	Burgos <i>et al.</i> , 1997a	Patterson	CETA, 1990
Beliana	Burgos <i>et al.</i> , 1997a	Pelese Giovanniello	Cappellini and Limongelli, 1981
Bergeron	Audergon <i>et al.</i> , 1988	Peluzzella	Burgos <i>et al.</i> , 1997a
Boccuccia	Burgos <i>et al.</i> , 1997a	Pepito del Rubio	Burgos <i>et al.</i> , 1993
Boccuccia Liscia	Cappellini and Limongelli, 1981	Piet Cillie	Burgos <i>et al.</i> , 1997a
Boccuccia Spinosa	Cappellini and Limongelli, 1981	Pisana	Burgos <i>et al.</i> , 1997a
Búlida	Egea <i>et al.</i> , 1991b	Portici	Burgos <i>et al.</i> , 1997a
Cafona	Cappellini and Limongelli, 1981	Precoce Boulbon	Burgos <i>et al.</i> , 1997a
Canino	Burgos <i>et al.</i> , 1997a	Precoce Pugget	Burgos <i>et al.</i> , 1997a
Canino Tardiva	Burgos <i>et al.</i> , 1997a	Polonais	Audergon <i>et al.</i> , 1988
Carrascal	Burgos <i>et al.</i> , 1993	Poppy	Egea, unpublished
Castelbrite	Ramming and Tanner, 1978	Quattova	Burgos <i>et al.</i> , 1997a
Comondor	Burgos <i>et al.</i> , 1997a	Robada	Ledbetter and Ramming, 1997
Cot 2579	Burgos <i>et al.</i> , 1997a	Rojo Pasión	Egea <i>et al.</i> , 2004a
Cot 3080	Burgos <i>et al.</i> , 1997a	Rouge de Fournes	Audergon <i>et al.</i> , 1988
Cot 3380	Burgos <i>et al.</i> , 1997a	Rouge de Rivesaltes	Audergon <i>et al.</i> , 1988
Cruzman	Burgos <i>et al.</i> , 1997a	Rouge de Roussillon	Audergon <i>et al.</i> , 1988
Currot	Burgos <i>et al.</i> , 1997a	Rouget de Sernhac	Audergon <i>et al.</i> , 1988
Desfarges	Burgos <i>et al.</i> , 1997a	Royal Rosa	Glucina <i>et al.</i> , 1990
Dulcinea	Burgos <i>et al.</i> , 1997a	Riffault	Glucina <i>et al.</i> , 1990
Erevani	Burgos <i>et al.</i> , 1997a	San Castrese	Cappellini and Limongelli, 1981
Farmingdale	Burgos <i>et al.</i> , 1997a	Sarituzu	Burgos <i>et al.</i> , 1997a
Florilege	Blane <i>et al.</i> , 2004	Screara	Burgos <i>et al.</i> , 1997a
Fracasso	Cappellini and Limongelli, 1981	Selene	Egea <i>et al.</i> , 2004b
Galtarotja	Burgos <i>et al.</i> , 1997a	Skaha	Burgos <i>et al.</i> , 1997a
Goldcot	Lamb and Stiles, 1983	Soldone	Burgos <i>et al.</i> , 1997a
Harlayne	Burgos <i>et al.</i> , 1997a	Soledane	Blane <i>et al.</i> , 2003
Harval	Burgos <i>et al.</i> , 1997a	Tardif de Bordaneil	Audergon <i>et al.</i> , 1988
Hatif Colomer	Burgos <i>et al.</i> , 1997a	Tardif de Tain	Burgos <i>et al.</i> , 1997a
Hasenbey	Burgos <i>et al.</i> , 1997a	Tadeo	Burgos <i>et al.</i> , 1997a
Helena	Ledbetter, 1994	Tilton	Burgos <i>et al.</i> , 1997a
HW411	Burgos <i>et al.</i> , 1997a	Timpurii	Burgos <i>et al.</i> , 1997a
Lorna	Ledbetter, 1998	Trevatt	Burgos <i>et al.</i> , 1997a
Malan Royal	Burgos <i>et al.</i> , 1997a	Tomcot	Burgos <i>et al.</i> , 1997a
Mamaia	Burgos <i>et al.</i> , 1997a	Tyrinthos	Burgos <i>et al.</i> , 1997a
Marculesti 12/5	Burgos <i>et al.</i> , 1997a	Valenciano-1	García <i>et al.</i> , 1988
Mauricio	Burgos <i>et al.</i> , 1997a	Valenciano-2	Burgos <i>et al.</i> , 1997a
Modesto	Burgos <i>et al.</i> , 1997a	Valenciano-4	Burgos <i>et al.</i> , 1997a
Monaco Bello	Cappellini and Limongelli, 1981	Wenatchee	Burgos <i>et al.</i> , 1997a
Mono	Burgos <i>et al.</i> , 1997a		

Table 5. Main self-incompatible apricot cultivars

Cultivars	Reference	Cultivars	Reference
A179 Delbard	Burgos <i>et al.</i> , 1997a	Moniquí	Burgos <i>et al.</i> , 1993
A202 Delbard	Burgos <i>et al.</i> , 1997a	Moniquí Azaraque	Andrés and Durán, 1998
Apache	Ledbetter, 2002	Moongold	Burgos <i>et al.</i> , 1997a
Arengi	Crossa-Raynaud, 1961	Nagykorosi Orias	Szabó and Nyéki, 1991
Aurora	Burgos <i>et al.</i> , 1997a	Nicole	Ledbetter, 2003
Avikaline	Burgos <i>et al.</i> , 1997a	NJA 54	Burgos <i>et al.</i> , 1997a
Bedri	Crossa-Raynaud, 1961	NJA 55	Burgos <i>et al.</i> , 1997a
Bergarouge	Blane <i>et al.</i> , 2003	Nonno	Burgos <i>et al.</i> , 1997a
Ceccona	Burgos <i>et al.</i> , 1997a	Orange Red	Burgos <i>et al.</i> , 1997a
Cegledi Orias	Szabó and Nyéki, 1991	Pancin	Burgos <i>et al.</i> , 1997a
Charmagz	Seth and Kuksal, 1977	Perfection	Schultz, 1948
Cluthagold	Glucina <i>et al.</i> , 1988	Priana	Burgos <i>et al.</i> , 1997a
Cot 2679	Burgos <i>et al.</i> , 1997a	Primula	Burgos <i>et al.</i> , 1997a
Cot 2779	Burgos <i>et al.</i> , 1997a	Riland	Schultz, 1948
Cot 2879	Burgos <i>et al.</i> , 1997a	Rival	Burgos <i>et al.</i> , 1997a
Cream Ridge	Burgos <i>et al.</i> , 1997a	Scronsniy	Burgos <i>et al.</i> , 1997a
Early Blusa	Burgos <i>et al.</i> , 1997a	Stark Early Orange	Burgos <i>et al.</i> , 1997a
Erevani	Burgos <i>et al.</i> , 1997a	Stella	Burgos <i>et al.</i> , 1997a
Fakoussi	Crossa-Raynaud, 1961	Sundrop	Austin <i>et al.</i> , 1998
Gitano	Burgos <i>et al.</i> , 1993	Sunglo	Burgos <i>et al.</i> , 1997a
Goldbar	Bassi <i>et al.</i> , 1995	Sungold	Lamb and Stiles, 1983
Goldrich	Lamb and Stiles, 1983	Szegedi Mammut	Szabó and Nyéki, 1991
Goldstrike	Burgos <i>et al.</i> , 1997a	Tokaloglu	Gulcan and Askin, 1991
Hamidi	Crossa-Raynaud, 1961	Vallero-1	Burgos <i>et al.</i> , 1997a
Harcot	Egea and Burgos, 1996	Vallero-2	Burgos <i>et al.</i> , 1997a
Hargrand	Egea and Burgos, 1996	Veecot	Burgos <i>et al.</i> , 1997a
Kermanshah	Burgos <i>et al.</i> , 1997a	Velázquez Fino	Burgos <i>et al.</i> , 1993
Lambertín-1	Egea and Burgos, 1996	Velázquez Tardío	Burgos <i>et al.</i> , 1993
Laycot	Burgos <i>et al.</i> , 1997a	Velvagio	Burgos <i>et al.</i> , 1997a
Ligeti Orias	Szabó and Nyéki, 1991	Venturina	Burgos <i>et al.</i> , 1997a

nators and the yield depends on abundant pollen transfer among the trees.

Genetic control

In *Prunus*, the incompatibility system operating in most of the studied species is controlled by one gene with several different alleles. Pollen is rejected when its *S*-allele is present in the genotype of the style. Hence, an incompatibility reaction will occur between two plants if their genotypes at the *S* locus do not differ in at least one allele (De Nettancourt, 1972; Heslop-Harrison, 1975).

Sweet cherry was the first *Prunus* species where this model was described (Crane and Brown, 1937). The same mechanism has been demonstrated in almond (Dicenta and García, 1993) and apricot (Burgos *et al.*, 1997b). However, a different mode of inheritance was

found in Japanese plum, for which it has been proposed that two genes with epistatic relationships control the trait (Arora and Singh, 1990).

In apricot, alleles for self-compatibility would allow pollen tube growth in any style (Table 6, reciprocal crosses of types I and II and crosses of types IV to VIII). Self-incompatibility alleles would stop pollen tube growth if the same allele was present in the pistil and the pollen grain (Table 6, crosses of types III, IV, VIII, X, XI and XII).

To determine the mode of inheritance of self-(in)compatibility in apricot, 19 families with a total of 948 seedlings, were evaluated (Table 7). Seedling segregation for the trait allowed it to be deduced that the parents used were heterozygous. Also, there were two families where segregation could only be explained if the parents shared one allele (Table 6, cross type IV). A similar situation had been found previously in almond when crossing 'Ferragnes' with the self-compatible cultivars

Table 6. Theoretical crosses and expected genotypes and phenotypes, depending on the self-(in)compatibility status of the parents

Parents genotypes	Expected genotypes	Expected phenotypes (SC:SI)	Type of cross
Crosses between SC and SI cultivars			
<i>(No common allele)</i>			
SC (ScS_1) × SI (S_2S_3)*	$ScS_2, ScS_3, S_1S_2, S_1S_3$	1:1	I
SC ($ScSc$) × SI (S_1S_2)*	ScS_1, ScS_2	1:0	II
<i>(Common allele)</i>			
SC (ScS_1) × SI (S_1S_2)	ScS_2, S_1S_2	1:1	III
SI (S_1S_2) × SC (ScS_1)	ScS_1, ScS_2	1:0	IV
Crosses between SC cultivars			
<i>(No common allele)</i>			
SC1 (ScS_1) × SC2 (ScS_2)*	$ScSc, ScS_1, ScS_2, S_1S_2$	3:1	V
SC1 ($ScSc$) × SC2 (ScS_1)*	$ScSc, ScS_1$	1:0	VI
<i>(Same genotype)</i>			
SC1 ($ScSc$) × SC2 ($ScSc$)	$ScSc$	1:0	VII
SC1 (ScS_1) × SC2 (ScS_1)	$ScSc, ScS_1$	1:0	VIII
Crosses between SI cultivars			
<i>(No common allele)</i>			
AI1 (S_1S_2) × AI2 (S_3S_4)*	$S_1S_3, S_1S_4, S_2S_3, S_2S_4$	0:1	IX
<i>(Common allele)</i>			
AI1 (S_1S_2) × AI2 (S_2S_3)	S_1S_3, S_2S_3	0:1	X
AI2 (S_2S_3) × AI1 (S_1S_2)	S_1S_3, S_1S_2	0:1	XI
<i>(Same genotype)</i>			
AI1 (S_1S_2) × AI2 (S_1S_2)	No seedlings produced		XII

* Reciprocal crosses are not included since the same genotypes are expected.

‘Genco’ and ‘Tuono’ (Dicenta and García, 1993). Further work on stylar proteins of almond (Boskovic *et al.*, 1997) and apricot (Burgos *et al.*, 1998) cultivars demonstrated the existence of a common *S*-allele.

Cross type XII in Table 6 could only happen if both self-incompatible parents have the same genotype. Two

groups of cross-incompatible cultivars have been described after controlled pollinations. One of them includes three Hungarian apricot cultivars (Nyéki and Szabó, 1995) and the other the North American cultivars ‘Lambertin’, ‘Goldrich’ and ‘Hargrand’ (Egea and Burgos, 1996).

Table 7. Rate of self-compatible and self-incompatible seedlings obtained in crosses among apricot cultivars, depending on the type of cross*

Type of cross	Number of tested families	Number of seedlings			Expected phenotypes (SC:SI)	χ^2	P
		SC	SI	Total			
I or III	10	328	297	625	1:1	1.5	<0.25
IV	1	221	3	224	1:0	—	
IX, X or XI	3	0	37	37	0:1	—	
V	4	39	15	54	3:1	0.2	<0.75
VIII	1	8	0	8	1:0	—	

* Elaborated from results published by Burgos *et al.* (1997b).

Molecular aspects of incompatibility

Within the Rosaceae, a correlation between known genotypes for self-(in)compatibility and bands resulting from electrophoresis of stylar extracts has been found in Japanese pear (Hiratsuka *et al.*, 2001; Sassa *et al.*, 1992) where the proteins have been characterised as glycoproteins with RNase activity (Hiratsuka, 1992; Sassa *et al.*, 1993; Hiratsuka *et al.*, 1995; Hiratsuka and Okada, 1995). Similar results have been found in apple (Sassa *et al.*, 1994), and European and Chinese pears (Tomimoto *et al.*, 1996).

In *Prunus*, similar studies have been carried out in sweet cherry (Mau *et al.*, 1982; Boskovic and Tobutt, 1996; Boskovic and Tobutt, 2001) and almond (Tao *et al.*, 1997; Boskovic *et al.*, 1997; Certal *et al.*, 2002). In our laboratory, a good correlation was established between RNases from stylar extracts and the available information on (in)compatibility genotypes of different apricot cultivars (Table 8). It was also demonstrated that these proteins were in-

herited as if they were the products of the *S* gene (Burgos *et al.*, 1998) and this methodology was used to genotype unknown cultivars and selections from the breeding programme (Albuquerque *et al.*, 2002b).

A further step in the molecular research on *S*-alleles in fruit trees was the use of a combination of *S*-allele-specific primers, designed from non-conserved sequences from each allele in apple, and the digestion of PCR products with *S*-allele-specific restriction enzymes (Janssens *et al.*, 1995). Results from this approach to the identification of *S*-alleles correlated perfectly with information on genotypes from phenotypic and RNases analyses and it is a rapid and useful method for determination of the genotype of different apple cultivars (Sakurai *et al.*, 1997 and 2000). A recent paper reports the identification of 15 different *S*-alleles in apple using this methodology (Broothaerts, 2003).

The same strategy, with or without modifications, has been used to design specific primers for *S*-alleles in almond (Tamura *et al.*, 2000; Channuntapipat *et al.*,

Table 8. Self-(in)compatibility genotypes proposed for apricot cultivars based on electrophoresis of stylar RNases. Segregation of stylar RNases in progenies from controlled crosses*

Cultivar	Genotype	Cultivar	Genotype
Beliana	<i>ScS</i> ₇	Lambertin	<i>S</i> ₁ <i>S</i> ₂
Canino	<i>ScS</i> ₂	Mauricio	<i>ScS</i> ₁
Colorao	<i>ScS</i> ₅	Moniquí	<i>S</i> ₂ <i>S</i> ₆
Currot	<i>ScSc</i>	Palabras (Clon V4)	<i>ScSc</i>
Gitano	<i>S</i> ₅ -	Pepito	<i>ScS</i> ₂
Goldrich	<i>S</i> ₁ <i>S</i> ₂	Priana	<i>S</i> ₂ <i>S</i> ₇
Harcot	<i>S</i> ₁ <i>S</i> ₄	Sunglo	<i>S</i> ₂ <i>S</i> ₃
Hargrand	<i>S</i> ₁ <i>S</i> ₂		

Family	Number of seedlings	Observed genotypes	Expected segregation for individual RNases	χ^2 (df)	P
Moniquí x Pepito	17	12 <i>ScS</i> ₂ 5 <i>ScS</i> ₆	(<i>S</i> ₂ : <i>S</i> ₆) 1:1	2.5 (1)	P < 0.25
Gitano x Pepito	38	17 <i>ScS</i> ₅ 5 <i>Sc</i> - 11 <i>S</i> ₂ <i>S</i> ₅ 5 <i>S</i> ₂ -	1:1:1:1	9.9 (3)	P < 0.025
Colorao x Pepito	7	4 <i>ScSc</i> 1 <i>ScS</i> ₂ 2 <i>ScS</i> ₅ 0 <i>S</i> ₂ <i>S</i> ₅	2:1:1	4.7 (2)	P < 0.1
Goldrich x Pepito	10	6 <i>ScS</i> ₁ 4 <i>ScS</i> ₂	(<i>S</i> ₁ : <i>S</i> ₂) 1:1	0.4 (1)	P < 0.75

* Elaborated from results published by Burgos *et al.* (1998) and Albuquerque *et al.* (2002b).

2003), pear (Zuccherelli *et al.*, 2002), sweet cherry (Tao *et al.*, 1999; Yamane *et al.*, 2000; Sonneveld *et al.*, 2001; Wiersma *et al.*, 2001) and Japanese apricot (Yaegaki *et al.*, 2001).

In apricot, the alleles S_1 and S_2 have been sequenced completely (Romero *et al.*, 2003) by using a bacterial artificial chromosome (BAC) library from the cultivar Goldrich (Vilanova *et al.*, 2003). This is a first step that will allow the design of primers from these sequences in order to amplify different S -alleles in apricot. The possibility of designing primers for the self-compatibility allele found in all self-compatible apricot cultivars tested to date (Alburquerque *et al.*, 2002b) is especially interesting (Burgos *et al.*, 1998). A similar strategy has allowed the design of molecular markers for this important trait in Japanese apricot (Tao *et al.*, 2000, 2002a and 2002b).

Conclusions

The study of the flower biology of apricot, described in this review, has had strong implications for the breeding programme of this species, which has been developed at the same time. First of all, the knowledge of the factors limiting fruit set in an important number of commercial cultivars has oriented the selection of parents. Some cultivar-dependent characteristics, like macro styles and flower bud density or drop, indicate that some cultivars would not be a good choice as parents in the breeding programme. Other factors, like ovule immaturity at anthesis, are signs of bad adaptation of the cultivars to local climatic conditions and these, therefore, would also be a wrong parental selection. In those cases when such parents must be used, the knowledge of these characteristics is important in order to evaluate the seedlings, paying much attention to the possible segregation of these traits within the progenies in order to select the ones that have not inherited the undesirable characters.

Determining the mode of inheritance of economically-important traits improves the efficiency of breeding. For instance, male sterility may produce up to 25% of male-sterile seedlings from crosses between fertile heterozygous cultivars. The selection of the appropriate parents is, again, the solution. Also, determining the inheritance of self-(in)compatibility and the parents' genotypes for this trait allows hybridisations to be planned which minimise or eliminate the production of self-incompatible seedlings. The

correlation between stylar RNases and different S -alleles has been a great advance for determination of the genotype of a good number of cultivars. With this methodology, homozygous self-compatible cultivars can be easily identified, which will produce 100% self-compatible progeny regardless of the other parent's genotype. If the necessity of evaluating the progenies generated within the breeding programme, to discard the self-incompatible seedlings, is eliminated, the programme is speeded up, which greatly reduces its cost.

Self-incompatibility phenotype determination by controlled crosses and evaluation of fruit set or pollen tube growth as well as RNase analysis, to determine the genotype at the S locus, need mature trees with flowers, which, for fruit trees, means at least three years after seeds are obtained. Using PCR with S -allele-specific primers allows detection of the self-incompatible genotype in the first stages of plant development, and therefore allows roguing of undesirable seedlings straight after germination of the seeds. Specific primers to amplify selectively the allele (or alleles) that determine self-compatibility are molecular markers for this trait with 100% efficiency, since they are located within the S locus. In apricot, these primers have not yet been identified nor efficient molecular markers developed. However, some recent papers on this species, and methodologies developed in related *Prunus* species, indicate that they will soon be available.

The number of publications in recent years indicates the interest in the different aspects of reproductive biology. This interest is, possibly, closely linked to the fact that this knowledge may avoid production failures and also allows the efficiency of the fruit breeding programmes to be increased.

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