Chapter 2
Apricots

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Abstract Several dozen publicly-sponsored breeding programs around the world are developing new fresh market and processing apricot cultivars. Apricots have a more limited environmental range than other tree fruits, and therefore, many breeders are interested in broadening adaptations for specific growing regions. Plum Pox Virus resistance is a widely pursued objective and there are ongoing efforts to identify molecular markers that are closely linked to disease resistance. Fruit sugars, acids, pigments and volatile aromatic compounds are being quantified in newly bred and historically important cultivars. Researchers have identified and characterized several stylar ribonucleases associated with self-unfruitfulness. Molecular phylogenetic studies are examining the dispersion routes of apricot germplasm from its centers of origin to those cultivars currently in production. Although several linkage maps have been developed using diverse parents and a wide variety of molecular markers from apricot and other *Prunus* crops, the scarcity of documented monogenic characters in apricot limits the effectiveness of marker assisted selection for economically important traits.

2.1 Introduction

Prunus armeniaca L. is not a true native to the plains of Armenia, but it has been continuously cultivated there since at least the first century AD. It was brought to Armenia from a more eastern center of origin much earlier as evidenced by archeological excavations at pre-Christian sites. Since those early times, Armenian foods, traditions and folklore have been influenced by the presence of apricot in the region. Perhaps due to its early ripening season, its unique and pleasant aroma, or its high nutritive content and ability to be processed into a non-perishable sustaining ration, early explorers and conquerors brought apricot with them to foreign

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lands. No attempt will be made here to convey what are currently accepted as the dissemination routes of apricot from its natural centers of origin, as an excellent review article on this subject was published recently (Faust et al. 1998).

Throughout the world apricot is considered to be among the most delectable of all fruits, with flowers, fruit and tree playing parts in various traditions of diverse human cultures. Fruit are used in both fresh and dry form, canned or otherwise preserved as jam and marmalade or pulp. Wines and distillates made from both cultivated and non-domesticated apricot are traditional beverages in parts of both Europe and Asia (Joshi et al. 1990, Genovese et al. 2004).

Since the early 1990s, both fruit tonnage and orchard area have been increasing in African and Asian countries, whereas European and South American countries have realized increased apricot production on fewer hectares of orchard. Fifty countries are listed by FAO as having annual production in excess of 1,000 Mt with Turkey being the largest current apricot producer (370,000 Mt). Three other countries (Iran, Italy and Pakistan) now have annual production in excess of 200,000 Mt. Half of the world's orchard area and nearly half of all apricot production comes from Asiatic countries. Fruit production and orchard area are both declining in North American and Oceanic growing regions. Taken as a whole, apricot fruit production and harvested orchard area are both increasing on a worldwide basis, with 2005 levels of fruit tonnage and orchard area standing at 2.8 million Mt and 434,000 ha, respectively (FAOSTAT, 2006).

2.2 Evolutionary Biology and Germplasm Resources

2.2.1 Taxonomy

Botanists in Western countries have historically placed apricots within the plant family *Rosaceae*, subfamily *Prunoideae*, tribe *Pruneae* and the genus *Prunus*. Depending on the botanical authority, opinions have been mixed on whether apricot should be placed within the sub-genera *Prunophora* or *Amygdalus*, as apricot shares some morphological and pomological characteristics of both (Zielinski 1977). Leaves emerging from dormant buds are open and in a whorl, or *convolute*, as described by Bailey (1916) for the plums, prunes and apricots of the *Prunophora*, whereas the leaves of almonds and peaches in the sub-genus *Amygdalus* have *conduplicate* leaves – folded along the midrib as they emerge from dormant buds. Genetic linkage maps based on several types of molecular markers have shown a high degree of colinearity between an F_1 progeny population from 'Polonais' × 'Stark Early Orange' apricots and an almond × peach F_2 population, indicating a very similar genomic structure between *Prunophora* and *Amygdalus* (Lambert et al. 2004). A recent investigation into the overall genetic diversity of *Prunus* based on random amplified polymorphic DNA (RAPD) analyses place apricots well within the subgenus *Prunophora* and apart from the sub-genus *Amygdalus* (Shimada et al. 2001).

Early botanical descriptions of the different apricot species were based primarily on leaf shape and pubescence, and these characters were not always consistent between specimens. Bailey's (1916) categorical distinctions of apricot species and botanical varieties used leaf characteristics. The classification by Rehder (1940) distinguished plums (Sections Euprunus and Prunocerasus) from apricots (Section Armeniaca) on the basis of ovary pubescence, being absent or glabrous in the plums and present or pubescent in apricots. *P. brigantina* Vill. (syn. *P. brigantiaca*), a glabrous apricot, was a noted exception to the Rehder (1940) scheme. Table 2.1 provides a comparison of the classification of apricots by Bailey (1916) and Rehder (1940).

The taxonomy of apricots by Chinese investigators was also based mainly on leaf characteristics. China's immense size and varied topography, as well as its numerous geographic and climatic zones provided enormous genetic diversity in many plant families to Chinese botanists that were unknown to their counterparts in Western countries (Hou 1983). Apricot classification in China parallels that of Western taxonomists to the subfamily level (*Prunoideae*). At this point, the *Prunoideae* is divided into nine genera: *Prinsepia, Pygeum, Maddenia, Amygdalus, Armeniaca* (apricots), *Prunus, Cerasus, Padus* and *Laurocerasus* (Gu et al. 2003). These authors point out the complexity of taxonomy within *Rosaceae*, and the fact that some of the listed genera within the *Prunoideae* have been grouped together by other authorities. The genus *Armeniaca* is divided into 10 species (Lingdi and Bartholomew 2003), with mention made of an 11th species that is not present in

Table 2.1 Comparison of apricot classification schemes as suggested by Bailey (1916) and Rehder (1940)

Bailey scheme	Rehder scheme
Prunus (genus)	*Prunus* (genus)
Prunophora (sub-genera) (plums, prunes & apricots)	*Prunophora* (sub-genera) (sections)
	Euprunus (European/Asian plums) Prunocerasus (N. American plums) Armeniaca (apricots)
P. armeniaca L.	*P. brigantina* Vill.
Var. *pendulata* Dipp.	*P. mandshurica* Maxim.
Var. *variegata* Hort.	*P. sibirica* L.
Var. *sibirica* Koch	*P. armeniaca* L.
Var. *mandshurica* Maxim.	pomological varieties:
Var. *Ansu* Maxim.	*P. a. variegata* Schneid.
P. mume Sieb. & Zucc.	*P. a. péndula* Jaeg.
Var. *Goethartiana* Koehne.	*P. a. Ansu* Maxim.
Var. *albo-plena* Hort.	*P. mume* Sieb. & Zucc.
other forms:	pomological varieties:
laciniata Maxim.	*P. m. alba* Rehd.
microcarpa Makino	*P. m. Alphandii* Rehd.
viridicalyx Makino	*P. m. albo-plena* Bailey
cryptopetala Makino	*P. m. Péndula* Sieb.
P. brigantiaca Vill.	*P. m. tonsa* Rehd.
P. dasycarpa Ehrh.	*P. dasycarpa* Ehrh.

China (*P. brigantiaca*). Five of the 10 listed species (Table 2.2) were not described by either Bailey (1916) or Rehder (1940).

The Desert apricot (*P. fremontii* S. Wats.) also deserves mention among the listed apricot species even though it is not mentioned by any of the above listed authorities. First described in 1880 during a geological survey of California, it was probably unknown or of no interest to Bailey (1916) and Rehder (1940) purposefully excluded North American trees and shrubs from subtropical and 'warmer temperate' regions in his classification key. Being a native to the Mohave and Sonoran deserts, it was naturally not mentioned by Gu et al. (2003) among the *Rosaceae* of China. *P. fremontii* has been represented in some recent molecular studies (Bortiri et al. 2001, 2002) where it has been classified within section Penarmeniaca, along with the desert dwelling species *P. andersonii* A. Gray. While *P. fremontii* differs in many ways from the other mentioned apricot species, it can hybridize freely with them and has morphological characteristics that resemble other apricot species.

From the breeding perspective, more important than the apricot species' placement in any particular classification key are their relevant characteristics that might

Table 2.2 Classification of Chinese apricot germplasm by Lingdi and Bartholomew (2003)

Rosaceae (family)
Prunoideae (subfamily)
Armeniaca (genus) – apricots
A. vulgaris L.
Var. *vulgaris* L.
Var. *zhidanensis* Qiao & Zhu
Var. *ansu* Maxim.
Var. *meixianensis* Zhang
Var. *xiongyueensis* Li
A. limeixing Zhang & Wang
A. sibirica L.
Var. *sibirica* L.
Var. *pubescens* Kostina
Var. *multipetala* Liu & Zhang
Var. *pleniflora* Zhang
A. holosericea Batal.
A. hongpingensis Li
A. zhengheensis Zhang & Lu
A. hypotrichodes Cardot
A. dasycarpa Ehrh.
A. mandshurica Maxim.
Var. *mandshurica* Maxim.
Var. *glabra* Nakai
A. mume Sieb. & Zucc.
Var. *mume* Sieb.
Var. *pallescens* Franc.
Var. *cernua* Franc.
Var. *pubicaulina* Qiao & Shen

make them useful in an apricot improvement program. Key traits and general geographic origins are listed by apricot type in Table 2.3 without regard to their particular classification as distinct species or pomological/botanical varieties.

2.2.2 Eco-Geographical Groups of Apricot

Plant exploration throughout Asia by scientists of the former Soviet Union during the early part of the 20th century led to the discovery of three centers of origin for apricot. One was located in the mountainous regions and adjacent lands of central and western China known as the 'Chinese Center'. Apricot was among the more than 30 temperate fruit-producing crops listed for this region. Nine *Prunus* species including *P. armeniaca* and *P. mume* Sieb. & Zucc. were identified, as were quinces, walnuts, pecans and hazelnut species. A second region with apricots, the 'Inner-Asiatic Center' was defined by the approximate boundaries of northwestern India, Afghanistan, Tajikistan, Uzbekistan and the western Tien-Shan mountains. Smaller in land area than the Chinese center, the Inner-Asiatic center still contained many fruit and nut crops. Besides *P. armeniaca*, this region is known as the center of origin to species of *Vitis, Pistacia, Pyrus*, *Malus* and *Juglans.* The last center of origin for apricot described by Vavilov (1992) was known as 'Asia Minor Center'. The region is defined as the lands of Transcaucasia, Iran and Turkmenistan. More than 15 genera of fruit crop plants were identified in this region.

The Transcaucasian lands were exemplified as being particularly rich in diversity of fruit crops, in all stages of evolutionary development. Forests composed almost entirely of wild fruit trees could be found throughout the region. When clearing forested areas for agricultural development or timbering, the most horticulturally valuable wild fruit trees would be left in place, and local growers were known to graft the most valuable forms onto less desirable seedling fruit trees. Particularly promising local selections were saved and sometimes named (i.e. 'Shalah' apricot) as their popularity increased and they eventually found their course into more mainstream horticulture (Mirzaev and Kuznetsov 1984).

A result of the Russian exploration and collection expeditions was the establishment of a large apricot collection from the different centers of origin at the Nikiti Botanical Garden near Yalta, Ukraine. Over 700 apricot accessions were established there for evaluation and breeding of better adapted types. Kostina (1936) developed a classification key that could characterize any given apricot accession on seed taste (sweet or bitter), type of skin (pubescent or glabrous), stone separation (cling or freestone), flesh color (white and/or cream or yellow and/or orange) and fruit size (small, medium or large). In studying the apricot collection at the Botanical Garden, Kostina (1936) originally described three distinct eco-geographical groups of apricot based on discrete fruit characteristics. Further work by Kostina (1969) re-divided the diverse apricot germplasm into the now well known four eco-geographical groups and 13 sub-groups (Table 2.4).

Table 2.3 Key botanical traits and geographic origin of currently accepted apricot species

Species	Tree stature	Leaf specifics	Bloom specifics	Fruit specifics	Geographic origin
ansu					
Ansu apricot	Bush-like	Broad & elliptic with characteristic cuneate base, highly glabrous	Usually pink petals	Sometimes cultivated, deep & distinct suture, sweet gray-brown flesh, freestone	1000–1500 m in Central China, humid climate
armeniaca					
Common apricot	5–12 m	Ovate to round-ovate, sl. cordate at base, sharply pointed	Variable bloom date	Small to v. large, variable flesh color, glabrous & pubescent forms, bitter to sweet kernels	0–3000 m in Central & West China, Mongolia Central Asia, Japan & Korea
brigantiaca					
Alpine apricot	Small bush to 3 m	Broad-oval to ovate, abruptly short-pointed	Light pink petals, clusters of 2 to 5 blooms	Barely edible, white to yellow skin, glabrous, kernel oil for cosmetics	Foothills of the French Alps
dasycarpa					
Black apricot	Small stature tree, 4–7 m	Elliptic-ovate with closely serrate margin, smaller than common apricot	Large, showy long-stalked flowers	Pubescent purple skin, plum-like with soft/sour flesh, rarely cultivated	A natural plum-apricot hybrid, perhaps only native to Manchuria
fremontii					
Desert apricot	deciduous shrub or small tree, 1–4 m	Roundish to ovate (2 cm) with short petioles, glabrous	Complex branching habit yields intense bloom, flowers small (1 cm) with white petals	Round to elliptic and small (15 mm dia.), yellow/orange with dry flesh, freestone	below 1200 m on dry slopes & canyons of Southern California deserts

Table 2.3 (continued)

Species	Tree stature	Leaf specifics	Bloom specifics	Fruit specifics	Geographic origin
holosericea					
Tibetan apricot	Small tree, 4–5 m	Plum-like form, large & ovate with short petiole, heavy red pubescence on leaf mid-rib underside	Currently unknown	Densely pubescent with a small amount of flesh, medium fruit size, non-dehiscent, large pitted stone	Qinghai, Shaanxi, Sichuan and Yunnan provinces of China 700–3300 m
hongpingensis	Up to 10 m	Elliptic to elliptic-ovate with densely pubescent petioles	Currently unknown	Edible and cultivated locally, 3 to 4 cm fruit width	Western Hubei and Hunan Chinese providences
hypotrichodes	Shrub up to 3 m	Long lanceolate blades with short glabrous petioles, small lanceolate & caducous stipules	Single flowers, glabrous pedicle, blooms before leaf emergence	Style base & ovary are velvety, – other fruit specifics are unknown	Calcareous mountains of China's Chongqing prov. approx. 1400 m
limeixing	Small spreading tree of 3–4 m	Elliptic to obovate-elliptic, glabrous petioles, pubescence in abaxial vein axes	Solitary or groups to 3, 1.5–2.5 cm dia., white petals	Variable skin & flesh color, deep suture, sweet-sour flesh, clingstone	Natural plum-apricot hybrid cultivated in northeastern China, now unknown in the wild

Table 2.3 (continued)

Species	Tree stature	Leaf specifics	Bloom specifics	Fruit specifics	Geographic origin
mandshurica					
Manchurian apricot	5–15 m	Round with long sharp tip, double serrate margin	Typically more pink than white	Generally edible, but flesh sometimes sour, bitter or dry, with apricot aroma	200–1000 m in N.E. China, Korea & Eastern Russia
mume					
Japanese apricot	4–10 m, variable bark color	Narrow to round-ovate, long pointed tip, leaves crinkle naturally	Smaller than common apricot, variable color, double-flower varieties, very early bloom	Generally small with dry flesh, clingstone, highly pitted stones	Below 3100 m in Western Sichuan & Yunnan prov. of China and in Tiawan
siberica					
Siberian apricot	Bush-like or small tree, 5 m	Small, ovate to round, glabrous, but sometimes with pubescence on lower surface	Early and profuse, white to pink color	Small, dry & typically splitting, generally inedible	400–2500 m in diverse habitat, North & Eastern China, Mongolia, Transbaikal Steppe
zhengheensis	Erect form, 35–40 m	Elliptic or oblong with red glabrous petioles, 2–6 nectaries mid-petiole	White petals, 3 cm dia., flowers open before leaves emerge	Typically yellow with possible red blush, succulent & sweet flesh, clingstone & non-dehiscent	Mountainous regions of China's Fujian provi.

Table 2.4 Eco-geographical groups and regional sub-groups of ordinary apricot as defined by Kostina (1969)

Eco-geographical group	Regional sub-group
Central Asian	Fergana Horezm Kopet-Dag Samarkand Sharessyabz Verhnezeravshan
Irano-Caucasian	Dagestan Irano-Transcaucasian
European	Eastern European Western European Ukrainian
Dzhungar-Zailij	Dzhungar Zailij

On an evolutionary timescale, the Dzhungar-Zailij group is said to be the most primitive whereas the European group is believed to be the most recently developed and the product of apricot dispersion from the other eco-geographical groups. Apricots from Central Asia are certainly the most diverse group in their fruit, vegetative and phenological characters. Central Asian apricot trees are generally very long-lived, and they have a longer juvenile period prior to fruit production. Kostina (1936) initially subdivided the Central Asian apricots into two regional subgroups (Fergana and Samarkand) and evaluations of nearly 300 apricots types from these regions in 1928–1929 exemplified the general diversity present in the apricots of these regions (Table 2.5). Two glabrous skinned Central Asian apricot accessions from the Vavilov Research Institute's Central Asian Station in Tashkent, Uzbekistan became available to US apricot breeders upon their release from quarantine in 1993 (Fig. 2.1). Apricots from the Irano-Caucasian group are also very diverse, but are generally shorter-lived than those from Central Asia. 'Shalah' (syn. 'Erevani'), a widely grown apricot from the Irano-Caucasian group, survived plant protective quarantine in the United States and became available to US breeders and interested growers in the late 1990s. Extremely late ripening apricot forms are also present in the Irano-Caucasian germplasm. 'Levent' apricot, from the Anatolia region of Turkey, is said to have a fruit development period of 190–200 days (Asma and Ozturk 2005). Importation and utilization of this germplasm in breeding programs would undoubtedly assist in the extension of the fruit maturation period.

2.3 History of Improvement

2.3.1 Historical Breeding/Selection Efforts

Selection of apricots with superior qualities and their clonal propagation began around 600 AD in China (Faust et al. 1998), and possibly as early in other regions. This is not to say that orchard establishment through the planting of apricot seed

Table 2.5 Differences in fruit characteristics of 273 Central Asian apricot accessions from the Fergana and Samarkand regional sub-groups of Uzbekistan as evaluated during 1928–1929

Fruit character	Percentages of sub-group with this character	
	Fergana sub-group	Samarkand sub-group
Glabrous skin	5	38
Pubescent skin	95	62
Sweet kernel	97	96
Freestone pit	90	85
Clingstone pit	10	15
Large fruit size (>35 g)	3	21
Medium fruit size (20–35 g)	55	57
Small fruit size (<20 g)	42	22

disappeared at this time, as seed-propagated apricot orchards are still commonplace today in some East Asian (Geuna et al. 2003) and North African regions (Khadari et al. 2006).

Apricot breeding perhaps began accidentally after the development of grafting and budding. An astute grower might have selected a few superior trees from seed-propagated orchards, and then passed them on to friends and/or neighbors who clonally propagated them. If this were to happen either simultaneously, or even over the course of many years within a geographical region, an individual grower might find numerous and distinct selected clones within his/her orchard area. Given that these early orchards probably contained self-incompatible trees, fruit within the orchard would have arisen from cross-pollinations only. Without any knowledge of plant

Fig. 2.1 Compared with 'Patterson' apricot (*lower right*), the glabrous skin of the other Central Asian apricot accessions is quite evident. F_1 hybrids between glabrous and pubescent skinned apricots are typically pubescent

breeding, the utilization of seed from the fruits in this sort of orchard would lead to growing out of seedlings from cross-pollination of selected clones. Since it has now been clearly shown that the selection of phenotypic superior parental choices leads to significant genetic gain in apricot (Couranjou 1995, Bassi et al. 1996), the next generation of trees from seed-propagated orchards should have yielded new and variable trees worthy of selection and further propagation.

Named cultivars of apricot began appearing in the European written record during the 1600s, although apricot had been introduced to these areas many centuries before. It appears that these named cultivars were the product of selection only, from seed propagated orchards, or by chance seedlings that developed on their own. Nonetheless, some of these apricots have been important in various regions since their discovery, and have now been used extensively as parents in planned hybridizations. A listing of some of the more important historical apricot cultivars is presented in Table 2.6.

2.3.2 Current Breeding Efforts

Breeding efforts on stone and pome fruits have historically been conducted by public institutions (Table 2.7), with European breeding programs accounting for the majority of apricot improvement. Nikita Botanical Gardens in Yalta, Ukraine, is the longest ongoing apricot breeding program, beginning in 1925, while the majority of the other breeding programs began their work on apricot between the 1960s and 1980s. New cultivar introductions from these programs have numbered 150 during the last 15 years. Besides improved fruit quality traits, environmental adaptation and resistance to diseases are major objectives in many breeding programs. The extension of the fruit ripening season is also a current breeding objective for several programs. Hybridizations between locally adapted apricot accessions and Central Asian germplasm are being performed in several programs to attain that goal (Benedikova 2004, Ledbetter and Peterson 2004).

2.3.3 Repositories and Research Institute Holdings of P. armeniaca *Germplasm*

Considerable amounts of apricot germplasm are being held in repositories for research purposes and conservation of the species. A recent (May 2006) search of the International Plant Genetic Resources Institute (IPGRI) database revealed 62 separate research locations with holdings of *P. armeniaca* germplasm (Table 2.8). Over 6,000 accessions (with duplications) reside at these institutions in the 30 listed countries.

Table 2.6 Notable apricot cultivars in recorded history

Cultivar	Year selected or discovered	Remarks	Reference
Roman	ancient Rome	Most widely grown until 'Moor Park'	Faust et al. 1998
Shalah	unknown	Progenitor of numerous later cultivars, landrace from Armenia, still widely planted in Ararat valley	Mirzaev and Kuznetsov 1984
Nancy	1755	Disc. near Nancy, France, progenitor of numerous later cultivars, many synonyms inc. 'Peach-Apricot'	Bordeianu et al. 1967
Moor Park	1760	Superior to all previously grown apricots, sel. by Admiral Lord Anson near Watford, Herefordshire, England	Faust et al. 1998
Royal	1808	French origin, disc. by M. Hervy, seedling of 'Nancy', named by King Louis XVIII, France	Bordeianu et al. 1967
Blenheim	Bef. 1830	Syn. 'Shipley', intro. by Miss Shipley Blenheim daughter to gardener of Duke of Marlborough Blenheim, England	Hedrick 1925
Luizet	1838	Chance sdlng. found by G. Luizet, widely adapted to Europe & N. Africa	Löschnig and Passecker 1954
Hungarian Best	1868	Disc. and named by E. Lucas in Enyed, Hungary	Löschnig and Passecker 1954
Bergeron	1820	Chance sdlng. of exceptional flavor, from seed obtained at St-Cyr au Mount d'Or, Rhône, France, sel. by M. Bergeron	Lichou and Audubert 1989
Stark Earli-Orange	1920	Disc. in Grandview, Washington by W. Roberts, late-blooming apricot used extensively for resistance to sharka	Brooks and Olmo 1972
Scout	1937	Intro. by Dominion Expt. Sta. in Morden, Manitoba, Canada, sel. from seed sent by Expt. Sta. of Eastern Siberian Railway, Echo, Manchuria	Brooks and Olmo 1952
Perfection	1937	Orig. in Waterville, Washington U.S.A., unknown parents, sel. from seed planted in 1911, progenitor of many N. American cultivars	Hesse 1952

Table 2.7 Current public–sponsored apricot breeding programs throughout the world[1]

Institution & Location	Year Program Began	Named apricot cultivars since 1991	Current Breeding Objectives
South Australian Research & Development Institute Loxton, South Australia	1982	Rivergem (1995), River Ruby (2005), Riverbrite (2005), Rivergold (2005)	For fresh, dry & processing markets, fruit quality traits (flavor, size, fruit color & firmness, high TSS)
Byelorussian Research Institute for Fruit Growing Samokhvalovitchy, Minsk Region, Belarus	1935	Znahodka (1995), Govorukhin's Memory & Memory Loyko (2004), Spadchyna (2005)	First objective is extreme winter-hardiness, tolerance to *Cladosporium carpophilum* & *Monilinia laxa*
Liaoning Institute of Pomology Yingkou, P. R. China	2000	Luotuo Huang (1995), Chuanzhi Hong (1997), Fengren & Guoren (2000)	Fruit quality traits for fresh and drying markets (firm flesh, strong aroma, attractiveness, freestone, high TSS)
Research & Breeding Institute of Pomology Holovousy Ltd., Horice, Czech Rep.	1972	Darina (1999), Kompakta (1999), M-HL-1 rootstock (2002)	New cvs. for fruit quality and appearance, resistance to late frosts and brown rot, compact growth.
Mendel Agriculture and Forestry University in Brno. Horticulture Faculty of Lednice, Lednice, Czech Rep.	1977	Leala & Lebela (1995), Ledana, Legolda, Lejuna, Lemeda, Lenova & Lesorka (1999), Marlen, Minaret, Palava & Svatava (since 2000)	First priority is PPV resistance, also frost hardiness & fruit quality traits (fruit size, firmness, attractiveness, high TSS)
National Agricultural Research Foundation – Pomology Institute, Naoussa, Greece	1982	Lito & Pandora (1991), Neraida, Niobe, Nomia, Nastasia, Nina, Nausika, Nefele, Nostos & Nereis (2001), Tyrbe (2002)	New cvs. for canning & fresh market, PPV resistance is 1st selection criteria Self-compatibility, local adaptation & fruit quality (size, flavor, firmness, color)
Instituto Sperimentale per la Frutticoltura – Sezione de Caserta, Caserta, Italy	1986	No introduced cultivars yet	New cvs. for fresh & processing markets, extended fruit ripening season, high & regular productivity, high quality, Sharka & *Monilinia* resistant.

Table 2.7 (continued)

Institution & Location	Year Program Began	Named apricot cultivars since 1991	Current Breeding Objectives
Instituto de Coltivazioni Arboree – University of Milan, Milano, Italy	1980	Cora & Ninfa (1993), Boreale (1995), Bora (2002), Ardore & Pieve (2004), Priscilla (2006)	Environmental adaptation (rain crack resistant, frost tolerance), PPV & *Monilinia* resistance, self fertility, fruit quality, wide ripening season
Dipartimento de Coltivazione e Difesa Delle Specie Legnose, University of Pisa, Pisa, Italy	1980	Dulcinea & Pisana (1992), Milady (1997), Ardenza, Bona, Cabiria, Kinzica, Maharani, Piera & Salambo (2001), Angela, Caludia, Gheriana & Silvana (2005)	Improved eating quality with good postharvest characters, extension of ripening period, late flowering & environmental adaptation, Sharka & *Monilinia* resistance.
National Agriculture and Food Research Organization, National Institute of Fruit Tree Science, Tsukuba / Ibaraki, Japan	1970	Hachirou & Kagajizou (1997) (these are Japanese apricot – *P. mume*)	*P. armeniaca*: High eating quality, disease & freeze resistance, longevity, self-compatibility. *P. mume*: Self-compatibility, disease resistance, late bloom, early fruit maturity, processing ability.
The Botanical Gardens of the University of Latvia, Riga, Latvia	Late 1940s	Lasma, Daiga & Velta (1999), Jausma & Rasa (2004)	Winter hardiness (late bloom & deep dormancy), fruit quality (freestone, large size, early harvest & attractiveness), tolerance to *Monilinia* & leaf spot
Horticulture and Food Research Institute of New Zealand Ltd., Havelock North, Hawke's Bay, New Zealand	1976	Cluthastar (1991), Cluthalate & Cluthasun (1992), Cluthaearly (1993), Alex, Benmore, Dunstan, Gabriel & Vulcan (1997), Cluthafire & Mascot (1998)	New cvs. with large size, attractive & with good eating quality, good adaptation, precocity & productivity, expansion of ripening season (both early and late).

Table 2.7 (continued)

Institution & Location	Year Program Began	Named apricot cultivars since 1991	Current Breeding Objectives
Baneasa Research & Development Station for Fruit Tree Growing, Bucharest, Romania	1967	Comandor, Excelsior, Favorit & Olimp (1994), Carmela, Dacia, Rares, Sirena & Viorica (2002), Adina, Andrei, Nicusor & Valeria (2004)	Variety development for fresh & industry, disease and pest resistance, climatic adaptability & productivity, extension of fruit ripening season
Irkursk State University, Botanical Gardens Irkursk, Russia	1968	Lubímii (1996), Solnishko (1998), Four advanced selections now in registration process	Cold hardiness & local adaptation are prime objectives, high fruit quality, late bloom, dwarf tree stature
Russian Academy of Science Main Botanical Garden, Moscow, Russia	1957	Aisberg, Alyosha, Favorit, Grafinya, Lel, Monastyrsky, Tsarsky & Vodoley (2005)	New variety development (fresh & processing) for climate of Moscow, reliable long-lived rootstocks
Research Breeding Station, Vesele Piestany, Slovak Rep.	1964	Vesna, Vegama, Veharda, Velbora & rootstock MY-VS-1 (1991), Vesprima & Barbora (1996), Vestar (1997), Veselka, Vemina & Velita (1999)	Resistance to spring frosts, late blooming, high fruit quality, extended fruit season, flesh firmness, processing suitability, disease resistance (*Monilinia*, *Gnomonia*, PPV, ESFY)
Agricultural Research Council of South Africa, Stellenbosch, South Africa	1940s	Ladisun (1991), Charisma (2005)	New variety development for fresh markets (enhanced postharvest quality), canning and drying
Centro de Edafología y Biología Aplicada del Segura. Consejo Superior de Investigaciones Científicas, Murcia, Spain	1986	Rojo Pasión (2001), Selene (2002), Murciana & Dorada (2003)	Self-compatible cultivars of high fruit quality and productivity, early ripening, Sharka resistance
Instituto Valenciano de Agrarias Investigaciones, Valencia, Spain	1993	Two advanced selections are currently in the registration process	Resistance to PPV of prime consideration, early season fruit, fruit quality traits (size, blush, firmness, attractiveness, Brix : Acid ratio)

Table 2.7 (continued)

Institution & Location	Year Program Began	Named apricot cultivars since 1991	Current Breeding Objectives
Estación Experimental de Aula Del Consejo Superior de Investigaciones Científicas, Zaragoza, Spain	1998	No introduced cultivars yet	Rootstock breeding, interspecific hybridization to obtain graft-compatible stocks adapted to heavy & calcareous soils
Institut National de Recherche Agronomiques de Tunis, Tunis-Ariana, Tunisia	1955	Asli, Atef, Fakher, Meziane, Ouafer & Raki (1995)	Combining early-ripening with fruit quality traits (color, firmness, size, sugar & aroma)
Alata Horticultural Research Institute, Mersin, Turkey	1944	Alata Yıldızı, Çağrıbey, Çağataybey, Dr. Kaşka, Şahinbey	New cultivars for fresh market, combine early-ripening with high fruit quality, *Capnodis* resistance
Apricot Research & Application Center of Inonu University, Malatya, Turkey	1996	No introduced cultivars yet	Both fresh and drying types, extended fruit ripening season, Sharka resistance
Mustafa Kemal University, Antakya, Hatay, Turkey	1995	No introduced cultivars yet	Combining superior fruit quality from 'Sakit' population with early – ripening (earlier than 'Ninfa')
Nikita Botanical Gardens National Scientific Center Yalta, Crimea, UKRAINE	1925	Burevestnik (1991), Forum (1992), Krympsk Amur (1993), Aviator (1995) Autok, Alyanc, Divnee Zorkee, Krokus, Pamyati Arevoy & Shedevr (2005)	Introduction of diverse germplasm for hybridization & selection in creating highly adaptable new varieties
Department of Plant Biology & Pathology Rutgers University New Brunswick, NJ UNITED STATES	1955	SunGem (1994), Earlyblush & NJA82 (1995), NJA97 (1996), NJA150 (2006)	Improved cold hardiness, bacterial resistance, Sel. for high quality & attractiveness, extend the ripening season, novel characters (cream flesh & glabrous skin)
USDA / Agricultural Research Service San Joaquin Valley Agricultural Sciences Center parlier, CA UNITED STATES	1955	Helena (1994), Robada (1997), Lorna (1998), Apache (2002), Nicole (2003), Kettleman (2005)	Fresh and processing markets Fruit quality is 1st criteria, wide adaptation, novel fruit characters (modified sugar profile, white flesh, glabrous skin), increased ripe season

[1]Apricot breeders at 27 public-sponsored programs around the world responded to a short query to gather information on new cultivars and breeding program objectives. Other public-sponsored breeding programs might certainly exist, but information is available from only those programs where a query response was received.

Table 2.8 *Prunus armeniaca* L. germplasm resource holdings at national repositories and research institutes as listed by the International Plant Genetic Resources Institute (IPGRI)

Country	No. Accessions	Last Updated[1]
Albania	28	September 1991
Argentina	50	May 2003
Australia	693	October 1990
Brazil	4	May 1999
Canada	294	January 1994
Chile	19	October 1990
Ecuador	7	August 1990
France	406	May 2002
Greece	18	February 2003
Hungary	472	February 1995
India	28	October 1990
Israel	132	March 1995
Italy	1358	March 1995
Macedonia	56	October 1990
Mexico	200	April 1999
Morocco	68	October 1990
Netherlands	4	August1994
Norway	2	April 2002
Pakistan	32	July 1994
Poland	76	March 2003
Portugal	97	November 1994
Serbia & Montenegro	65	April1995
Slovakia	319	April 2002
South Africa	73	October 1990
Spain	212	July 2002
Switzerland	87	April 1995
Turkey	109	May 2002
Ukraine	873	August 1995
United Kingdom	2	April 1995
United States	417	April 2002

[1]Indicates date when IPGRI was last contacted by respective country. IPGRI repository database was queried during May 2006 for accession counts. Current database queries are found at: http://web.ipgri.cgiar.org/germplasm/default.asp

2.4 Problems of Genetic Significance

2.4.1 Fruit Quality

Enhanced fruit quality is the universal goal of all tree fruit breeders. Fruit quality must be sub-divided and specific characteristics evaluated by the breeder in order to measure genetic gain from planned hybridizations. Individual characters that collectively comprise fruit quality include fruit size and the degree of flesh firmness, aroma and flavor characters, color of flesh, skin and overcolor (blush) and fruit juiciness. Each of these characters can be measured objectively with appropriate instrumentation. Couranjou (1995) demonstrated that good genetic gain is possible

in apricot breeding by choosing parents based on fruit phenotype. Thus, parental apricots used in hybridizations that are markedly superior in specific aspects of fruit quality (high overcolor, strong aroma & flavor, or large fruit size) generally pass along those quality characteristics to the next generation of seedlings (Fig. 2.2). Breeding programs based on apricots from the European eco-geographic group could benefit substantially in the development of higher quality fruit by utilizing germplasm from the other eco-geographical groups.

In a principal component analysis of 55 European apricot cultivars, Badenes et al. (1998) demonstrated a significant negative correlation between harvest season and fruit acidity. The lack of sweetness in early season apricots is a common consumer complaint, and a fact that can limit repeat sales of apricots later in the season. Similarly, fruit cracking was also found to be most common in the early maturing apricots. The lack of appropriate parental choices for these characteristics among European apricot clones limits genetic gain. Fruit with lower acidity and a lower potential for cracking in the early harvest season will be common only when parental germplasm with these potentials are identified and utilized in the breeding program's hybridizations.

2.4.2 Self-Compatibility

The self-(in)compatibility status of a tree is an important consideration for both breeders and producers. Opinions are divided with regard to the utility of this trait. Fully self-compatible cultivars can be grown as a monocultural system, eliminating potential problems at bloom and during the harvest period(s) that one might have growing two or more self-incompatible varieties. However, excessive fruit set can

Fig. 2.2 Utilizing apricot accession 'Habiju' (Central Asian germplasm) in hybridizations with California adapted 'Lorna' apricot, and the effect on fruit size. The 'Hibiju' fruit weigh 14 g, while the fruit of 'Lorna' weigh 117 g and the F_1 hybrids weigh 80 g

sometimes be a problem in a self-compatible orchard, and thinning costs can reduce the producer's profit margin significantly. At the same time, fruit set might be ensured in an orchard with a self-compatible cultivar during bloom periods when poor weather conditions limit bee pollination. Through trial and error, fruit set in self-incompatible apricot varieties can be manipulated by the relative number and distribution of pollinator trees in the orchard; however, weather conditions must allow adequate bee visitation.

Self-compatibility in apricot is determined by a single allele, S_c, in a multiallelic series of a monofactorial system (Burgos et al. 1997), analogous to the well-defined system in *P. dulcis* (Mill) D.A. Webb. The locus is found on linkage group 6 (Vilanova et al. 2003). The status of self-compatibility in a given tree can be determined by numerous means, and methods have grown increasingly more complex with advanced methodology. Pre-anthesis bagging of blooming branches with insect-proof bags was probably the first method employed as a means of identifying those trees capable of self-pollination. Self-pollinations and fluorescence microscopy have been utilized very effectively (Burgos et al. 1993), in particular when poor weather conditions might question the validity of bagging studies in the field.

Cross-incompatibility in apricot was first detected amongst three American apricot cultivars all having 'Perfection' apricot in their parentage (Egea and Burgos 1996). Being the first incompatibility group described in apricot, these three cultivars ('Goldrich', 'Hargrand' and 'Lambertin-1') received an identical genotype with the allelic designation S_1S_2. This information is used as a starting point for further testing to find other alleles for self-incompatibility.

2.4.3 Bloom Period and Frost Tolerance

The early bloom period of apricot has limited its cultivation in some areas where it is safe to grow other stone fruits. Freezing temperatures of only a few hours in the late spring can diminish the chance for an economic yield. Breeding programs in regions where this is a problem typically have late blooming periods as major breeding priorities. Bloom date for a given cultivar is determined by both its chilling requirement and its necessary heat unit accumulation after the chilling requirement is fulfilled (Brown 1957, Cesaraccio et al. 2004). Germplasm that is consistently productive in a region where late spring frosts are problematic typically have high heat unit requirements. Selections made from native seedling populations in a region where frequent late frosts occur would undoubtedly be good starting points in hybridization programs.

Apricot germplasm collected from the Hunza region of northern Pakistan was brought to the United States in 1988 for evaluation and breeding (Thompson 1998). While not well adapted to the hot dry conditions present in California's San Joaquin Valley, the imported Hunza apricot accessions did flower significantly later than California adapted apricots. The full bloom date of some of the Hunza apricots

averaged 30 days later than that of apricots typical to California (Ledbetter and Peterson 2004). Hybridizations between California adapted apricots and the Hunza types yielded F_1 trees that segregated widely in bloom date.

An extended bloom period is another means of achieving fruit set in regions plagued by less than optimal spring weather (Benedikova 2004). Sufficient variability in bloom period exists in specific germplasm within some of the eco-geographical groups such that exploitation through breeding could benefit apricot producers in regions where late frosts are ever-present. Irano-Caucasian apricot germplasm collected from Anatolia, Turkey varied greatly in average bloom date, with approximately one month difference between early and late-blooming cultivars (Asma and Ozturk 2005). The Erzincan plain of Turkey is also said to have large native seedling apricot populations from which late blooming forms can be selected (Ercisli 2004).

2.4.4 Disease Resistance

Numerous diseases plague apricot trees in the various growing regions of the world, and the development of resistance to these diseases is a major goal for many breeding programs. Some of these diseases are very widespread, while others are restricted to specific growing regions. *Monilinia laxa* Honey (Brown rot) is perhaps the most widespread and damaging fungal disease for apricot and numerous cultivars have been noted from the different eco-geographical groups that tolerate or resist the disease. A new brown rot fungus, *Monilinia mumecola* Harada, Sasaki & Sano, was recently isolated and characterized from *P. mume* trees infected in Oita Prefecture, Kyushu, Japan (Harada et al. 2004). The seriousness of this new *Monilinia* outside of its point of discovery, and its effect on *P. armeniaca* cultivars is not yet known. Powdery mildew (*Podosphaera tridactyla* DeBary) is also a widespread disease with far fewer sources of resistance or tolerance available. *Xanthomonas campestris* pv. *pruni* Young et al. is responsible for bacterial spot, a disease affecting both foliage and fruit. 'Harcot' and 'Harglow' are two apricots from Ontario, Canada that are said to be resistant to both foliar and fruit infections (Layne 1984). *P. salicina* × *P. mume* hybrids 'PM-1-1' and 'PM-1-4' have also been described as tolerant of bacterial spot (Kyotani et al. 1988). Shothole (*Stigmina carpophila* Ell.) is a prevalent fungal disease in Eastern Europe, and field observations of an apricot collection under disease pressure have shown wide variability in symptom expression (Smykov 1978). The viral disease plum pox or Sharka is becoming increasingly more important in apricot growing regions, as it is disseminated to previously Sharka-free regions by unknowing nursery persons or careless producers. Bacterial canker (*Pseudomonas syringae* van Hall) and Eutypa dieback (*Eutypa lata* Tul.) are two other serious diseases capable of killing trees with a single infection. While both diseases are limited geographically in distribution, there are few cultivars currently known that adequately resist infection.

'Blackheart' of apricot, caused by the fungus *Verticillium dahliae* Kleb., is particularly troubling in orchards where the land was previously occupied by susceptible agronomic crops. Several apricot species (*P. armeniaca, P. ansu, P. mandshurica* and *P. siberica*) have shown susceptibility to this soilborne fungus in controlled greenhouse tests (Gathercole et al. 1987). However, apricot orchards can be easily protected from *Verticillium dahliae* through the use of widely available plum (resistant) rootstocks. *P. armeniaca* is generally regarded as being uniformly immune to root knot (*Meloidogyne* sp.) nematode species and several selections of *P. mume* and *P. dasycarpa* have also shown resistance in field trials (Day 1953, Yoshida 1981). In addition, the use of *P. mume* as a rootstock protects against crown gall (*Agrobacterium tumefaciens*), whereas the *P. armeniaca* seedling rootstock 'Manicot GF 1236' has been proven to be very susceptible (Lichou and Audubert 1989).

2.5 Genetics of Important Traits

2.5.1 Male Sterility

Evidence presented by Burgos and Ledbetter (1994) indicated that male sterility is controlled by a single recessive gene, as in peach. Seedling populations segregating for this character demonstrated that the fresh market cultivar 'Helena' was heterozygous for male-sterility. The Spanish cultivars 'Gitano' and 'Pepito' (syn. 'Pepito del Rubio') have also been shown to be heterozygous for male sterility in controlled crosses (Burgos et al. 1998). Similar to the male sterile peach cultivar 'J.H. Hale', apricot cultivars 'Arrogante' and 'Colorao' have been found to be male sterile and require cross pollination with a pollen compatible male fertile apricot (García et al. 1988).

Male sterility is considered by most breeders to be a fatal flaw for an otherwise superior apricot clone. Since heterozygous individuals have fully functional pollen and are indistinguishable from homozygotes, crossing amongst heterozygotes might be quite common in some breeding programs. Seedling progenies might be left unscreened for male sterility as other duties during the bloom period could have priority over examining whole progenies for this visually apparent character. It might be only at a time near variety release that a breeder discovers that an elite clone is pollen sterile. The fact that male sterility is a discrete trait discernible only after the tree becomes reproductive makes it an excellent candidate for marker assisted selection in a breeding program. Bulk segregant analysis (BSA) was used by Badenes et al. (2000) on a segregating seedling population of apricot in an attempt to identify RAPD markers linked to the male sterility trait. Out of 228 primers used in the analysis, only primer 'M4-950' (Operon Technologies) was found to be loosely linked to male fertile trees.

2.5.2 Amygdalin Content of Seed

Amygdalin content of apricot seed was evaluated by Gómez et al. (1998) in a study designed to examine correlation between phenotypic expression (sweet or bitter seed) and actual amygdalin content. The chromatographic data revealed large numeric differences between sweet and bitter seeded apricot cultivars. Among the bitter phenotypes, significant differences did exist in actual amygdalin content, indicating that perceived bitterness could be influenced by factors other than amygdalin concentration. However, the extent of the numeric differences in amygdalin content between sweet and bitter seeded accessions indicated discrete classes or apricot seed, and not continuous variation. The inheritance of apricot seed taste had been studied previously by Kostina (1969), who determined it to be a simply inherited single gene trait with sweet kernel being dominant to bitter kernel.

2.5.3 Sugars, Acids and Nutrient Composition

Sucrose is the primary sugar present in apricot fruit. Several other sugars such as glucose, fructose, maltose, sorbitol and raffinose are also present to lesser and varying degrees (Witherspoon and Jackson 1995). Collectively, the combined concentrations of the sugars present in apricot are known as the sugar profile, and while absolute concentrations of each sugar in a given accession changes year to year, their relative ratios remain quite constant within any given variety (Bassi et al. 1996). The glucose: fructose ratio has been suggested as an indicator of juice/pulp authenticity for apricot, and glucose: fructose ratios higher than 3.3 suggest adulteration with other less expensive juice/pulp additives (Lo Voi et al. 1995). However, this study was conducted with pulp from 11 apricot varieties common to Italy. Authentic samples of ‘Lorna’ apricot, developed in Central California, have higher fructose levels that boost the glucose: fructose ratio to 4.6 (Ledbetter et al. 2006).

Malic and citric acids are both typically present in apricot fruit, but the predominant acid is dependent on the particular apricot accession. Gurrieri et al. (2001) studied the patterns of sugars and acids in fruit from 51 diverse apricot varieties and found that they differed greatly with respect to the levels of malic and citric acids. Malic acid predominated in 14 of the 51 sampled varieties, and no significant correlations were noted between the levels of malic and citric acids. These authors suggested that taste panels should be used in conducting correlation studies between organoleptic quality and both the levels and kinds of sugars and acids present in fruit. Apricot breeders could also exploit the observed diversity in sugar and acid contents through the employment of appropriate instrumentation as a part of the fruit evaluation procedures.

The consumption of carotenoids in the diet is associated with a degree of protection against cancers and cardiovascular diseases. Total carotenoid content of fruit was associated with the general flesh color class of the apricots (white, yellow, light orange or orange), with light orange and orange apricots having significantly more carotenoid than the white or yellow fleshed accessions sampled in the

study (Ruiz et al. 2005a). The absolute determination of carotenoids in fruit requires precise extraction procedures and a diode array detector equipped HPLC. These analyses can be both time consuming and expensive for a breeding program. Ruiz et al. (2005a) however was able to find a very strong correlation between fruit flesh hue angle and carotenoid content. In a study involving 37 diverse apricot accessions, white fleshed apricots (hue angle $\sim 88^{\circ}$) were found lowest in total carotenoids (2,450 mg/100 g fresh fruit). Apricots of the orange flesh class (hue angle $\sim 72^{\circ}$) were highest in total carotenoids (12,750 mg/100 g fresh fruit). Given these reported findings, it seems reasonable that the apricot breeder can utilize color meter readings to identify those apricot accessions most rich in carotenoid content.

A similar study attempted to correlate apricot fruit color coordinates with the absolute phenolic content of the fruit. Unlike carotenoids, phenolic content of fruit was not related to flesh color. Neither total phenolics nor any specific class of phenolic compounds (procyanidins, hydroxycinnamic acid derivatives, flavonols or anthrocyanins) could be correlated with flesh hue angle or other color coordinates (Ruiz et al. 2005b). Therefore, if the breeder's intention is to identify apricots with particularly high or low levels of phenolic compounds, direct extractions of these compounds are the only reliable means of determining their specific quantity. While extraction procedures for phenolics are not difficult or involved, analysis of phenolic extracts requires authentic samples of the phenolics in question, and a HPLC equipped with diode array detection capability. Levels of both rutin (quercetin-3-*O*-rutinoside) and astragalin (kaempferol-3-*O*-glucoside) were found to differ significantly among apricot accessions in both mature and meristematic leaves harvested and extracted during the active growth season (Ledbetter et al. 2000). Rutin and astragalin are both important diagnostic phenolic compounds in the authentication of jams, marmalades and nectars produced from apricot fruit (Tomás-Lorente et al. 1992, Dragovic-Uzelac et al. 2005).

2.5.4 Aroma and Flavor in Apricot

Tang and Jennings (1967 and 1968) were among the first to conduct research on the aroma profiles of apricot. Several methods of extraction were used by these researchers so that chromatographic profiles could be compared and volatile artifacts detected that were products of any given extraction procedure. Headspace analysis of volatiles from intact fruit as well as simultaneous vacuum steam distillation-extraction of fresh fruit slurries have been used by other researchers to identify and quantify the compounds responsible for typical apricot aroma (Takeoka et al. 1990, Gómez 1993).

The specific methods used for extracting volatiles from apricot determine what will be separated by the gas chromatogram. Heating of the fruit slurry at any time during the extraction procedure increases detection of low-boiling point compounds whereas solvent elutions (without heat) of trapped headspace volatiles favor higher boiling point constituents. Regardless of the extraction methods used, researchers

are in agreement that natural apricot aroma is complex, and the profile of volatile constituents is composed of dozens of compounds from many different classes of chemicals. A wide variety of hydrocarbons, ketones, alcohols, aldehydes, esters and lactones have been identified from both apricot headspace gasses (Gómez and Ledbetter 1993) and solvent extraction-distillation procedures (Guichard and Fournier 1990). Further, there is no clear consensus of the exact mixture of aroma constituents that is responsible for a 'typical' apricot aroma (Guichard 1990).

Varietal differences in apricot aromatic profiles have been documented by comparing apricots grown with the same cultural management/environment and extracted under similar conditions (Guichard 1995, Ledbetter et al. 1996a). When comparisons of aromatic profiles are made between fruit varieties, careful consideration must be taken for having fruit of equal maturity, as many of the key volatile constituents responsible for apricot aroma increase dramatically as fruit approach full maturity (Gómez and Ledbetter 1997). From the perspective of apricot breeding, Couranjou (1995) estimated heritability of fruit aroma for apricot at $h^2 = 0.603$, similar to that of fruit size, fruit firmness and flesh color. Thus, appropriate parental choices of apricots based on their specific phenotype (i.e. high perceived aroma) generally leads to overall improvement of that selected characteristic in the successive seedling population. As a specific example, Gómez et al. (1993) observed paternal transmission of specific volatile constituents from apricot to plum × apricot progeny. Levels of g-decalactone and g-dodecalactone were quantitatively high in plum × apricot seedlings' fruit when the apricot parent's fruit was also high in these important apricot volatiles.

2.5.5 Plum Pox Virus Resistance

Plum pox virus (PPV) or sharka disease has been devastating to the stone fruit industry in Europe during the 20th century. It was originally described in Bulgaria around 1918 and spread throughout Eastern Europe. Two major isolates or forms of PPV, Dideron (D-type) and Marcus (M-type), have been described and characterized in Europe (Candresse et al. 1994), and four other forms are now known to exist. D-type isolates of PPV have recently been identified in apricot and plum accessions at a germplasm repository in Kazakhstan (Spiegel et al. 2004), as well as from a commercial apricot/plum orchard in San Juan Providence, Argentina (Zotto et al. 2006). A mixed infection of PPV (PPV-D and PPV-Rec) has recently been detected in an orchard from Pakistan's Hunza region (Baltistan District) and characterized by ELISA and RT-PCR (Kollerová et al. 2006). The disease is naturally vectored by aphids in the orchard environment.

Because of the ease of spread and severity of sharka disease on economic losses to European growers, much emphasis has been placed on control measures as well as on developing new varieties that resist the virus. Resistance was present in some North American apricot cultivars, and Karayiannis and Mainou (1994) cite Syrgiannidis (1979) for being the first to identify 'Stark Early Orange' and

'Stella' as type-M PPV resistant apricot cultivars. Poor fruit quality and a high chill requirement of these varieties prevented their adoption into traditional European growing regions. However, they were soon used as progenitors in breeding programs to develop new PPV resistant apricot varieties.

The initial breeding efforts in the development of new PPV resistant apricot varieties were tedious and expensive. Quantities of PPV infected GF305 seedling peach rootstock would be needed for each individual seedling coming from planned hybridizations between PPV resistant and PPV susceptible apricots. Each seedling would be budded, many times in replicate, onto PPV infected GF305. The budded stocks would be placed in a darkened cold chamber (7°C) for approximately two months to simulate a dormancy period. Upon return to a greenhouse environment, the budded rootstocks would begin to grow, and symptoms *could* then develop on the emerging leaves. After several months in the greenhouse, plants would many times be pruned back and returned to the cold chamber for another round of simulated dormancy. A subsequent second cycle of growth could then stimulate symptom development in budded seedlings that had not shown symptoms in the first cycle. ELISA could be used to confirm the presence of the virus, and it would generally be employed after symptom development. Seedlings that demonstrated characteristic PPV symptoms and tested positive for ELISA would naturally be scored as PPV susceptible. PPV tolerant seedlings would be those showing no visible symptoms after a given number of growth cycles on PPV infected GF305 rootstock, but testing positive by ELISA. Resistant seedlings would neither test positive in an ELISA nor demonstrate visible symptoms after repeated growth cycles.

Current research on PPV resistance in apricot follows several paths. As evidenced in the current breeding objectives column of Table 2.7, many European programs are attempting to develop PPV resistant varieties that are adapted to their local conditions and tastes. Variety development populations are also used by some researchers to assist in developing inheritance models for PPV resistance as well as in molecular mapping studies for targeting the location of PPV resistance gene(s) in the apricot genome.

Numerous PPV resistant apricot varieties were discovered through large field screenings at the Pomology Institute's orchards in Naoussa, Greece (Karayiannis and Mainou 1994). Natural transmission by aphids spread the virus from infected peach orchards to the adjacent replicated apricot plots. After at least four years of growth, the resistant cultivars were evident amongst the mostly susceptible apricot germplasm. Resistance of both 'Stark Early Orange' and 'Stella' was re-confirmed, and apricot cultivars 'Goldrich', 'Harlayne', 'Henderson', 'NJA2', 'Sunglo' and 'Veecot' were deemed resistant to PPV through a lack of symptoms in field trials as well as subsequent ELISA. Concurrently, hybridizations had been undertaken at this Institute with PPV resistant Stark Early Orange and the traditional Greek cultivar 'Tirynthos'. Selection from the seedlings of this population led to two new PPV resistant apricots 'Lito' and 'Pandora', both introduced in 1991.

It has been of considerable interest that the original sources of PPV resistance in apricot came from North American cultivars. Since the PPV susceptible European

apricot cultivars lack molecular markers common to the Asian apricot germplasm, it has been suggested that perhaps PPV resistance in the North American apricots came from *P. mandshurica* germplasm that was used in the distant pedigrees of North American cultivars (Badenes et al. 1996). A single accession of *P. mandshurica* was used in hybridizations with 'Currot' (PPV susceptible) to determine the worthiness of *P. mandshurica* in transmitting resistance to seedlings (Rubio et al. 2003). All seedlings from the cross were found to be PPV susceptible. However, given the diversity of this species in its center of origin and the unknown nature of the single examined accession, it is still quite possible the PPV resistance does reside within this botanical form.

Different isolates of PPV have complicated some analyses, and at least one case of differential resistance in apricot cultivars has been reported. 'Harcot' was determined PPV susceptible in Greek field tests with the predominant 'M' isolate (Karayiannis and Mainou 1994) whereas this same cultivar was found PPV resistant when the 'D' type Spanish isolate was employed under greenhouse conditions (Martínez-Gómez and Dicenta 2000). A survey of popular *Prunus* rootstocks was also conducted recently to identify those resistant to the Spanish D-isolate. After artificial inoculation and four complete cycles of growth/artificial dormancy under controlled conditions, *Prunus* rootstocks 'GF677' and 'Myrobalan 29C' were found free of PPV symptoms as well as being negative in ELISA and PCR assays. Rootstocks 'Marianna 2624' and 'Nemaguard' were both PPV susceptible based on symptom expression and laboratory assays (Rubio et al. 2005). In a test with six different PPV isolates, apricot cultivars 'Harlayne' and 'Betinka' were shown to be highly resistant or immune to all isolates during the three year examination period (Polák et al. 2005).

There are several published accounts of the inheritance mode for resistance to PPV in apricot. All associate resistance with the presence of one or more dominant genes. Nearly 300 seedlings segregating for PPV resistance and susceptibility from 20 different cross combinations led Dicenta et al. (2000) to believe PPV resistance was controlled by a single dominant gene. Symptoms were recorded after one or two cycles of growth/artificial dormancy and corroborated with laboratory ELISA. Vilanova et al. (2003) used a 76 seedling population from the self-pollination of 'Lito' apricot for molecular mapping of SSRs and AFLPs as well as for establishing the segregation of resistant: susceptible seedlings. These researchers observed a 46:30 ratio (resistant : susceptible) which deviated significantly from a 1:1, but fit a 9:7 ratio that could be expected if resistance were controlled by two dominant genes.

A three dominant gene model was proposed recently by Salava et al. (2005) using 'Stark Early Orange' as a PPV resistance donor. This study differed from the two previously mentioned ones in that the more aggressive M-type PPV isolate was used. Salava et al. (2005) allowed at least 3 complete cycles of active growth/artificial dormancy prior to final scoring of segregation ratios. Resistant plants were only considered as those with visual symptoms and either positive ELISA or PCR during the last three growth cycles. This study documented changes over time in the ratio of resistant to susceptible seedlings. Higher numbers of resistant (symptomless) plants were observed after the first growth cycle as compared to after the third cycle.

As was discussed by these researchers, there might be variability in the amount of time necessary for any particular genotype to express PPV symptoms. Furthermore, a 'false susceptible' might be a result of an insufficient time period after inoculation, prior to the plant's recovery and elimination of the virus. The combined results of three specific crosses with over 200 segregating seedlings yielded a 1:7 segregation ratio (resistant : susceptible), indicating a tri-genic mode of inheritance.

Linkage of a molecular marker to PPV resistance would be a huge benefit for apricot breeding programs in sharka infested areas or even in areas where the disease is not yet present. PPV resistance has been mapped with a diversity of molecular markers in several studies (see section on genetic mapping and QTL analysis). Soriano et al. (2005) has also characterized apricot resistance gene analogs (RGAs) for the development of specific AFLPs that are tightly linked to PPV resistance. An RGA marker, SEOBT101, has recently been identified as an amplification product only in PPV resistant apricots. The marker was present in the six tested PPV resistant accessions ('Stark Early Orange', 'Lito', 'Pandora', 'Stella' and two breeding selections from the Department of Tree Culture, University of Bologna, Italy) and failed to amplify in the 10 examined PPV susceptible apricot cultivars (Dondini et al. 2004).

2.6 Crossing and Evaluation Techniques

2.6.1 Pollen and Seed Management

Pollen is typically collected from flowers in the 'balloon' stage, prior to the unfurling of petals and anther dehiscence. This is best done when the flowers are dry, after any morning dew has evaporated. The harvested flowers can be brought back to the laboratory in small paper bags, and dozens of samples can be collected from the orchard and stored in a small cold ice chest prior to laboratory handling. A coarse metal-wire kitchen sieve is used to render the anthers from the bulk of the floral tissues. The anthers are collected on clean paper as the flowers are carefully rubbed through the sieve. The anthers are then dried overnight at room temperature. A 60–100 watt incandescent lamp placed approximately 30 cm above the sample aids in the drying process.

Dried anthers are then placed in a smaller nylon fine-mesh sieve to remove any dry floral tissues and to break open the anthers. The pollen and anthers are again collected on clean paper. Rubbing the dry sample is seldom necessary as the sample can be easily separated with a few light taps to the side of the sieve. Pollen/anthers are stored in appropriately labeled vials in the freezer.

Viability of pollen can be easily examined with a germination test. Petri dishes are prepared for this purpose with a 12% sucrose solution and 0.5% gelling agent. When dishes are cool, pollen can be distributed over the medium by gently tapping a pollen coated brush on the dish's edge to release and distribute the pollen on the surface of the medium. These dishes are stored under refrigeration (2–7°C) and

Fig. 2.3 Emasculated apricot flowers receive pollen in a planned hybridization. Given favorable weather conditions, emasculated flowers are receptive to pollen for approximately one week

the samples are scored in 12–36 hours with the aid of a dissecting microscope. Pollen samples are kept in the freezer when not in use and in a cold ice chest when being transported to and from the orchard. In this manner, samples can normally be used with good results for two seasons. Pollen can be applied successfully with the fingertip (Fig. 2.3), a small paint brush or with other small applicators.

Fruits containing hybridized seed are handled in a manner similar to the other stone fruits. There are many variations in the specifics of handling seed of *Prunus* (Grisel 1974), and procedures are typically modified to suit the individual program and its resources. In our situation, we prefer to harvest fruit with hybridized seed while still a bit under-ripe. This is actually for sanitation, to reduce the amount of free sugar available for contaminant bacteria and fungi. Seed are cut from the pits, taking care not to cut the seed open or damage the seed coat. They are then surface disinfested with a cleaning agent and rinsed thoroughly with sterile water to remove any residue. Seed are stored in zip-closure bags containing a pre-moistened/autoclaved filter paper to provide moisture during the stratification period. The bags are then held in a common household refrigerator (1–2° C) and checked periodically during the stratification period for contamination and whether they require more moisture. A high percentage of the seed germinate during the stratification period.

The early fruit ripening period of apricot can benefit to the breeder as it is possible to perform hybridizations, collect and stratify the seed and plant the seedlings in the same calendar year. However, one or more things might limit this possibility,

with a large part depending on a program's resources and length of the growing season. For example, in Central California where we harvest our hybridized seed during May and June, it is possible to have seed ready for greenhouse planting by September 1. However, certain constraints and responsibilities limit our field transplanting possibilities until after the bloom period. Therefore, we choose to hold stratified seed under refrigeration until November. Seed planted in the greenhouse at this time produce healthy and vigorous seedlings, and are ready to field transplant in the March time frame. The greenhouse planting of seed prior to November leads to larger and more root-bound seedlings that can complicate the field transplanting procedure.

Seed are generally planted in the greenhouse when most of their roots extend 2–5 cm. The seed are planted at the soil level in flats with a soil depth of approximately 10 cm. Typically, each seedlot is allowed to soak in a shallow pan of water before it is planted. Seed coats are removed prior to planting, and the soaking period greatly facilitates this procedure. Apricot seed coats have been shown to have an inhibitory effect on seed germination (Chao and Walker 1966) however, they are primarily removed to allow easier emergence of the elongating shoots. Seed are commonly planted on a 2.5 cm grid within the flat and initially drenched with a fungicide to reduce pressure from damping off organisms. The flats are watered deeply and infrequently, and allowed to dry down prior to re-wetting.

Apricots exhibit hypogeal germination, and the cotyledons remain in place at the soil surface as the seedling begins to develop. When the seedlings attain approximately 15 cm in height, they are pruned back to their 4th or 5th true leaf. This is done to strengthen the small stem and to allow a higher rate of survival upon field transplanting. The seedlings will typically produce multiple shoots with this treatment, and they are pruned individually to their single strongest shoot. The general pruning treatment is performed again after the majority of the seedlings have again attained 15–20 cm in height. The pruning cut is made to allow just a node or two to grow above the level of the first pruning cut. This cycle can occur from four to six times prior to field transplanting, with the seedling stem diameter increasing with each pruning cycle.

Recent advances in breeding for earlier ripening apricots has led to a situation where there is a noted reduction in viability for seed from very early ripening cultivars. Seed from cultivars such as 'Apache' and 'Poppy' appear normal compared to seed from later-ripening cultivars, and will germinate after sufficient stratification. However, seedling emergence is reduced, and many of the emerged seedlings of very early-ripening cultivars die in the seedling flat. The in vitro culture of apricot embryos is a solution to this problem (Burgos and Ledbetter 1993).

2.6.2 Fruit Evaluation

The unlinking of visual appeal and fruit taste can be accomplished through the use of a formal tasting panel. For apricots and the other stone fruits, taste can be ranked by

participants on the panel in specific descriptive categories: sweet, sour, astringency, flavor, texture and juiciness. Visual appeal and all the descriptive categories of taste in apricot can be directed and improved through selective breeding.

The evaluation of fresh fruit quality in new apricot accessions requires a knowledge of the quality characteristics of competing cultivars, or those that would be available during the same maturity season. The competing cultivars should be grown with similar orchard conditions and cultural practices, and harvest maturity of the different apricot accessions must be very similar in order to have valid comparisons. Flesh firmness is often used as a measure of harvest maturity, as it can be measured objectively and quickly with widely available instruments. Thus, when apricot samples from two different accessions do not differ significantly in flesh firmness, other quality characteristics such as Brix, acidity and flesh color can be compared validly with appropriate measures. Prior to the release of 'Lorna' apricot (selection K505-50), its fruit were compared objectively with fruit from 'Katy', a cultivar that ripens during the same period. Fruit from K505-50 had significantly higher Brix, significantly lower juice acidity and flesh with a significantly lower hue angle (deeper orange flesh) than fruit from 'Katy' in samples that did not differ significantly in flesh firmness (Ledbetter et al. 1996a).

There are other quality characteristics that are important for processing apricots, depending on the particular industrial use. Because large quantities of apricots are typically processed for any given industrial use, the percentage of usable flesh in a given apricot shipment is of primary importance to the processor. Brix, acidity and juice pH are also important measured parameters for apricots at any industrial starting point. It is extremely important to examine the particular industrial product and evaluate quality during what would be considered a normal storage period.

Fruit softening during storage is a major problem in canned apricots, and citrates present in the apricot juice have been implicated in chelation reactions that lead to an unacceptably soft canned product (French et al. 1989). Developing new apricots specifically for canned product might therefore involve a closer examination of the acids present in newly selected accessions. Citric and malic acids are most predominant in apricot fruit, and within a collection of apricot accessions, large differences exist in the levels of each acid per each accession. However, the ratio of malic to citric acids remains relatively constant year to year in a given accession, allowing for selection of germplasm with the desired contents of specific acids (Bassi et al. 1996). Hence, fruit evaluation of new apricot material for canned product might involve measurements of malic and citric acids in order to identify those types less prone to fruit softening after canning.

In dried apricot, color retention during storage is a major concern. Storage at a higher temperature exasperates the problem, but even during cold storage, darkening of the product affects marketability (Ledbetter et al. 2002). The degree of darkening during storage is influenced by the particular apricot accession, and one discovers whether or not an accession is suitable for drying by actually putting it through the drying process. Immediately after drying, baseline color coordinates such as

Luminosity and Chroma can be established with one of the available tristimulus colorimeters, and the dry product can then be sampled periodically during storage to establish rates of change. Stone freeness is certainly of equal importance in selecting new apricots for drying quality. Processing speed and efficiency is severely affected on both mechanical and hand-cut lines if the apricot stone clings to any of the cavity flesh.

2.6.3 Trialing and Variety Introduction

Trialing of advanced selections with a commercial grower is extremely important to fully realize a clone's performance. The trial of experimental apricot selection(s) and competing cultivars should be sized realistically with both the grower and breeder in mind. In some cases the trial grower might choose to top work the experimental selection into trees of an existing orchard, perhaps within the orchard of the competing cultivar. Doing so provides the opportunity to observe both the new selection and the competing cultivar throughout the year, and eases comparisons of growth habit, as well as seasons of bloom, fruit maturity and fall senescence. New orchards for trialed varieties can also be established, and in this case evaluations can be conducted on trees grown on the same rootstock, an important consideration for self-incompatible varieties where bloom matching with another variety is critical. Whatever the makeup of the trial orchard, it must be sufficiently large such that commercial quantities of fruit can be treated in an identical manner with other varieties. When it is probable that a new variety's destiny is predominantly for large scale producers or export markets, then it is important to have sufficient fruit to be convinced that the new selection performs adequately during the harvest and packing operation. Pre-conditioning and/or cold storage, and perhaps quarantine treatments might be applied to determine the effects on the new apricot selection(s). If the new variety's destiny is intended for smaller growers who would market fruit locally, then a smaller-scale orchard trial might be more appropriate. Fruit of greater maturity is typical of the local 'farmers markets', so a breeder could get an indication the bruising potential of new selections as well as customer opinions on fruit quality, by conducting smaller-scale trials where fruit of higher maturity is handled.

If there is insufficient fruit from the trialed trees for marketing, the grower will not be motivated to harvest the fruit, or work the trees in a manner similar to the existing varieties that are being commercially productive. Hence, the breeder must allow trials to be large enough such that the producer has the opportunity for a successful commercial harvest. A single flaw in an experimental selection can make the apricots unworthy for market, so trials should not be so large as to produce an unnecessary financial burden on the producer. A large failure with a grower who is new at trialing experimental selections might sour the grower's opinion of providing such assistance to the breeder again in the future.

2.7 Biotechnological Approaches to Genetic Improvement

2.7.1 Cultivar Fingerprinting and Phylogenetic Studies

The analysis of plant isozymes was an early technique used for hybrid verification (Byrne and Littleton 1989a) and to characterize apricot germplasm (Byrne and Littleton 1989b), but low numbers of useful (segregating) loci limited the effectiveness of the technique in establishing precise relationships among closely related accessions of a given species (Badenes et al. 1996). DNA fragment based analyses have proven more useful in discerning similarities or differences between apricot cultivars or between accessions in different eco-geographical groups. There are many examples of apricot cultivars having different names that have been successfully 'fingerprinted' to demonstrate their genetic origin. Other examples of the technique's usefulness are the ability to identify mistakes in a cultivar's pedigree, or to demonstrate genetic identity of new cultivars, and thereby insist or provide evidence regarding the protection of plant breeder's rights. As a key example, Ahmad et al. (2004) utilized a set of 28 single sequence repeat (SSR) primers on a specific set of apricot, Japanese plum, plumcot and pluot™ cultivars. Developed by a private plant breeding company in California, a pluot™ is said to be the product of backcrossing a plumcot (*P. salicina* × *P. armeniaca*) with a plum, thereby creating a novel new fruit type with 25% apricot germplasm in its pedigree. Since their arrival in California nurseries in 1989, the pluot™ cultivars have steadily increased in acreage and fruit volume to present levels of approximately 5 million 12 kg boxes (California Tree Fruit Agreement, 2002). Consumers have paid dearly at the marketplace for this novel new fruit type, but the SSR analysis made by Ahmad et al. (2004) found no alleles specific to apricot amongst any of the six tested pluot™ cultivars. This research has the potential to affect the marketing of these 'novel' fruits as well as pesticide labeling information.

When genetically and geographically diverse germplasm has been compared in molecular phylogenetic studies, good separation is usually discovered between species and/or subgenera. Such was the case with a RAPD analysis conducted by Shimada et al. (2001), on 40 *Prunus* accessions representative of four subgenera (*Prunophora*, *Amygdalus*, *Lithocerasus* and *Cerasus*). In another RADP analysis of a genetically diverse group of 35 apricot cultivars that included a large group of Japanese cultivars, as well as cultivars from China, Europe, Nepal, Turkey and North America, all the Japanese germplasm grouped together and separate from apricots of other origin (Takeda et al. 1998). Apricots from China, Europe, Nepal, Turkey and North America were represented together in another single group. These researchers also found that the Turkish cultivars 'Hajihaliloulu' and 'Hasanbay' were probably identical, being inseparable in both plant morphology and RAPD analysis. In an early restriction fragment length polymorphism (RFLP) study using chloroplast DNA, Uematsu et al. (1991) successfully discriminated non-domesticated *Prunus* species (*P. mira* Wilson., *P. davidiana* Maxim.) from several diverse cultivated varieties (*P. persica* Sieb and Zucc., *P. domestica* L., *P. armeniaca* and *P. mume*).

Hagen et al. (2002) examined genetic diversity within a group of 53 apricot accessions (47 diverse cultivars and 6 related apricot species accessions) with AFLP markers. *Prunus ansu*, *P. mume*, *P. dasycarpa* and *P. brigantiaca* were well separated from *P. armeniaca* in their analysis. All cultivars had unique AFLP profiles and segregated into four clusters. In another AFLP study of 118 apricot accessions representing Europe, China and North America, Geuna et al. (2003) provided evidence that the North American cultivars were created from a complex blend of germplasm from both Europe and China, although only 17% of the total observed variation was described by the first three principal components. In agreement with the AFLP-based study by Hagen et al. (2002), they found the cultivars 'Erevani' ('Shalah'), 'Stella' and 'Veecot' to be located in close proximity, but they differed in the placement of 'Goldrich' and 'Harcot'. These two cultivars were clustered together by Hagen et al. (2002), whereas they found themselves in completely different clusters in the study by Geuna et al. (2003). In a much smaller AFLP-based study designed specifically to examine genetic variability within the progenitors of a breeding program with the objective of developing sharka resistance, 'Goldrich' and 'Harcot' clustered together amongst other sharka resistant accessions (Hurtado et al. 2002). In another unrelated study 'Goldrich' and 'Harcot' were placed in different clusters by Khadari et al. (2006) in an AFLP study examining the uniqueness of Tunisian apricot germplasm relative to cultivars from other geographical regions.

Peach and cherry SSRs were used to study a group of 48, 40 and 74 apricot accessions by Hormaza (2002), Romero et al. (2003) and Zhebentyayeva et al. (2003), respectively. Hormaza (2002) studied European and North American apricot germplasm, along with a single Chinese cultivar, 'Piu Sha Sin'. Romero et al. (2003) focused their analysis on a somewhat geographically wider collection including apricots from Europe, North America, and Central Asian origin, as well as Central Asian × European apricot hybrids. Zhebentyayeva (2003) compared 74 apricot accessions representing both domesticated and wild forms from all four eco-geographic groups. The French traditional cultivar 'Bergeron' was the only apricot accession common to all three studies. The total number of SSR primers analyzed in these studies were 20, 16 and 14 for the studies by Hormaza (2002), Romero et al. (2003) and Zhebentyayeva et al. (2003), with only a single SSR (98–406) common to all three studies. While these studies separated most cultivars successfully, there were some dissimilarities. In the Hormaza (2002) study, 'Bergeron' and 'Gönci Magyar' appeared quite similar whereas 'Canino' and 'Pandora' were very different. This contrasts with results from Romero et al. (2003) where 'Bergeron' and 'Gönci Magyar' were similar and 'Canino' and 'Pandora' resided as neighbors on the dendrogram.

An even larger SSR phylogenetic study was conducted in by Maghuly et al. (2005) with newly developed SSRs from the apricot genome. Over 130 apricot cultivars representing Europe, Central Asia, Irano-Caucasian region and North America were screened with the broad goal of grouping the accessions by their eco-geographic origin. Ten of the 120 then known apricot-derived SSRs (Lopes et al. 2002, Messina et al. 2004) were used to elucidate the similarities and/or differences between the accessions. The UPGMA dendrogram presented is complex, and the authors

broadly state that the position of Central Asian cultivars in the dendrogram supports the notion that most of the tested cultivars are of Asiatic origin. Based on their analysis, numerous cases of synonymous cultivars could be identified ('Alberna' = 'Andormaktájai Magyar kajszi' = 'Crvena ungarska' = 'Gönci Magyar kajszi' = 'Naggyümölcsü vagyar kajszi'; 'Kalasek' = 'Krasnoshchokijiz Nikolajeva 1486'; 'Cacansko zlato' = 'Magyar kajszi 235'; 'Chershonskij 1469' = 'Paksi Magyar kajszi'; 'Ceglédi óriás' = 'Ligeti óriás' = 'Szegedi mamut' and 'Kecskemet early' = 'Rosensteiner'); however, other reportedly synonymous cultivars did not group as genetically identical ('OrangeRed' and 'Bahrt', 'Erevan' and 'Shalah').

Other noteworthy placements of cultivars in the UPGMA dendrogram of Maghuly et al. (2005) are of practical breeding interest. 'Morden 604' and 'Kletnice' were placed as virtual outliers to all others in the chart. While 'Kletnice' is of Czech origin and of unknown parentage, 'Morden 604' was developed in Canada, from the cross 'Scout' × 'McClure'. 'Scout' was selected from seed of Siberian origin, perhaps being *P. manchurica* or *P. sibirica* (Brooks and Olmo 1952). The broadly adapted cultivar 'Goldrich' clusters with six other cultivars including the French cultivar 'Bergeron', two seedling selections and 'San Castrese' from Italy, and the Eastern European apricots 'Marille Bauer' and 'Spätblühende Koch'.

Overall, the researchers working on SSR-based phylogenetic studies of apricot germplasm have concluded that European cultivars have a narrower genetic base compared to the other eco-geographic groups. Most have also pointed out the need for diversification in apricot breeding programs. Cultivars and/or landraces from the other eco-geographic groups (Central Asian, Dzhungar-Zailij, Irano-Caucasian) need to be used in variety development (North American or European) in order to obtain wider climactic adaptation, disease resistance, lengthened fruit developmental periods and other diverse and interesting characters. These same suggestions were put forward by Kostina (1936) in her comprehensive whole plant studies of the 1920s and 1930s. Central Asian apricot germplasm has been utilized to breed higher quality California-adapted apricots since the early 1990s (Ledbetter and Peterson 2004, Ledbetter et al. 2006). High Brix, long fruit development period, strong fruit attachment, white flesh and glabrous skin are all available to the breeder with access to this germplasm. Fruit sizes of many Central Asian apricots are small, but good progress can be made in a single round of hybridization with a European parent.

2.7.2 Stylar Ribonuclease Characterization

Beyond bagging trials at bloom time to exclude bee visitation and fluorescent microscopy to examine pollen tube growth in planned self-pollinations, the next advancement in analyzing self-(in)compatibility in apricot involved characterizing stylar ribonucleases. Emasculated flowers were gathered from numerous apricot cultivars and evaluated on polyacrylamide gels for ribonucleases activity. Among the initial examined cultivars were 'Goldrich', 'Hargrand' and 'Lambertin-1'.

Non-equilibrium pH gradient electrofocusing was used to separate the stylar proteins, and after staining identical banding patterns were observed for 'Goldrich', 'Hargrand' and 'Lambertin-1' (Burgos et al. 1998). Stained bands migrated differently for other cultivars, and it was possible to assign other allelic designations on the basis of these tests. In other studies, the male-sterile cultivar 'Colorao' was determined to carry alleles for self-compatibility, although it was incapable of effecting self-pollination. When it was used as a female parent in a cross with self-compatible 'Pepito', several seedlings were obtained that proved to have the S_cS_c genotype (Alburquerque et al. 2002). These authors pointed out the general interest of apricot breeders in possessing germplasm homozygous for self-compatibility.

The self-(in)compatibility locus has been examined and characterized in a number of *Prunus* species including almond, both sweet and sour cherry, and Japanese apricot. Similarities exist in each of these species: stylar ribonucleases genes all contain two variable sized introns, five conserved regions (C1, C2, C3, RC4 and C5) and a hypervariable region (RHV) that is putatively recognized as the recognition site for the S-determinant in a pollen tube. An area known as the S-locus F-box (SFB) gene, is tightly linked with the stylar ribonucleases gene such that the two genes are inherited as a single unit. Recombination between the two is thought to be suppressed by a high content of repetitive sequences present between them (Ushijima et al. 2003). SFB genes are expressed specifically in the developing pollen.

The identification of self-(in)compatibility alleles from leaf tissue (as opposed from floral organ tissues) would aid breeders by allowing selection for self- compatibility (or any specific known allelic structure) at a very early stage of development. To achieve this, stylar ribonucleases DNA sequences specific to certain self- incompatibility groups needed to be characterized so that PCR-based primers could be developed for further identification of new self-incompatible alleles. Romero et al. (2004) characterized three stylar ribonucleases alleles (S_1, S_2 and S_4) from 'Goldrich' and 'Harcot' apricots and confirmed that they were linked closely to SFB genes as had been found in other *Prunus* species. Amino acid identity amongst these three alleles averaged 75.3%, indicating a high level of sequence diversity. Using other cultivars, Vilanova et al. (2005) characterized the other four known self-incompatibility alleles (as well as S_c) with consensus primers developed from stylar ribonucleases genomic sequences of apricot and sweet cherry. These developed protocols are now a tool available to the apricot breeder, and self-compatibility can be determined in the seedling flat with meristematic tissues.

2.7.3 Genetic Mapping and QTL Analysis

Several genetic linkage maps of have been generated for apricot. Hurtado et al. (2002) used AFLP, RAPD, RFLP and SSR markers to map 81 F_1 individuals from the cross of 'Goldrich' × 'Valenciano'. A total of 132 markers were placed on eight linkage groups of 'Goldrich', with a coverage of 511 cM. A total of 80 markers were placed into seven linkage groups of 'Valenciano' that defined 467.2 cM. Two

codominant markers were located on linkage group 2 that flanked sharka resistance. Vilanova et al. (2003) generated a map using 76 individuals from a self-pollination of 'Lito' ('Stark Early Orange' × 'Tyrinthos'). A total of 212 markers (180 AFLPs, 29 SSRs and two agronomic traits) were assigned to 11 linkage groups spanning 602 cM. Plum pox resistance was mapped to linkage group 1 and the self-incompatibility trait to linkage group 6. Twenty two loci were held in common with other *Prunus* maps and most of them showed the same linkage relationships. Lambert et al. (2004) examined 142 F_1 apricot hybrids from the cross 'Polonais' × 'Stark Early Orange' using 83 AFLPs, 88 RFLP and 20 SSRs from the *Prunus* reference map of almond 'Texas' × peach 'Earlygold' (T × E). A total of 110 markers were placed on a map of 'Polanais', covering 538 cM, and 141 markers were located on a map of 'Stark Early Orange', defining a length of 699 cM. Almost all markers could be aligned with those from the T × E map. Salava et al. (2007) developed an integrated genetic linkage map with 316 molecular markers (290 AFLPs, 26 SSRs) using a backcross progeny of 'LE-3246' × 'Vestar'. They assigned markers to 8 linkage groups covering 574 cM and found several markers linked to the *PPVres1* locus conferring resistance to PPV. Vilanova et al. (2006) hybridized sixteen SSRs to a BAC library and were able to identify clones belonging to the G1 linkage group.

2.7.4 Micropropagation/Plantlet Production

Literature reports of apricot micropropagation first appeared in the late 1970s (Skirvin et al. 1979). Pérez-Tornero et al. (1999a) developed successful techniques for meristem tip culture as a means of eliminating the persistent endophytic bacteria typically found in field-grown trees. Snir (1984) utilized growth chamber grown 'Canino' apricot shoots rather than field grown materials to avoid the heavy infestation problems of the later. Snir's work was focused on simply developing an effective in vitro rooting technique for apricot, as both softwood and hardwood cuttings of *P. armeniaca* L. are difficult to root, and other research at that time had demonstrated that fruit trees grown on their own root had better nutrient uptake and higher productivity (Couvillon 1982, Thibault and Herman 1982). Snir (1984) observed that MS medium was completely unsuccessful in supporting vegetative growth of 'Canino' apricot, but Woody Plant Medium (WPM) (Lloyd and McCown 1980) allowed a high percentage (70%) of the buds to elongate into shoots. Rooting of the elongated shoots was effectively accomplished on ½ strength MS medium supplemented with 0.5 mg L-1 1-naphthaleneacetic acid (NAA).

Marino et al. (1993) studied proliferation and rooting ability of apricot cultivars San Castrese and Portici in modified MS medium. Specifically, these researchers examined differences in the in vitro growth rate as related to different carbon sources. Sorbitol as a carbon source enhanced the proliferation rate of both cultivars, as did increasing the 6-benzyladenine (BA) concentration. However, high levels of BA (8.8 mM) in the proliferation medium caused hyperhydricity in the explants, in particular when sucrose was the medium's carbon source. While sorbitol enhanced

the proliferation rates of both apricot cultivars, lower percentages of rooting were reported when sorbitol was used as the carbon source in the rooting medium. A 70% success rate of rooted plantlets was reported by Marino et al. (1993) using a modified MS rooting medium with the inclusion of indolebutyric acid (IBA). Hyperhydricity in proliferating in vitro cultures has been a reported problem in other *Prunus* species (Rugini and Verma 1982, Ledbetter et al. 1996b). Basal cooling for several weeks during proliferation has been an effective treatment in reducing hyperhydricity among cultured apricot genotypes. The level of hyperhydric explants in specific apricot cultivars is also influenced by the medium's gelling agent (Pérez-Tornero et al. 2001).

Further work on the traditional Spanish apricot cultivar 'Canino' was carried out by Pérez-Tornero et al. (1999a and 2000b) to identify a more suitable nutrient medium to support healthy growth and enhance proliferation. Working on four apricot cultivars, they found meristem survival in culture was significantly affected by cultivar, as well as interactions of the cultivar with BA concentration, and the interaction between BA and gibberellic acid (GA). Establishment medium prepared without BA prevented all cultivars from developing rosettes of leaves and elongating into usable explant shoots (Pérez-Tornero et al. 1999a). A subsequent study by Pérez-Tornero and Burgos (2000) involved the development of a medium designated as 'M3' that provided a significantly superior number of proliferated shoots and total shoot length as compared to MS, QL (Quoirin and Lepoivre 1977) and WPM. Optimum medium BA concentrations were found to be highly cultivar dependent. In vitro productivity of 'Búlida', 'Helena' and 'Lorna' apricot explants was highest with a concentration of 4.44 mM BA in the medium whereas 'Canino' proliferation was optimal when BA concentration was 1.78 mM (Pérez-Tornero and Burgos 2000). The inclusion of a low BA concentration in the rooting medium was beneficial in alleviating apical necrosis of the proliferated explants; however, lower percentages of rooted explants were also associated with the BA inclusion. As a remedy, a sterile 22–44 mM BA solution was used as a dip treatment for explant shoot apices prior to insertion into the BA-free rooting medium.

2.7.5 Regeneration

Early research studies on vegetative regeneration in apricot utilized embryonic tissues. Pieterse (1989) observed that Stage 2 embryos (50 percent fill) produced the most regeneration buds, and that a MS medium modified with 2, 4-dichlorophenoxyacetic acid (2, 4-D) and BA could provide the stimulus for cultured embryos to produce shoots, some of which could spontaneously root in the culture medium. Similar results were obtained by Goffreda et al. (1995), again working with MS medium supplemented with either BA or thidiazuron (TDZ), where Stage 2 embryos (30–60 percent fill) from the cultivars 'Zard' and 'NJA82' produced shoot primordia. Transgenic regeneration of *P. armeniaca* L. was first reported by Laimer da Câmara Machado et al. (1992), with the successful insertion into 'Kecskemeter'

of a marker gene, ß-glucuronidase (GUS), and the coat protein gene for Plum Pox Virus (PPV). Cotyledonary tissues were the actual explants used in this work, and regeneration rates were highest for embryos harvested and utilized between 68 and 89 days after full bloom.

At the turn of this century, the Department of Breeding at the Centro de Edafología y Biología, Aplicada del Sugura (CEBAS) in Murcia, Spain began investigations on improving the efficiency of apricot regeneration. Studies were conducted on the establishment of explants in vitro and storage conditions that would allow high rates of regeneration after prolonged semi-dormant storage (Pérez-Tornero et al. 1999b). Numerous factors significantly affected the rate of shoot regeneration in apricot: leaf age and leaf position on the explant source, light and darkness regime during culture, specific gelling agent of the medium and plant growth regulator regime. As was found with the proliferation phase of micropropagation, apricot genotypes responded differently to culture conditions and medium composition (Pérez-Tornero et al. 2000a). TDZ at 9.0 mM was the most effective growth regulator at improving regeneration rates, across genotypes. Silver thiosulphate (STS) at 30–60 mM increased regeneration rates significantly in 'Helena' and 'Canino', whereas incorporating 8.6–17.1 mM kanamycin in the culture medium increased significantly the regeneration rate in 'Helena', but not in 'Canino' apricot. Utilizing STS and a low level of kanamycin in the regeneration medium resulted in regeneration rates 200% over what had previously been reported (Burgos and Alburquerque 2003).

The first successful genetic transformation in apricot from clonal vegetative material was reported by Petri et al. (2004) with 'Helena'. These CEBAS researchers succeeded in inserting a marker gene (green fluorescent protein – *gfp*) into 'Helena' through *A. tumefaciens* mediated transformation. It was no accident that cultivar 'Helena' was used as the test subject as this genotype responded favorably to in vitro systems. Subsequent work from this research group refined further the culture conditions necessary to increase transformation events during regeneration and select transgenic explants from regenerating cultures. Four day pulses of 2, 4-D and spermidine/STS in the regeneration medium increased stable *gfp*-producing calli in 'Helena' apricot (Petri et al. 2005a). Paromomycin has been recently suggested as an improved antibiotic alternative to kanamycin for selecting transformed explants in regenerating cultures (Petri et al. 2005b).

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