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Application of Biotechnology Tools to Apricot Breeding

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ABSTRACT

Apricot is a worldwide grown *Prunus* species originated in China with very appreciate fruit. In this study strategies and methodologies for the breeding of this species are described including germplasm description, classical breeding, main objective breeding, molecular marker application, and improved micro-propagation and genetic transformation protocols. Despite the plasticity expressed by the apricot species and its great diversity, there is a great specificity in the cultivation of the cultivars in each region. In addition, the typical long generation time, along with the extensive space requirements and other limitations to generating the required large segregating apricot progeny population, have restricted the development and testing of new cultivars. The main objectives of apricot breeding are to decrease production costs (pest and disease resistance with special emphasis in *Plum pox virus*, sharka, resistance), to increase yield (introducing self-compatibility and studying the chilling requirements for adequate adaptation) and to improve fruit quality. Additional advantages encouraging the utilization of new genomic technologies to apricot tree crop improvement include a small genome size, high levels of synteny between genomes, and a well-established international network of cooperation among researchers. To date, different genes and QTLs controlling traits such as self-compatibility, sharka resistance or male sterility have been mapped in genetic linkage maps. More recent efforts are being oriented to the elaboration of physical maps which can be the beginning for the complete genome sequencing of the species. Finally, the increasing availability of other biotechnological techniques such as genetic transformation further complements *in vitro* culture opportunities. In this sense, at this time several apricot genotypes genetically modified are being assayed.

Keywords: apricot, breeding programs, genetic transformation, *in vitro* culture, marker assisted selection, molecular markers, new cultivars, *Prunus*, quality

Abbreviations: AFLP, amplified restriction fragment length polymorphism; **BAC**, bacterial artificial chromosome; **CGA**, cloned gene analog; **cM**, centimorgan; **CU**, chill unit; **DNA**, deoxyribonucleic acid; **EST**, expressed sequence tag; **FAO**, Food and Agriculture Organization; **GDR**, Genome Database for Rosaceae; **GDH**, growing degree hour; **GFP**, green fluorescent protein; **LG**, linkage group; **MAS**, marker-assisted selection; **NCBI**, National Center for Biotechnology Information; **PCR**, polymerase chain reaction; **PPV**, *Plum pox virus*; **QTL**, quantitative trait locus; **RAPD**, random amplified polymorphic DNA; **RFLP**, restriction fragment length polymorphism; **SFB**, S-haplotype-specific F-box; **SNP**, single nucleotide polymorphism; **SSR**, simple sequence repeat

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ORIGIN, HISTORY AND ECONOMIC IMPORTANCE OF THE CROP

Apricot (*Prunus armeniaca* L.) is a species originated in China and Central Asia, from Tien Shan to Kashmir (Vavilov 1951). These regions are also two primary centers of domestication of apricot cultivars together with the Near Eastern (from Iran to Turkey) also described as the secondary centers of origin and diversification (Bailey and Hough 1975; Faust *et al.* 1998).

The apricot crop was already known in China in the year 2000 BC. This crop came from Central Asia to Iran, as part of military economic and cultural handle of Alexander the Great in its insights into Turkistan during the fourth century BC. The expansion of the species into Europe (a third center of diversification) seems that occurred at two different times. The Romans knew it through their wars with the Persians in the first century AC. From Rome it spreads throughout the rest of the continent, reaching Spain between the second and the fourth century AC. Apricot was also introduced into Spain by the Arabs to the seventh century from North Africa, from it spread to America, South Africa and Australia to the seventeenth century (**Fig. 1**) (Kostina 1969; Bailey and Hough 1975; Mehlenbacher *et al.* 1991; Lichou and Audubert 1992). It has been suggested that some present day cultivars originated directly from the primary centers, while others may have arisen from the hybridization of genotypes generated in the secondary

Received: 1 June, 2009. Accepted: 20 May, 2010.

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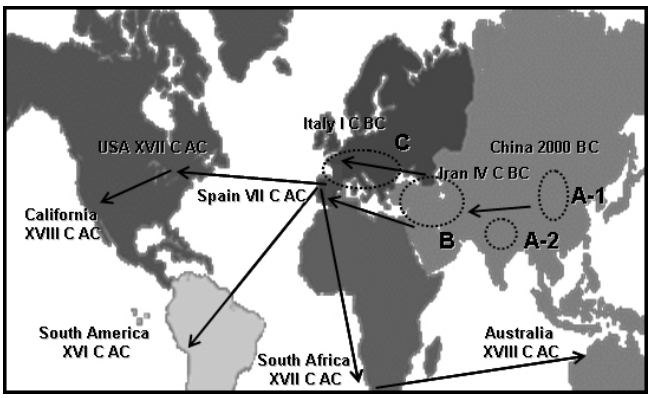

Fig. 1 Map of world showing the origin for apricot and primary center of domestication in China (A-1) and Kashmir (A-2), the secondary center for diversification in Iran, the Caucasus and Turkey (B) and the third center of diversification in Europe (C). A schematic representation of the dispersion of the species around the world from China to Turkey and to Europe, and from Spain and Portugal to North and South America, South Africa and Australia is also indicated by arrows.

centers (Bailey and Hough 1975; Mehlenbacher *et al.* 1991; Lichou and Audubert 1992; Layne *et al.* 1996; Faust *et al.* 1998).

From a horticultural point of view, apricots are classified as a drupe in which the edible mesocarp is the commercial product. Apricot species is grown worldwide, but most production is concentrated in the Mediterranean area, being Turkey the main producing country. **Table 1** shows the evolution of the apricot production in the main producing countries during the period 2002 and 2007. Worldwide annual apricot production exceeds 3,000 thousand metric tons, including 508 thousand metric tons in Turkey on average in the period 2002-2007. The second major apricot-producing area includes the European countries bordering the Mediterranean Sea, mainly Italy (the third leading country with 198 thousand metric tons), France (166 thousand metric tons), Spain (126 thousand metric tons), Algeria (116 thousand metric tons), Morocco (100 thousand metric tons) and in less degree Greece (82 thousand metric tons). On the other hand, emergent areas exist in central and southwestern Asia including Iran (the second leading country after Turkey with 262 thousand metric tons), Pakistan (189 thousand metric tons), Uzbequistan (150 thousand metric tons) and China (82 thousand metric tons). Finally we can include some of the traditional countries producing apricots with a lower production in comparison with the emergent countries such as USA, South Africa, Japan or Syria (FAO 2007).

BOTANICAL DESCRIPTION, KARYOTYPING AND GENETIC RESOURCES

Apricot (*Prunus armeniaca* Linnaeus syn. *Armeniaca vulgaris* Lammarck) is a species of genus *Prunus* (*Rosaceae* family, subfamily *Prunoideae*), subgenus *Prunus* and Section Armeniaca that is commercially grown world-wide (Mehlenbacher *et al.* 1991; Faust *et al.* 1998). This section Armeniaca includes eight different species including *P. ansu* and *P. holosterica* (from North areas of China and resistance to frost), *P. mume* (from humid areas of China and resistance to fungus disease), and *P. sibirica* and *P. manshurica* (from North of China and characterized by its resistance to low temperatures) as the most related species to apricot (Bailey and Hough 1975; Mehlenbacher *et al.* 1991; Lichou and Audubert 1992).

Chromosome studies are an important prerequisite for fruit genetic and breeding studies. These techniques have been used in apricot and other *Prunus* in the study of interspecific crosses, the characterization of germplasm and the establishment of taxonomic and phylogenetic relationships (Singh *et al.* 1984; Schuster 1996). *Prunus* fruit species, including apricot, are characterized by small chromosomes that are difficult to karyotype (Oginuma 1987; Schuster 1996). In apricot species, chromosome studies by slide preparation and staining have been reported (Kliphuis and Barkoudah 1977; Medeira and Warden 1986). All these apricot and related species are diploids with eight pairs of small, but distinguishable, chromosomes ($2n = 16$, $x = 8$) although some tetraploid mutants have been found (Bailey and Hough 1975; Layne *et al*. 1996).

The apricot is a deciduous fruit tree to bloom in spring, with intermediate chilling requirements within the *Prunus* species. Apricot fruit, as well as *Prunus* species, is a drupe where the mature, stony endocarp together with the seed forms a propagation unit comparable to a botanical seed with a seed coat (testa). Apricot trees present a globose bearing from 3 to 7 m with a pivoting root (**Fig. 2**). This species has hermaphrodite white and pink flowers. The fruit weights range from 20 to 90 g and is characterized by its sweetness, which is around 13-15 °Brix, combined with a good level of acidity around 2 g of malic acid per 100 mL (Mehlenbacher *et al*. 1991; Lichou and Audubert 1992). Apricot is a predominantly self-compatible species although some cultivars present a self-incompatibility of the gametophytic type controlled by a single locus with multiple codominant alleles.

In general, apricot species is characterized by a large phenotypic diversity due to its genetic and phylogeographic origin. However, despite the plasticity expressed by the apricot species (grown in places as diverse as South Africa or Canada) and the mentioned great diversity, more than

Table 1 Evolution of the apricot production in tons (t) in the world (FAO 2007).

Country	Year						Average
	2002	2003	2004	2005	2006	2007	2002-2007
Turkey	352,000	499,000	350,000	860,000	460,182	528,295	508,246
Iran	284,000	285,000	166,373	275,578	280,000	280,000	261,825
Italy	200,110	108,320	213,425	232,882	221,994	211,808	198,090
Pakistan	129,700	210,882	214,800	197,239	189,533	190,000	188,692
France	169,418	123,814	166,136	176,950	179,568	180,000	165,981
Uzbekistán	97,000	82,000	162,000	170,000	235,637	155,300	150,323
Spain	127,549	143,840	121,486	137,167	141,400	87,100	126,424
Algerie	73,733	106,469	87,991	145,097	167,017	116,000	116,051
Japan	112,600	88,300	113,600	123,000	119,800	125,000	113,717
Morocco	86,200	97,950	85,000	103,600	129,440	100,000	100,365
Syria	100,902	104,900	75,700	65,513	85,000	87,000	86,503
China	72,218	81,874	86,509	77,937	83,001	93,000	82,423
Greece	70,272	59,854	89,,538	84,135	93,709	95,000	82,085
Egypt	103,070	70,424	72,523	73,000	74,000	78,500	78,586
USA	81,647	88,541	91,716	74,070	40,530	80,070	76,096
Ukraine	68,500	110,500	99,300	94,200	28,000	36,900	72,900
South Africa	56,509	50069	97,774	43,741	83,639	85,000	69,455
World (Total)	2,670,234	2,887,956	2,861,184	3,498,820	3,221,416	3,067,952	3,034,594

Fig. 2 Apricot trees, flowers, and fruits showing a detail of mesocarp and encocarp with the seed inside.

Table 2 Main apricot germplasm banks in the World according to FAO 2009.

Country	Research center	City	Number of accessions
EUROPE			
Czech Republic	Mendel University	Lednice	320
France	INRA-Avignon	Avignon	450
Hungary	Enterprise Extension and Research	Budapest	442
Italy	ISF-Roma	Roma	350
Italy	Instituto de Cultivazione Arborea	Florence	258
Italy	University of Bologna	Bologna	650
Romania	Fruit Research Institute	Pitesti	685
Slovak Republic	Research Breeding Station	Pietsany	145
Spain	CEBAS-CSIC	Murcia	60
Spain	IMIDA	Murcia	111
Spain	IRTA-Mas Bove	Reus	96
Ukraine	Institute for Fruticulture	Kiev	300
ASIA			
China	Jilin Academy of Agricultural Science	Jilin	140
Japan	National Institute of Agrobiological Science	Tokio	91
Iran	National Plant Gene Bank	Karaj	173
Turkey	Plant Genetic Resources Institute	Izmir	327
AMERICA			
Argentina	INTA-Mendoza	Mendoza	52
Canada	Summerland Research Station	Summerland	150
Mexico	INIFAP	Mexico	165
USA	National Germplasm Repository	Fresno	165
AFRICA			
Morocco	Station de Recherche sur Arbres	Rabat	68
South Africa	Fruit Technology Research Institute	Stellenbosch	44
OCEANIA			
Australia	Loxton Research Center	Loxton	615
New Zealand	Plant & Food Research	Auckland	150

1,300 different cultivars growth in Europe and around 10,000 accessions described in the 225 Germplasm Banks of the world according to the Plant Genetic Resources for Food and Agriculture Commission of FAO (**Table 2**) (FAO 2009), there is a great specificity of the cultivars in each area. However, around 80% of global production is based on fewer than 30 cultivars (Bailey and Hough 1975; Mehlenbacher *et al.* 1991; Lichou and Audubert 1992).

In addition, cultivated apricot is characterized by a diverse genetic origin and it is among the most polymorphic of all cultivated fruit and nut species (Byrne and Littleton 1989 Martínez-Gómez *et al*. 2003a). Apricot cultivars in the world are classified into four major eco-geographical groups: Central Asian (the most ancient and with higher diversity), Iran-Caucasian (less vigorous trees than the other group), European (the most recent group with less diversity and including cultivars from North and South America, South Africa, Australia or New Zealand), and Dzhungar-Trasylian (from the border of Kazakhstan and China and characterized by its high chilling requirements). In general, the Central Asian, the Iran-Caucasian and the Dzhungar-Trasylian groups show the richest variability, while the youngest European (including cultivars from North and South America, Australia, New Zealand and South Africa) is the group with the least diversity (Bailey and Hough 1975; Mehlenbacher *et al.* 1991; Badenes *et al.* 1998). We have to note the great diversity present in China. In this country there are nine native species of apricot (*Armeniaca* Mill.) and above 2,000 local apricot cultivars (Liu *et al.* 2010).

CLASSICAL BREEDING PROGRAMS

Apricot production has been traditionally based on local cultivars although the last two decades the number of new varieties has increased significantly. The introduction of new apricot cultivars on the market is of great interest to get better products and to attract consumers. Breeding and marketing should find closer relationships to allow new released apricot cultivars to maintain long term premium prices. Apricot breeding programs started in the sixties in France, Italy and USA. During this period have been developed hundreds of new cultivars enlarging the seasonality in the harvest calendar (Bailey and Hough 1975; Mehlenbacher *et al.* 1991; Lichou and Audubert 1992). However, the introduction of new cultivars has encouraged greatly in the recent years; almost 60% of all new cultivars have been released from the year 2000. The public sector is still more important than the private one (concentrated mainly in four countries: USA, France, Italy and Spain) with the 75% of

the new releases (Fideghelli and Della Strada 2010).

Apricot cultivars have been mainly generated through controlled crosses, open pollination, and in very few cases bud mutation and genetic transformation. The most common method for producing new cultivars is still the cross of chosen parents and the selection within the progeny of those genotypes that share the most appropriate combination of traits, which are evaluated superior to the original parents and to the reference cultivar harvested on the same period. Selection of individuals to be considered as candidates for introduction as new cultivars is largely subjective, based in the experience of the breeder and the characteristics of established cultivars (Bailey and Hough 1975; Lichou and Audubert 1992). In this sense, developing new apricot cultivars is a long and tedious process involving the generation of large population of seedlings from which are selected the best genotypes. Whereas the capacity of breeders to generate large populations from crosses is almost unlimited, the management, study and selection of these seedlings are the main limiting factors in the generation of new releases. In this sense, marker-assisted selection (MAS) is a very interesting strategy for increasing selection gains (Arús and Moreno-González 1993; Luby and Shaw 2001) (see **Marker-assisted selection** section).

The broad crossing of standard or reference cultivars with new genotypes carrying novel genes is the basic strategy for producing genetic variability over a known basement that could give in short time enriched genotypes, or even potentially new cultivars. Genetic heterozygosity is sustained in two methods: crossing unrelated genotypes, and selection for heterozygous polygenic systems that yield desirable forms. Most of apricot breeding programs adjust permanently their strategy for reaching their genetic goals (Bailey and Hough 1975; Mehlenbacher *et al.* 1991). Recent studies using molecular markers (see **Molecular characterization** section) demonstrated the low heterozygosity observed in some of the European apricot cultivars studied producing a consequent loss of variability in these genotypes. Mechanisms to promote out crossing with other high-genetic-heterozygosity genotypes could improve the genetic heterozygosity level, as we have observed in the case of complementary crosses. As in the case of apricot cultivars, the genetic heterozygosity level in some transgressive or complementary offspring is the result of the geographic and genetic origin of the parents (Sánchez-Pérez *et al.* 2006).

Apricot, among crop species, could be considered as one of the hardest for reaching breeding goals in rationale time. This species has a long juvenile phase that could delay the observation of reproductive traits; on the contrary it is possible to induce a seedling to bear fruit after two years on the orchard. The shortening of breeding cycles would be possible mainly through a proper management of the nursery/orchard and an efficient application of assisted selection protocols to the progenies (see **Marker-assisted selection** section). Four bearing seasons would be enough to maintain or discard a seedling from a segregating population. From seed germination to first seedling selection it should pass 4-5 years. Further, these selections would be propagated onto proper rootstocks and be evaluated for other 4-5 years. The nature of these inescapable steps, particularly considering the year to year variability on fruit quality traits, obligates to evaluate crops of selected individuals during consecutive seasons, if robust results are pursued. This consideration imposes a condition that make difficult to hasten these specific steps of a breeding program.

The efficiency of crossbreeding program depends on the information available on the transmission of those traits we want to improve. In this sense, there has been a considerable progress in the study of inheritance of agronomic traits in apricot. In this species most of the important agronomical characteristics are quantitative although some monogenic or oligogenic traits have been described.

MAIN BREEDING OBJECTIVES

The main objectives of apricot breeding in this moment are to decrease production costs (pest and disease resistance with special emphasis in *Plum pox virus* (PPV), sharka, resistance), to increase yield (introducing flower self-compatibility and studying the chilling requirements of the cultivar in relation to the adaptation) and to improve quality (increasing fruit quality) (Fideghelli and Della Strada 2010).

Sharka (*Plum pox virus*) resistance

Among all the new cultivars, one of the most interesting traits is the resistance/tolerance to sharka (Fideghelli and Della Strada 2010). Sharka disease, caused by PPV (**Fig. 3**), is one of the most serious viral diseases affecting apricot production in those areas that are affected. Described for the first time in Bulgaria in 1917, it spread throughout all Europe, North Africa, India and Chile (Kölber 2001). More recently, PPV was detected in the USA in 1999, Canada in 2000, and China in 2005. No control means have been developed yet except for the complete destruction of infected trees and the application of quarantine measures (Martínez-Gómez *et al.* 2003a).

The development and cultivation of new, resistant cultivars could be the definitive solution to this problem (Martínez-Gómez *et al.* 2000). To date all the apricot cultivars traditionally cultivated in Europe which have been evaluated have been susceptible to the virus. However resistant cultivars have been described in North America (including 'Stella', 'Stark Early Orange', 'Goldrich', 'NJA2', 'Harlayne' or 'Veecot') although they have a number of negative characteristics such as self-incompatibility, low fruit quality and high chilling requirements which preclude their cultivation in traditional European cultivation areas. In the 1980s such a situation gave rise to a series of breeding programmes in different European countries leading to the development of new resistant cultivars such as 'Lito' and 'Pandora' in Greece, 'Avilara' in France and 'Leronda' in the Czech Republic (Martínez-Gómez *et al*. 2000). More recently, other new resistant cultivars have been released in Spain such as 'Rojo Pasión' (Egea *et al*. 2004a) and 'Murciana' (Egea *et al.* 2005b).

In the breeding programs for PPV resistance, the availability of a reliable method for the evaluation of resistance is of great importance. In this sense, different authors described the important handicaps of the evaluation of sharka resistance in controlled conditions, due to the different source of inoculum used, the inoculation method, the detection method, etc. (Rubio *et al.* 2008a). First results about sharka resistance evaluation in stone fruits, concretely in apricot, started in seventies in Greece where the plantations were very damaged by the disease. These studies were performed in natural infection conditions cultivating the plants in orchards severely affected by the disease (Syrgiannidis 1980). In this moment, in countries in which PPV is widely spread including Greece, and Centre and East Europe, PPV resistance evaluation is still carried out in natural infection conditions grafting the cultivars onto infected trees. This is the case of Bulgaria, Greece, Czech Republic, Turkey, Romania or Germany. On the other hand, in other countries with a significant level of infection like Spain or France, evaluation is carried out in controlled isolated conditions in greenhouses (Martínez-Gómez *et al.* 2000) or in *in vitro* conditions (Martínez-Gómez and Dicenta 2000) (**Fig. 3**). Finally, in those countries where sharka introduction is recent, like Canada, USA, China or Argentina, or in countries like Australia where sharka has not been detected yet, studies about PPV resistance are carrying out in collaboration with European countries.

The evaluation of the resistance and susceptibility against sharka through symptoms observation in controlled conditions has been contradictory due to different factors including the irregular distribution and low PPV concentration in *Prunus* species, and the environment influence in the

Fig. 3 Sharka (*Plum pox virus*) symptoms in apricot leave, fruit and endocarp, and evaluation program for *Plum pox virus* resistance at CEBAS-CSIC of Murcia (Spain). Detail of the sealed insect proof greenhouse and the cold chamber used for the artificial period of lethargy in the controlled evaluation process; and detail of the evaluation in *in vitro* conditions.

symptom expression (Dicenta *et al.* 2000a; Martínez-Gómez and Dicenta 2001). For these reasons, susceptible genotypes sometimes do not show symptoms of the disease in some cycles in some replications. This situation make difficult the evaluation of the level of resistance/susceptibility to PPV of a new cultivar, which makes necessary to evaluate a high number of replications for several cycles of growth in order to obtain reliable results. New more accurate methods of evaluation would be interesting for breeders, reducing the number of cycles and replications necessary to classify as susceptible or resistant each genotype. These new methods would improve the selection of resistant seedlings, the studies of inheritance and the search of molecular markers linked to resistance, increasing the efficiency of the breeding program. The study of the ability of a variety to allow the long distance movement of the

virus through its vascular vessels could be an alternative method to the symptoms observation for the evaluation of PPV resistance in *Prunus* (Rubio *et al.* 2008b).

In order to plan an efficient breeding program to obtain cultivars resistant to sharka, it is important to know the genetic control of this resistance. Although there is controversy about the genetic control of the resistance to PPV in apricot, all authors consider that resistance could be transmitted from resistant progenitors to offspring. However, Dosba *et al.* (1988) found that descendants from crosses between susceptible and resistant cultivars segregated in a complex way. Later, Dosba *et al.* (1991, 1992) suggested that two genes controlled this trait, the resistance being dominant. Dicenta and Audergon (1998), studying a family from open pollination of the resistant cultivar 'Stella', found all seedlings to be resistant. They suggested that resistance to sharka could be dominant, 'Stella' being homozygous for this trait. Later, Dicenta *et al.* (2000b), studying 291 descendants from 20 combinations between susceptible and resistant progenitors, observed that their data fitted quite well to the hypothesis of a one gene, dominant trait.

On the other hand, Moustafa *et al.* (2001) and Krska *et al.* (2002), studying different crosses of their breeding programmes, established the hypothesis of two dominant genes. Guillet-Bellanger and Audergon (2001) confirmed the dominance of the resistant allele, postulating that at least three genes could be involved. Hurtado *et al.* (2002a) and Vilanova *et al.* (2003), studying apricot progenies, suggested that resistance to PPV could be controlled by two genes. They also identified several molecular markers associated with this trait. More recently, Dondini *et al.* (2004), after a study of RGA (Resistance Gene Analogues), also indicated the oligogenic nature of the PPV resistance in apricot. Lambert *et al.* (2007) indicated that PPV resistance is controlled by a major dominant factor located on linkage group 1 (see **Marker-assisted selection** section). Although the hypothesis of recessive factors with lowers effect affecting this resistance is also exposed. Finally, Rubio *et al.* (2007) described the resistance as dominant and monogenic with an error of 25% in the evaluation test which some time interfere in the final analysis of the results.

Self-compatibility and floral biology

Apricot is a predominantly self-compatible species although some cultivars present a self-incompatibility of the gametophytic type, a genetically controlled mechanism enabling styles to reject self pollen (Schultz 1948; Egea *et al.* 1991; Halász *et al.* 2010). This trait is controlled by a single *locus* with pistil *S-ribonuclease* (*S*-RNase) activity and multiple codominant alleles (*S*-alleles) (Burgos *et al.* 1997a; Halász *et al.* 2010). More recently, new genes apart of the *S*-RNase allelic series have been described involved in the expression of self-compatibility in apricot. Romero *et al.* (2004), Vilanova *et al.* (2006a) and Halász *et al.* (2007) indicated that the loss of pollen-*S* function in apricot self-compatible mutants is associated with deletions or insertions in S-haplotype-specific F-box (SFB) genes. In this sense, in China, the centre of origin, apricot is self-incompatible. However, most European varieties are self-compatible. This self-compatibility is due to a loss-of function mutation within the pollen gene of the *Sc*-haplotype (Vilanova *et al.* 2006a; Halász *et al.* 2007, 2010).

Apricot self-incompatibility alleles (*S*-alleles) were initially identified in the field through controlled crosses with a series of known *S*-genotypes studying the fruit set and the pollen tube growth through optical microscopy (Egea and Burgos 1996; Burgos *et al.* 1997b). Molecular methods have been developed in two areas: identification of stylar *S*-RNases by electrophoresis in vertical polyacrilamide gels, and the amplification of specific S-alleles using appropriately designed primers for PCR and electrophoresis in horizontal agarose gels (Burgos *et al.* 1998; Alburquerque *et al.* 2002; Halász *et al.* 2005; Vilanova *et al.* 2005) (see **Marker-assisted selection** section). More recently DNA sequencing of the *S*-RNase and the SFB alleles are the method used (Romero *et al.* 2004; Vilanova *et al.* 2006a; Halász *et al.* 2007, 2010).

On the other hand, in apricot species, erratic yields are frequent (Egea and Burgos 1998). Weather conditions influenced the fruiting process because pollination, stigma receptivity, ovule fertility, ovule longevity and fruit set are directly affected (Burgos *et al*. 1993). However, there are many genotype-dependent factors related with the floral biology that influenced fruit set and, consequently, productivity such as flower bud production, flower bud drop, flowering time, and ovule development stage at anthesis, pollen germination, height difference between the stigma and the superior plane of the anthers, aborted pistils and the autogamy level (Ruiz and Egea 2008a). For this reason breeding for productivity mean in a great part breeding for floral biology traits and self-compatibility. These floral traits are in most of cases polygenic with the exception of self-compatibility which is a well known monogenic trait.

The floral biology of apricot has been studied by different authors. Alburquerque *et al*. (2004) and Ruiz and Egea (2008a) found important differences among cultivars concerning flower bud density, flower bud drop and fruit set. Flower quality is defined as the capacity of the flower to become a fruit. This parameter is related with the starch reserves in the ovule (Rodrigo and Herrero 1998). Flower bud abscission is the main factor responsible for low yields in some cultivars. A strong influence of the cultivar on flower bud drop in apricot has been found (Legave *et al*. 1982). The competition between vegetative and floral buds, appear to be of great importance in relation with this topic (Martínez-Gómez *et al*. 2002; Ruiz *et al*. 2005). Egea *et al*. (2004b), studying the apricot cultivar 'Orange Red', found a significant flower bud drop when chilling requirements were not adequately satisfied.

In addition, abnormally small pistils have also been described in different apricot cultivars (Layne *et al*. 1996). Abnormally underdeveloped pistils are caused generally by abortion of floral primordia due to cellular necrosis, which is related to cultivar adaptation to different cropping areas. Warm pre-blossom temperatures also have been described as the cause of the underdevelopment of the pistil at the time of flower opening in apricot (Rodrigo and Herrero 2002). An important influence of weather conditions has been observed, as well as a significant variability among cultivars (Guerriero and Monteleone 1988; Legave *et al*. 2006; Ruiz and Egea 2008a). In apricot cultivars with very high flower density, pistil abortion could produce an early beneficial flower thinning. On the contrary, a high percentage of aborted pistils could limit the fruiting excessively and reduce the yield (Legave *et al*. 2006).

Height difference between the stigma and the superior plane of the anthers is a genotype-dependent characteristic in apricot cultivars (Ruiz and Egea 2008a), although weather conditions, especially temperatures just before or after flower opening, have a great influence on this parameter. Egea and Burgos (1993), working with apricot cultivars, observed that when the stigma position is some millimeters above the plane of the anthers, in the absence of bees, a lot of stigmas have few or no pollen grains some days after flower opening. Self-compatible cultivars with long styles may behave like self-incompatible cultivars in these conditions. In addition, low percentage of pollen germination also could affect fruit set in apricot cultivars. In general, the percentage pollen germination is lower in apricot than in other *Prunus* species, such as almond, and differences among cultivars have been observed (Egea and Burgos 1993). The autogamy level is defined as the capacity of flowers of self-compatible cultivars to set fruits in the absence of bees. It seems to be related among others, with the height difference between the stigma and the superior plane of the anthers (Egea and Burgos 1993).

Chilling requirements

Apricot is a temperate fruit species which is grown in climates with well differentiated seasons (Bailey and Hough 1975; Mehlenbacher *et al.* 1991). Mechanisms against the impact of low winter temperatures and frost damage have been developed by this species growing under these conditions. For this reason breeding for low or high chilling requirements is an important objective for the adaptation of this species in different areas guarantying the stability of the production. Dormancy and freezing tolerance are the main mechanisms developed against these difficulties and, although they could be independent, freezing tolerance cannot be developed adequately without growth cessation, which marks the onset of dormancy. Dormancy in temperate-zone deciduous fruit trees is a phase of development that allows trees to survive unfavorable conditions during winter. Although in temperate-zone deciduous fruit trees such as apricot enter dormancy at the end of the growth stage without need of cold conditions, during the correlative inhibition stage strained in autumn, cold accelerates dormancy induction (Samish 1954; Irving and Lamphear 1967).

The knowledge of the chilling requirement of a cultivar has significant practical and economic impacts on the control, maintenance and production of woody plants (Fennell 1999), and is necessary for cultivating each apricot cultivar in its most suitable area. In this way, if a cultivar is established in an area where its chilling requirements are not satisfied adequately, the vegetative and productive behavior of the cultivar will be affected negatively. On the contrary, in the case of cultivars with low chilling requirements (i.e. early-flowering cultivars) growing in cold-winter areas, blooming happens too early because chilling requirements are quickly satisfied and low temperatures can induce an important loss of yield by frost. On the other hand, in mild areas the early ripening of apricot has special economic importance and low chilling requirements is a main trait for it. This could be also enhanced with the use of chemical breaking agents which bring forward the date of dormancy breaking (Ruiz *et al.* 2005; Campoy *et al.* 2010a). Chilling requirements for the breaking of dormancy of apricot cultivars have to be fully satisfied for obtaining the desired vegetative growth and the best fruit-bearing capacity. Depth of rest, and consequently the chilling requirement, is apparently a specific parameter of each cultivar. Important differences between cultivars have been reported (Guerriero *et al*. 2002; Ruiz *et al*. 2007; Viti *et al.* 2010).

The first data available for the apricot species, which is one of the least-studied temperate fruit with regard to chilling requirements, show that this parameter range from 800 to 1,200 chill units in most apricot cultivars, with extreme values of 500 and above 1,400, when are calculated in not extreme climatic conditions. (Bailey *et al.* 1978, 1982; García *et al.* 1999; Guerriero *et al.* 2002). More recently, Ruiz *et al.* (2007) described in the apricot species a range of chilling requirements (chill units, CU) between 596 CU ('Currot') and 1,266 CU ('Orange Red'), though most of the cultivars showed chilling requirements between 800 and 1,200 CU. In addition, the heat requirements for flowering, which represent the thermal integral required for flowering after breaking of dormancy, ranged between 4,078 and 5,879 GDH (growing degree hour). These results were confirmed by a study of chilling requirements in central Italy and southeastern Spain (Viti *et al.* 2010). The apricot cultivars assayed by these authors showed important differences concerning flowering date, and the results indicate in apricot a high positive correlation between chilling requirements and flowering date, as well as a negative correlation between chilling requirements for breaking of dormancy and heat requirements for flowering (Ruiz *et al.* 2007). However, flowering date seems to be determined basically by chill requirements (Couvillon and Erez 1985; Ruiz *et al*. 2007). Recently, Olukolu *et al*. (2009) in a molecular study described several QTL linked to chilling requirement trait (see **Genetic linkage analysis** section) which corroborated the quantitative control of this trait.

Fruit quality

A major varietal renewal process is currently underway in apricot in order to satisfy consumers and industry demands. Consumers appreciate the beauty and aromatic flavor of high quality apricots, those with orange ground color, intense red blush, orange and juicy flesh and high organoleptic qualities. Fruit quality is fundamental for the acceptance of apricot cultivars by consumers, especially due to the current situation of high competition in the markets with the presence of numerous new cultivars and other fruits and foods. Consumers cherish the beauty and aromatic flavor of high-quality apricots, while other parameters, such as size, resistance to manipulation and good conservation aptitude, are especially taken into account by the apricot industry.

New apricot cultivars must be characterized by fruit quality attributes which satisfy the consumers. However, an evolution of the apricot fruit quality parameters has been observed in the last decades such as the increase of the fruit size, the increase of the firmness, the attractiveness of the blush color and the soluble solids and titratable acidity (Tricon *et al.* 2009). Numerous pomological traits influence the fruit quality in apricot (Bailey and Hough 1975; Bassi and Selli 1990; Audergon *et al*. 1991). Souty *et al*. (1990) proposed the size, color, firmness, resistance to manipulation, taste, aroma and texture as the fundamental quality attributes in apricot fruit. According to Parolari *et al*. (1992), sensorial properties in apricot fruits are influenced mainly by the sugars and organic acids content, volatile compounds content, color, size and texture. Firmness, attractiveness and taste are the principal parameters affecting apricot fruit quality in the opinion of Gurrieri *et al*. (2001).

An important genetic diversity has been observed in the apricot species regarding some quality parameters, this, fundamentally due to the different genetic origins of the cultivated apricot cultivars (Byrne and Littleton 1989; Audergon *et al.* 1991; Mehlenbacher *et al.* 1991; Ledbetter *et al.* 1996; Badenes *et al.* 1998; Hagen *et al.* 2002; Hormaza 2002; Asma and Ozturk 2005). However, there is limited information on the global evaluation of fruit quality attributes in apricot and on the relationship among pomological traits linked to the fruit quality. Ruiz and Egea (2008b) studied fruit quality attributes for two consecutive years in forty-three apricot cultivars and selections grown in a Mediterranean climate. Authors described a high variability in physical (weight, size, flesh and skin colour, percentage of blush, firmness and percentage of dry matter), chemical parameters (total soluble solids content and acidity) and sensory parameters (attractiveness, taste, aroma and texture). Year-by-year variations were observed for some pomological traits such as harvest date, flesh colour, fruit weight, firmness and soluble solids content. A high correlation was found among some apricot quality attributes. In addition, principal component analysis (PCA) made it possible to establish similar groups of genotypes depending on their quality characteristics as well as to study relationships among pomological traits in the set of apricot genotypes evaluated.

Regarding fruit quality assessment, new and rapid analytical techniques for assessing fruit quality are being currently evaluated for using on phenotyping, such as near infrared spectroscopy (Bureau *et al.* 2009a) and mid-infrared spectroscopy (Bureau *et al.* 2009b) for sugars and acids assessment, or reflectance colorimeter measurements for carotenoid evaluation (Ruiz *et al.* 2008). This can support more efficient selection of breeding lines based on quality traits. Sensory techniques can also be of help in understanding fruit quality, since apricot appreciation is based on specific eating traits that can be studied to develop standard protocols of sensory evaluation.

Finally, different studies are being performed to the identification of genomic regions involved in fruit quality

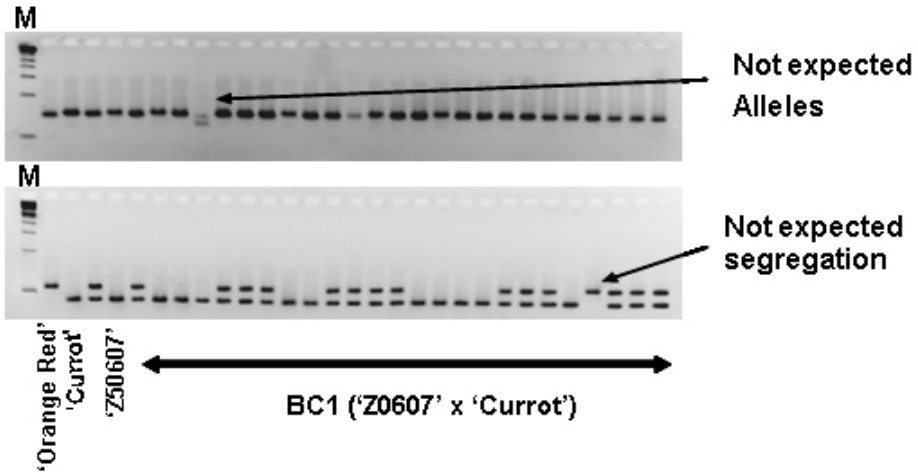

Fig. 4 Identification of accidentals pollination in the apricot BC1 progeny from the cross between the F1 selection 'Z506-07' ('Orange Red' × 'Currot') and 'Currot' using SSR markers. M, 1Kb DNA Ladder (Invitrogen, Madrid, Spain).

traits by joining the phenotypic data with the molecular characterization of different apricot progenies (Audergon *et al*. 2009). The related QTL identification linked to quality traits is the first step in order to develop specific molecular markers which will offer the opportunity of optimize apricot breeding programs for fruit quality by introducing an early marker assisted selection (MAS) strategy (see **Genetic linkage analysis** and **Marker-assisted selection** sections).

MOLECULAR BREEDING STRATEGIES AND GENOME SEQUENCING

Molecular characterization

Characterization of genetic variability in apricot species has been traditionally based on morphological traits. However, such traits are not always available for analysis, and do not provide sufficient information to trace the expansion of apricot from the centers of origin; to expose the major genetic events in the evolution of cultivars or to identify and characterize genotypes during the breeding process. Molecular marker technology offers several advantages over the sole use of morphological markers, it have proved to be a powerful tool for solving the above problems, and have become an essential tool for the molecular studies (Wünsch and Hormaza 2002; Martínez-Gómez *et al.* 2003b).

Isoenzymes were the first molecular markers to be utilized in apricot (Byrne and Littleton 1989; Battistini and Sansavini 1991; Major *et al.* 1999). Nevertheless, the utilization of these markers is limited due to the small number of *loci* that can be analyzed with staining methods and the low variation observed. In this sense, the availability of DNA markers provides a new opportunity to evaluate plant diversity (Gupta *et al.* 1996; Powell *et al.* 1996). Restriction fragment length polymorphism (RFLP) markers are based on the differential hybridization of DNA fragments from restriction-enzyme digestion (Tanksley *et al*. 1989). RFLP markers are codominant and can detect a virtually unlimited number of markers. These markers have been used in the molecular characterization of apricot genotypes (De Vicente *et al.* 1998) and also in the development of the first genetic linkage map in apricot (Hurtado *et al.* 2002a).

More recently, the utilization of PCR-based markers has increased the opportunities for molecular characterization and mapping of populations. Random amplified polymorphic DNA (RAPD) markers are based on the PCR amplification of random locations in the genome (Welsh and McClelland 1990). RAPDs are characterized by using arbitrary primers. A single oligonucleotide is utilized for the amplification of genomic DNA. RAPD techniques have been successfully used in apricot for identifying cultivars (Gogorcena and Parfit 1994; Baránek *et al.* 2006) and also for mapping population (Hurtado *et al.* 2002a). In contrast to isoenzymes and RFLPs, RAPDs are dominant markers. This feature, as well as their variable degree of repeatability and problems in transferring across populations, limits their utilization to map construction. These difficulties can be overcome by converting RAPDs to sequence-characterized amplified regions or SCARs (Paran and Michelmore 1993). On the other hand, amplified restriction fragment length polymorphism (AFLP) technology is a powerful DNA fingerprinting technology based on the selective amplification of a subset of genomic restriction fragments using PCR developed later than RAPD (Vos *et al*. 1995). These markers have been mainly used in apricot for molecular characterization of varieties (Hagen *et al.* 2002; Hurtado *et al.* 2002b; Geuna *et al.* 2003) and genetic linkage mapping (Hurtado *et al.* 2002a; Vilanova *et al.* 2003; Zhebentyayeva *et al*. 2008a; Olukolu *et al.* 2009). AFLP has a number of advantages over the RAPD: more *loci* analyzed and better reproducibility of banding (Powell *et al*. 1996) although presents the inconvenient of the difficulty to the use in routine (Sorhkeh *et al.* 2007).

Presently, simple sequence repeat (SSR or microsatellite) markers, also based on the PCR technique but with specific primers, are the best suited markers for the assessment of genetic variability within crop species because of their high polymorphism, abundance, and codominant inheritance (**Fig. 4**) (Gupta *et al*. 1996). SSRs are extremely abundant and dispersed relatively evenly throughout the genome (Tautz 1989). DNA flanking SSRs is often well conserved in related species, which allows the cross-species amplification with the same primer pairs in related species (Huang *et al.* 1998; Martínez-Gómez *et al.* 2003c, 2003d). This situation gave the first application of SSR in apricot using primers developed in other *Prunus* species such as peach (Hormaza 2002; Romero *et al.* 2003; Zhebentyayeva *et al.* 2003). More recently, primer sets using apricot sequence information have been developed (Lopes *et al*. 2002; Hagen *et al.* 2004; Messina *et al.* 2004; Maghuly *et al.* 2005; Vilanova *et al.* 2006b). These markers have been mainly used in apricot for molecular characterization of apricot varieties (Sánchez-Pérez *et al.* 2005, 2006; Pedryc *et al.* 2009), identification of accidental pollination in apricot progenies (**Fig 4**) and genetic mapping (Vilanova *et al*. 2003, 2006b; Soriano *et al.* 2008; Zebentyayeva *et al*. 2008; Olukolu *et al.* 2009).

Genetic linkage analysis

Genetic linkage analysis was initially performed in apricot using the combination of different molecular makers including RFLPs, RAPDs, AFLPs and SSRs (Hurtado *et al.* 2002a). The use of RFLPs provided a virtually unlimited

Table 3 Markers associated to main monogenic, oligogenic and polygenic traits in apricot.

Trait	Symbol	Marker[1]	LG[2]	Reference
Chill requirements	*CR*	AFLP	1	Olukolu *et al.* 2009
Chill requirements	*CR*	AFLP	5	Olukolu *et al.* 2009
Chill requirements	*CR*	AFLP	7	Olukolu *et al.* 2009
PPV resistance	*Ppv*	AFLP	1	Hurtado *et al*. 2002b
PPV resistance	*Ppv*	SSR	1	Hurtado *et al*. 2002b
PPV resistance	*Ppv*	AFLP	1	Salava *et al*. 2001
PPV resistance	*Ppv*	AFLP	1	Vilanova *et al*. 2003
PPV resistance	*Ppv*	SSR	1	Vilanova *et al*. 2003
PPV resistance	*Ppv*	SSR	1	Lambert *et al*. 2007
PPV resistance	*Ppv*	SSR	3	Lambert *et al*. 2007
PPV resistance	*Ppv*	SSR	5	Lambert *et al*. 2007
PPV resistance	*Ppv*	SSR	1	Sicard *et al*. 2007
PPV resistance	*Ppv*	SSR	1	Dondini *et al*. 2010
PPV resistance	*Ppv*	SSR	1	Lalli *et al*. 2008
PPV resistance	*Ppv*	SSR	1	Soriano *al*. 2008
PPV resistance	*Ppv*	SSR	1	Zhebentyayeva *et al*. 2008a
Self-incompatibility	*SI*	RAPD	6	Badenes *et al*. 2000

[1] RAPD: Random amplified polymorphic DNA, SSR: Simple sequence repeat, RFLP: Restriction fragment length polymorphism, AFLP: Amplified restriction fragment length polymorphism, EST: Expressed sequence tags, STS: Sequence tagged site, SCAR: Sequence characterized amplified region.
[2] LG: Linkage group.

source of high quality markers, located all over the genome although the difficulty to the routine application made that for the development of new apricot linkage maps researchers have used more easy application markers. In this sense, the most recent utilization of PCR-based markers has increased the opportunities for mapping and tagging a wide range of traits. New genetic linkage maps in apricot has been developed using a reduced type of markers. AFLPs have been other wide used markers allowing the detection of a higher level of polymorphism in apricot than RFLPs or RAPDs (Vilanova *et al.* 2003; Zhebentyayeva *et al.* 2008a). Finally, SSR markers are currently becoming the markers of choice for genetic mapping in apricot (Vilanova *et al.* 2003; Lambert *et al.* 2004; Vilanova *et al.* 2006; Dondini *et al.* 2007; Lambert *et al.* 2007; Soriano *et al.* 2008; Zhebentyayeva *et al.* 2008a).

A saturated linkage map of for *Prunus* was obtained parallel to the development of genetic linkage maps in apricot using an almond (cv. 'Texas', syn. 'Mission') × peach (cv. 'Earlygold') F2 progeny (TxE map), the *Prunus* reference map (Arús *et al*. 1994). All markers studied mapped in the eight linkage groups found, with an initial distance of 491 cM. The TxE map has been progressively improved with the addition of more markers of good quality (Aranzana *et al*. 2003). The current version includes 562 markers which cover a total distance of 534 cM with high density (average density 0.92 cM/marker and largest gap of 7 cM) (Dirlewanger *et al*. 2004). A recent strategy for the location of new markers in an established genetic linkage map is the "selective" or "bin" mapping approach have been developed in the *Prunus* reference linkage map. This technique allows mapping with the use of a subset of plants of a population from which a map is already available. The advantage of this strategy is that allows mapping with less time and cost and is adequate for simplifying the construction of high density maps. The use of this set of 6 individuals promises to be a very useful resource for peach genetic linkage studies in the future. The reference map has been divided in 67 "bins" or regions (from 8 to 25 cM) to locate the future markers (Howad *et al*. 2005). For example, these authors have incorporated 151 SSRs to the *Prunus* reference map using only 6 individuals from the TxE ('Texas' × 'Earlygold') *Prunus* reference population of 65 individuals. The similar order of molecular markers observed in different *Prunus* maps when compared to the *Prunus* reference map (Aranzana *et al*. 2003), suggests a high level of synteny within the *Prunus* genus (Dirlewanger *et al*. 2004; Arús *et al*. 2006) and should also facilitate the successful transfer of sets of markers and coding sequence to apricot.

One of the traits more studied in apricot in the genetic linkage analysis using molecular markers is the PPV resistance. PPV resistance has been mapped as a phenotypic marker in apricot by Hurtado *et al.* (2002a) and Villanova *et al*. (2003). Hurtado *et al.* (2002a) used a F_1 progeny obtained from a cross between 'Goldrich' and 'Valenciano'. Villanova *et al.* (2003), used a F_2 progeny derived from a self-pollination of 'Lito', an F_1 individual originated from a cross between 'Stark Early Orange' and 'Thyrinthos'. These two authors, who belong to the same group, mapped the resistance to linkage group 1 of 'Goldrich' and 'Stark Early Orange' respectively, but the locations they obtained were not homologous. More recently, Decroocq *et al.* (2005) used F_1 progeny derived from a cross between *Prunus persica* cv. 'Summergrand' and *P. davidiana*, and performed QTL analysis for PPV resistance, with several methods. These authors identified 6 minor genomic regions of *P. davidiana* involved in PPV resistance. Two of these (PPV1-1 and PPV1-2) mapped to linkage group 1, as in apricot, but the main QTL was detected on linkage group 6. More recently, Lambert *et al.* (2007) identified four genomic regions involved in PPV resistance, accounting for different effects. One of these mapped to the upper region of linkage group 1 of 'Stark Early Orange', and accounted for 56% of the phenotypic variation. Two putative QTLs were detected on linkage groups 3 of 'Polonais' and 5 of both 'Polonais' and 'Stark Early Orange'. The last QTL was only detected in the early stage of the infection. Finally, last studies also indicated the presence of a gene strongly associated to the major quantitative trait loci (QTL) described in linkage group 1 and related to PPV infection (Vilanova *et al*. 2006; Lalli *et al*. 2008; Soriano *et al.* 2008; Zhebentyayeva *et al*. 2008a; Dondini *et al.* 2010) (**Table 3; Fig. 5**).

More recently, Olukolu *et al.* (2009) performed a cross between two cultivars with high ('Perfection') and low ('A1740') chilling requirements to develop a molecular study for QTL linkage analysis. A high density map was constructed in this study using around 500 SSR and AFLP markers spanning 537 cM with marker interval of 0.87 cM. In this map, several QTLs linked to chilling requirements were found in linkage groups (LGs) 1, 5 and 7. Additionally, a two new genetic linkage maps have been developed inside the European project ISAFRUIT in two apricot populations 'Goldrich' × 'Moniquí' and 'Lito' × 'BO81604311'of about 120 seedlings each. Both populations have been characterized over two consecutive years for several physical and chemical characters including blooming and maturity dates, flesh weight, firmness, color, dry matter, kernel weight, flesh adhesion, and acid and sugar contents. Genetic linkage maps of each population were constructed using SSR markers and QTL analysis are being performed for all the investigated traits related to fruit quality (Audergon *et al.* 2009) (see **Marker-assisted selection** section) (**Table 3;**

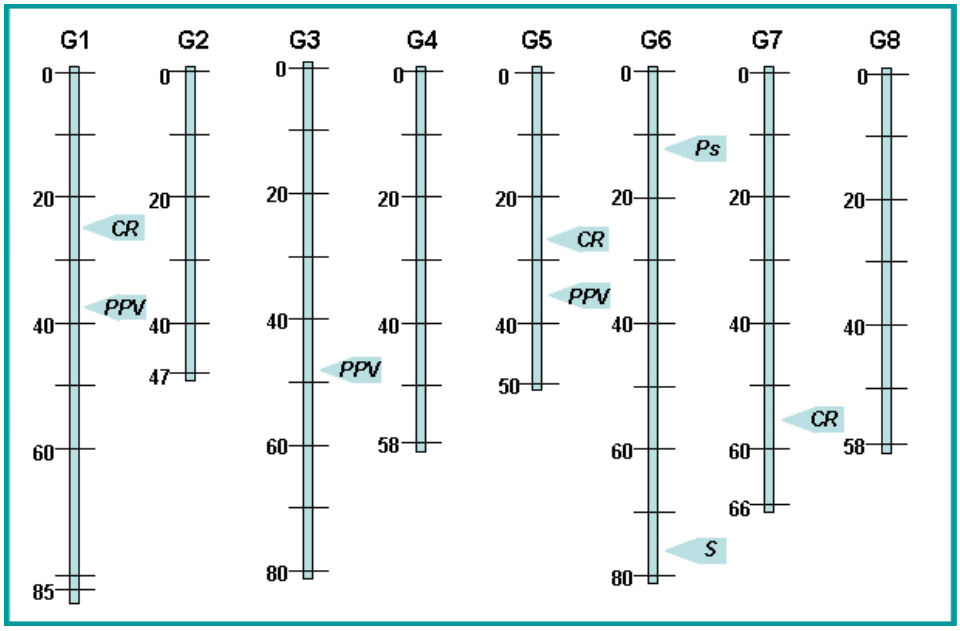

Fig. 5 Schematic representation of the major genes and QTLs mapped in different apricot populations indicated in Table 3 and located on the framework of the *Prunus* reference map (Aranzana *et al*. 2003; Dirlewanger *et al*. 2004). Gene abbreviations correspond to: *CR*, Chill requirements; *PPV*, *Plum pox virus* (Sharka) resistance; *Ps*, Male sterility; *S*, Self-incompatibility.

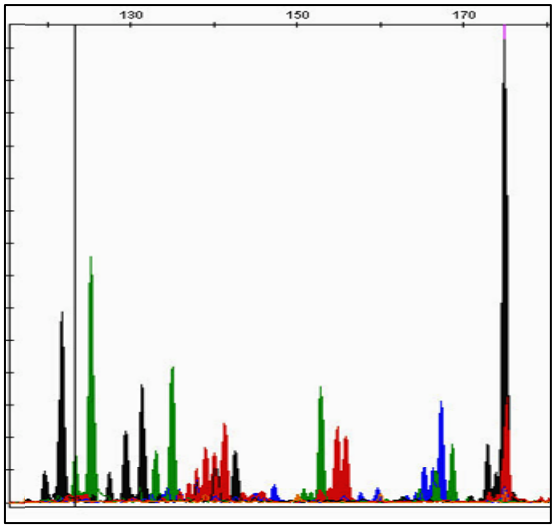

Fig. 6 Distribution of amplified products in a megaplex PCR reaction after the automated capillary electrophoresis. The observed peaks [blue (6-FAM dye), black (NED), red (PET) and green (VIC)] correspond to the different SSR markers included in the megaplex (adapted from Campoy *et al*. 2010b).

Fig. 5).

Recently, Campoy *et al*. (2010b) set up a new more efficient strategy developing microsatellite multiplex and megaplex PCR systems for high throughput characterization of breeding progenies and linkage maps spanning the *Prunus* genome (**Fig. 6**). This work illustrated the possibility of analyzing 20 SSR markers, within initial set of 120 markers with a range of 92-249 bp, in only one reaction. Seeing that the 120 markers set had not been tested in our population prior to selecting the markers, the optimization of the process can be further increased if the exact size of the amplified product of the markers in the progeny is known in advance. Thus, the choice and grouping of primers in the megaplex could be more accurate. Moreover, the higher the range of the amplified sequences the more markers could be included in a single megaplex. The optimization and use of the megaplex can open new dimensions in the multifunctional use of microsatellites for breeders and geneticists, multiplying the efficiency and significantly reducing the cost of the analysis.

Genome databases and sequencing

More recent markers being used in the development of markers associated to agronomic traits in apricot are those based on obtained from either cDNA sequences (expressed sequences tags, ESTs) or databases (cloned gene analogs, CGAs) and those based on single point mutations (Single Nucleotide Polymorphisms, SNPs). In addition, complete genome sequencing is to date a long term objective. All these new strategies are framed within the joint approach of different *Prunus* species given the great similarity and synteny within the *Prunus* genomes (Arús *et al*. 2004; Dirlewanger *et al*. 2004).

The EST analysis have provided a first picture of the numerous apricot genes potentially involved in fruit and tree development also providing an extensive reservoir for gene cloning and genetic mapping. A recent collection of ESTs from different *Prunus* (mainly peach but also almond and apricot) based on cDNA libraries has been released to public databases, and more than 83,751 putative unigenes have been detected (http://www.bioinfo.wsu.edu/gdr/), the

Genome Database for Rosaceae (GDR). In this data base, a comparison of *Prunus* map is performed together with the development of EST. In addition, current studies about mapping EST, physical mapping and transcriptomic are being development (Zhebentyayeva *et al*. 2008b). This data base is a valuable resource in genomic and genetic research in peach. Single Nucleotide Polymorphisms (SNPs) are the most frequent molecular marker in the genome with a co-dominant inheritance. Unigene contigs have been searched for SNPs in this GDR database with a total of 5,284 SNPs, a frequency of 0.65 SNPs/100 bp (Meneses *et al*. 2007). The above mentioned research is complementary to the other works regarding EST development in *Prunus* performed by different research groups in Italy as part of the work of the Italian National Consortium for Peach Genomics (http://www.itb.cnr.it/estree/) (Pozzi *et al*. 2007). In this data base is also presented a collection of 75.404 sequences analyzed from different cDNA libraries obtained in peach. In addition, approximately 200 ESTs were selected for mapping on a physical framework map. In addition, a total of 33,189 SNPs was identified and further analysis concentrated on a subset of different SNPs representing genes putatively involved in important aspects of secondary metabolism.

Analysis of different ESTs using the National Center for Biotechnology Information (NCBI) databases (http://www.ncbi.nlm.nih.gov) indicated significant similarity to protein coding sequences in the database and open interesting new possibilities in peach genomics. As part of the effort of these two groups to increase and enrich the genomics resources in different *Prunus* species, it is starting the fabrication of different peach microarrays using unigene sets as probes (Pozzi *et al*. 2007). The development of microarrays could be also a good tool in the study of fruit quality in apricot with an important potentiality in the development of marker associated to these important horticultural characteristics. Microarray analysis is a powerful way to use sequence information allowing the simultaneous monitoring of many genes in a single experiment. Recently, transcriptomic studies has been carried out in apricot in the study of fruit development and ripening using the available peach microarray that contains around 5,000 oligonucleotides, each correspond to a single unigene (Manganaris *et al.* 2010). On the other hand, 13 006 expressed sequence tags (ESTs) were generated from a transcriptomic study of apricot fruit ripening (Grimplet *et al.* 2005).

Several works have made possible the identification of candidate genes coding for enzymes involved in apricot ripening such as ACC oxidase which is involved in ethylene biosynthesis (Mbéguié-A-Mbéguié *et al.* 1999), polyphenol oxidase (Chevalier *et al*. 1999), enzymes involved in cell wall modifications related to the softening process (Mbéguié-A-Mbéguié *et al.* 2002), carotenoid biosynthesis (Marty *et al*. 2005), and enzymes involved in apricot aroma (González-Agüero *et al.* 2009). In addition, Geuna *et al*. (2005) identified candidate genes involved in cell wall, sugar, lipid, organic acids, and protein metabolism, as well as genes participating in hormonal signalling and in signal transduction.

Candidate gene approaches have proven to be useful for finding associations between genes involved in the resistant to PPV. Recessive resistance against Potyviruses has been described in different species, particularly in *Solanaceae* where half of the 16 well-characterized major resistance genes are recessive. In addition the eukaryotic initiation factor 4E (*eIF4E*) has been identified as a recessive inherited resistance locus in pepper, lettuce and pea against several *Potyvirus*. In the case of PPV, Decroocq *et al*. (2005) and Sicard *et al.* (2007) mapped several candidate genes involved in the eukaryotic translation initiation complex in peach and apricot and suggested the involvement of some of them in resistance to PPV. In addition, a total of twenty candidate genes among those described in Decroocq *et al*. (2005) had been mapped to the 'Polonais' x 'Stark Early Orange' apricot maps (results not shown), including the *eIF4e(iso)* and the *Sde3* RNA helicase gene, but none of these genes co-localized with a region included in the limits of the confidence interval of any of the QTLs detected in this study. A stronger linkage was found between PPV resistance and the *AG51 a* allele. The *AG51* RFLP probe was an almond gene which was positioned on the map of 'Texas' × 'Earlygold'. Its sequence (NCBI N°BH023809) has similarity with an O-linked GlNAc transferase of *Arabidopsis thaliana*, but no record exists about its possible involvement in resistance. However a protein kinase (Cd199) was mapped by Decroocq *et al*. (2005) to the F2 peach map in the vicinity of *AG51*. This gene could be a potential candidate for PPV resistance. On the other hand, other candidate gene approach is in relation to RGAs (resistance gene analogues). Some authors described some of these RGAs (Dondini *et al.* 2004) or NBS-LRR RGAs (Soriano *et al.* 2005) as candidate gene for labeling PPV resistance in apricot.

Using high-information content fingerprinting (HICF) the first physical map has been generated in peach (Zhebentyayeva *et al*. 2008b). This map is composed of 2,138 contigs containing 15,655 BAC (Bacterial artificial chromosome) clones and it can be considered the first approach to the complete genome sequencing of peach opening the door to the complete sequencing of other *Prunus* species such as apricot. This physical map also integrates 2,633 markers including peach ESTs, cDNAs and RFLPs. This physical map is anchored to the *Prunus* reference map (T×E) using 152 core genetic probes. On the other hand, although gene cloning studies in apricot are very scares in this moment different authors are starting this task. In the case of apricot, some of this BAC contings are being used to study the apricot genomic region in LG1 (revised in **Genetic linkage analysis** section) containing the PPV resistance locus (fine genetic mapping) using as a reference the peach physical map (Vera-Ruiz *et al.* 2008; Dondini *et al.* 2010).

Finally, the International Peach Genome Initiative (IPGA) has recently released the complete peach genome sequence which ate this moment is available in the web page (http://www.rosaceae.org/peach/genome). Peach v1.0 currently consists of 8 pseudomolecules (the first 8 scaffolds) representing the 8 chromosomes of peach and are numbered according to their corresponding linkage groups (a total of 202 scaffolds were generated). The genome sequencing consisted of approximately 7.7 fold whole genome shotgun sequencing employing the accurate Sanger methodology, and was assembled using Arachne. The assembled peach scaffolds (total of 202) cover nearly 99% of the peach genome, with over 92% having confirmed orientation. To further validate the quality of the assembly, 74,757 *Prunus* ESTs were queried against the genome at 90% identity and 85% coverage, and we found that only ~2% were missing. Gene prediction and annotation, is an ongoing process that may take years to complete, but current estimates indicate that peach has 28,689 transcripts and 27,852 genes. Peach v1.0 was generated from DNA from the doubled haploid cultivar 'Lovell' which means that the genes and intervening DNA is "fixed" or identical for all alleles and both chromosomal copies of the genome. This doubled haploid nature was confirmed by the evaluation of >200 SSRs, and has facilitated a highly accurate and consistent and assembly of the peach genome. This complete peach sequence will be of great interest in the next molecular studies in *Prunus* species such as apricot.

Marker-assisted selection

Selection by molecular markers is particularly useful in fruit tree crops such as apricot with a long juvenile period, and when the expression of the gene is recessive or the evaluation of the character is otherwise difficult, as with resistance to biotic or abiotic stress (Arús and Moreno-González 1993; Luby and Shaw 2001). If sufficient mapping information is known, MAS can dramatically shorten the number of generations required to "eliminate" the undesired genes of the donor in backcrossing programs. Marker *loci* linked to major genes can be used for selection, which is

sometimes more efficient than direct selection for the target gene (Knapp 1998).

The use of mapping populations segregating for the characters of interest has been the principal approach for the analysis of marker-trait association in apricot (**Table 3; Fig. 5**). The analysis of cosegregation among markers and characters allows establishing the map position of major genes and QTLs responsible for their expression. In this sense, several genetic linkage maps have been developed using different molecular markers to identify major genes and QTLs associated to resistance to PPV in apricot. Decroocq *et al*. (2005) mapped a QTL (PPV-1-2) to the orthologous region of linkage group 1 in the map of *Prunus davidiana*. However this one was detected with multiple regression analysis (ANOVA) only and the effect was quite low (R^2 = 8%). Another QTL (PPV1-2s), with lower effect, was detected in the same linkage group of 'Stark Early Orange', with IM only, contrary to PPV1-1s. Its location seemed very similar to that obtained by Decroocq *et al.* (2005) for PPV-1-1 on linkage group 1 of *P. davidiana*. PC35 A, the closest marker from PPV1-2s has been placed on the map of 'Texas' × 'Earlygold' only 4 cM from pchgms3 the closest marker from PPV-1-1. In Decroocq *et al*. (2005), this QTL explained between 7 and 14% of the phenotypic variation, but was not detected in all years of evaluation. In another study, Villanova *et al*. (2003) mapped a PPV resistance locus to linkage group 1 between two anchor SSR markers (CPPCT 19 and CPPCT 26) in the apricot F2 map, at about the 2/3 from the upper distal end of the group, compared to the map of 'Texas' × 'Earlygold'. This location is quite far from PPV1-2s in 'Stark Early Orange' or PPV-1-1 in *P. davidiana* but could be explained by mapping imprecision. Lambert *et al.* (2007) detected on homologous regions of linkage group 5 of both 'Polonais' and 'Stark Early Orange' (PPV5-p and PPV5-s) and epistatic interactions were demonstrated with PPV1-1s. PPV5 was detectable in the early stage of the infection only.

Soriano *et al.* (2008) and Zhebentyayeva *et al*. (2008a) also described some SSR markers in linkage group 1 which can be used for assisted PPV resistance selection. A recent study (Sicard *et al*. 2007) also detected QTL associated to symptom severity (SYMP) and virus accumulation (ACC) for PPV infection in *A. thaliana*. One of these (*PPV.RD-1.1*) is homologous to the main dominant QTL mapped in linkage group 1 in apricot in the neighbourhood of PaCITA5 SSR marker (Lambert *et al.* 2007; Sicard *et al.* 2007; Lalli *et al.* 2008; Soriano *et al.* 2008).

Other important application of MAS is the selection of self-compatibility in apricot. As we indicated above, apricot *S*-alleles were initially identified in the field through controlled crosses (Egea and Burgos 1996; Burgos *et al.* 1997b). Molecular methods have been developed in two areas, identification of stylar *S*-RNases by electrophoresis in vertical polyacrilamide gels (Burgos *et al.* 1998; Alburquerque *et al.* 2002) and the amplification of specific S-alleles using appropriately designed primers for PCR and electrophoresis in horizontal agarose gels (Sutherland *et al.* 2004; Halász *et al.* 2005; Vilanova *et al.* 2005). This PCR technique is being routinely used for the identification of cross-incompatibility groupings for current almond cultivars and for efficiently breeding self-compatibility into new cultivars allowing earlier and more accurate selection of the most common self-incompatibility or self-compatibility alleles.

In the case of assistant selection for chilling requirements, only preliminary results have been obtained by Olukolu *et al.* (2009) performed in an cross between two cultivars with high ('Perfection') and low ('A1740') chilling requirements, where several QTLs linked to chilling requirements have been identified in LG 1, 2, 3, 5, 6, 7 and 8 although the most significant were found in LG 1, 5 and 7. In this moment different groups are working in this field.

Regarding the assisted selection for fruit quality traits, two apricot populations 'Goldrich' × 'Moniquí' and 'Lito' × 'BO81604311' segregating for these traits are being studied. Four genetic linkage maps have been constructed by SSR markers and QTL analysis for all the investigated traits related to fruit quality are being carried out at present (Audergon *et al*. 2009).

IN VITRO CULTURE AND GENETIC ENGINEERING

Micropropagation

Different techniques based on *in vitro* culture allow breeders to increase the efficiency of breeding programs and the selection of improved cultivars. Meristem culture has been used to obtain virus-free plant material (Kartha 1984) and to introduce and establish plant material *in vitro* (Pérez-Tornero *et al.* 1999a, 1999b). Somaclonal variation and *in vitro* selection have the advantage that they allow direct selection of novel phenotypes from large populations of physiologically uniform cells, under defined conditions, within a limited space and in a short period of time. Embryo culture has been used to obtain early maturing *Prunus* cultivars (Ramming 1990). Also, major efforts are being devoted to develop efficient plant regeneration systems for *Prunus* species via either organogenesis or somatic embryogenesis, which are important for genetic transformation.

Micropropagation has been employed for commercial production of fruit and nut crops since the late 1970s. Initially, for several fruit trees, especially peach, it was used for rootstocks production. According to Murashige (1974), micropropagation comprises three steps that represent not only different processes of plant propagation, but also changes in the culture medium. Step 1 consists of the introduction and establishment of plant materials *in vitro*, where stem nodal segments have been most commonly used in *Prunus*. However, meristem tip culture has also been used in cases where internal contamination becomes problematic (Boxus and Quoirin 1974; Pérez-Tornero *et al*. 1999a). Step 2 involves shoot multiplication and proliferation, while the aim of step 3 is to induce root formation from regenerated shoots. It has also been suggested to include step 0 for the preparation of the donor plants prior to *in vitro* culture (Debergh and Maene 1981) and step 4 for acclimatization of rooted shoots to the external environment (George 1996).

Clonal propagation of apricot *in vitro* has been described previously (Snir 1984; Murai *et al*. 1997; Pérez-Tornero and Burgos 2000; Pérez-Tornero *et al*. 2000a, 2000b) and a complete procedure describing factors affecting the different micropropagation steps is available (Pérez-Tornero and Burgos 2007) (**Fig. 7**). Most commercial production of micropropagated fruit trees has focused on the production of rootstocks. Studies on the behavior of trees grafted onto these tissue-cultured rootstocks revealed that grafting did not significantly influence fruit quality or yield (Zimmerman and Debergh 1991). Although grafting of scions onto seedling rootstocks is a common practice, the production of scions on their own roots has been limited. Nevertheless, the results of comparative studies have showed that own-rooted peach (Martin *et al*. 1983; Hammerschlag and Scorza 1991) and apricot (Pérez-Tornero *et al*. 2004) were found to fruit earlier and heavier than budded trees. Furthermore, micropropagated trees of 'Montmorency' sour cherry and 'Lambert' sweet cherry produced more yield than when grafted onto a commercial rootstock (Quamme and Brownlee 1993), but there was no difference in European plum (Webster and Wertheim 1993). These results suggest that own-rooted trees should be used when they are commonly grafted onto seedlings, since it allows a better rooting system, as micropropagated trees usually consist of several well-developed roots.

Embryo culture

In vitro methods can be used to grow embryos that normally would abort. Embryo abortion may be due to incomplete development in early maturing cultivars or genetic incompatibility in interspecific hybrids. In these cases, the embryos generally are small and not developed enough for

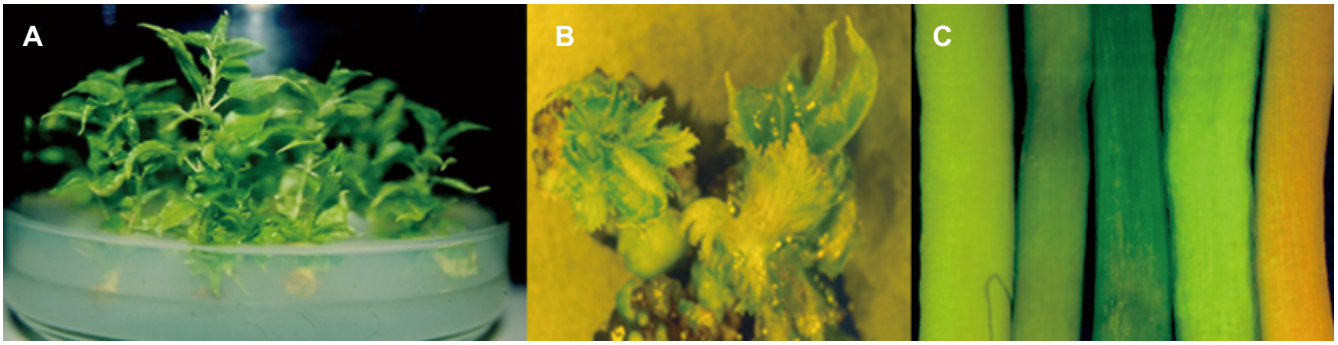

Fig. 7 Apricot tissue culture. (**A**) Shoots cultured in micropropagation medium. (**B**) Adventitious shoot regeneration from an apricot leaf explant. (**C**) Different GFP expression in roots of independent apricot transgenic lines. Non-transformed control shoot root on the right (reddish color).

normal germination in soil. The culture of immature embryos from *Prunus* cultivars permits the use of early maturing cultivars as both seed parents and pollen parents in breeding programs. Methodologies that improve the rescue of peach, plum and apricot embryos have been described previously (Ramming 1985; Burgos and Ledbetter 1993; Emershad and Ramming 1994; Ramming *et al*. 2003).

Adventitious regeneration

The choice of appropriate explant is critical for morphogenesis in *Prunus* as it greatly affects the embryogenic or organogenic potential. Commonly, leaves from micropropagated shoots are used as explants (Laimer da Câmara Machado *et al*. 1988; Bassi and Cossio 1991; Hammatt and Grant 1998; Ainsley *et al*. 2000; Pérez-Tornero *et al*. 2000a). Also cotyledons (Mante *et al*. 1989), embryos (Schneider *et al*. 1992) and protoplasts (Ochatt and Power 1988) have been used. Generally, micropropagated shoots at stage 2 are employed as the source of explants. Explant physiological status is an important factor. Published results indicate that regeneration can be improved by using explants derived from shoot cultures grown in the absence of or low concentrations of cytokinin during the last subculture (Escalettes and Dosba 1993; Miguel *et al*. 1996). The medium, where shoots used as explant donors are micropropagated, seems to exert a strong influence on the formation of adventitious buds (Burgos and Alburquerque 2003).

In apricot, explant age influenced regeneration rates. In general, young, actively growing tissues are more regenerative (Pérez-Tornero *et al*. 2000a). Other factors affecting apricot regeneration include the carbon source, growth regulators (Pérez-Tornero *et al*. 2000a, 2000b), ethylene inhibitors and polyamines (Escalettes and Dosba 1993; Burgos and Alburquerque 2003; Petri *et al*. 2005), gelling agents (Pérez-Tornero *et al*. 2000a; Burgos and Alburquerque 2003) and culture conditions (Pérez-Tornero *et al*. 2000a).

The main issue is that all factors and conditions in a regeneration/transformation protocol are highly genotype-dependent. In order to make genetic engineering techniques a useful tool for apricot improvement it is imperative to develop a genotype-independent system for regeneration and transformation. This could be achieved by using procedures based on meristematic cells, with a high regeneration potential, and/or using regeneration-promoting genes (Petri and Burgos 2005).

Genetic engineering

Plant transformation is a process whereby DNA is introduced into plant cells and subsequently integrated into the plant genome. Transgenic plants can be produced by *Agrobacterium*-mediated transformation or direct gene transfer techniques, such as particle bombardment. However, these transformation methods rely on the availability of efficient tissue culture systems, in which shoots or plants can be regenerated from cultured cells or tissues via either organogenesis or somatic embryogenesis. In *Prunus*, the efficiency of plant regeneration of most species is generally low or genotype-dependent. This is due, at least in part, to the lack of understanding of basic developmental processes that underlie organogenesis and somatic embryogenesis. Utilization of tissue culture systems for genetic transformation ultimately must depend on an improved understanding and control of developmental mechanisms.

The virulence of the *Agrobacterium* strain varies with plant species and several environmental factors, including pH, temperature and osmotic stress (Alt-Mörbe *et al*. 1989). Stachel *et al*. (1985) reported that the addition of the phenolic compounds, such as acetosyringone (3,5-dimethoxy-4 hydroxyacetophenone), to the culture medium stimulated transcription of virulence genes in *Agrobacterium*. Similar positive effect of acetosyringone on bacterial virulence has been observed in apricot (Laimer da Câmara Machado *et al*. 1992; Petri *et al*. 2004).

Different factors affecting survival of transformed buds, including possible toxicity of green fluorescent protein (GFP) and time of exposure to high cytokine concentration in the regeneration medium had to be optimized in order to produced transgenic apricot plants of a commercial cultivar for the first time (**Fig. 7**) (Petri *et al*. 2008a).

Selection of transformed plants is a critical step in transformation. Commonly, antibiotics have been used as selection agents since marker genes that confer antibiotic resistance have been integrated into the transgenic plants. The concentration of the selective agent and timing of its application must be optimized for each species. In apricot, a progressive selection pressure allowed regeneration of transgenic plantlets more efficiently than an early one-step selection (Petri *et al*. 2008b). On the other hand, apricot transformation with a vector containing the *ipt* gene (a regeneration-promoting gene from *Agrobacterium* involved in cytokinin biosynthesis) improved transformation efficiency between 3 to 9 fold (López-Noguera *et al*. 2009) compared to the standard transformation procedure where antibiotic selection was performed (Petri *et al*. 2008a).

The genotype-dependency of apricot transformation could be overcome by transforming meristematic cells and therefore avoiding the bottleneck of most transformation procedures. Such a procedure has been developed for apricot allowing the transformation of four different cultivars. Although only marker genes have been introduced in apricot commercial cultivars (Petri *et al*. 2008a, 2008b), transgenic apricots plants expressing the *Plum pox virus* (PPV) coat protein gene were successfully developed from seed-derived tissue (Cámara Machado *et al*. 1992, 1994). In this case, the coat protein gene of PPV was used to introduce coat protein mediated resistance. In plum (*Prunus domestica* L.), PPV resistance has been successfully achieved by post trascripcional gene silencing (Scorza *et al*. 1994; Hily *et al*. 2007). One highly PPV resistant plum clone regenerated in 1994 (Scorza *et al*. 1994), patented as 'Honey-Sweet', has been widely studied and characterized and its

resistance to PPV infection through aphid vectors and by graft inoculation has been clearly demonstrated (Hily *et al*. 2004; Malinowski *et al*. 2006). 'HoneySweet' was deregulated by APHIS in 2006 (Scorza *et al*. 2007), more recently by the U.S. Food and drug administration (FDA) and it is currently being evaluated by the Environmental Protection Agency (EPA) (R. Scorza, pers. comm.). These results demonstrate the impact that could have in *Prunus* resistance to virus induced by gene silencing.

FUTURE WORK, PERSPECTIVES

A key point for apricot breeding is to maintain production and consumers confidence by assuring acceptable production and quality levels through the introduction of new apricot cultivars on the market. Until now, apricot cultivars have been mainly generated through controlled crosses and open pollination. Additional advantages encouraging the utilization of the new biotechnologies to apricot breeding include high levels of synteny between genomes and a well-established international network of cooperation among researchers. In this sense, future works regarding marker assisted selection (MAS) of apricot breeding must include the comparative mapping of different progenies. Genomic methodologies including expressed sequences tags (ESTs) cloned gene analogs (CGAs) and single point mutations (single nucleotide polymorphisms, SNPs) may make it possible to discover genes of interest in quality selection in apricot. More recent efforts are being oriented to the elaboration of physical maps, the development of quick gene sequencing and cloning tools, and the complete sequencing of the peach genome to develop efficient molecular markers applicable to assistant selection in peach breeding programs. Finally, the increasing availability of biotechnological techniques such as genetic transformation further complements *in vitro* culture opportunities. In this sense, at this time several apricot genotypes genetically modified are being assayed although to date there is no commercial varieties.

ACKNOWLEDGEMENTS

This work has been supported by the research project "Mejora Genética del Albaricoquero" (AGL2007-60694/AGR) from the Comisión Interministerial de Ciencia y Tecnología (CICYT) of Spain. In addition, Antonio Larios gratefully acknowledges the reception of a fellowship from the Fundación Séneca of the Region of Murcia (Spain) to support his stay at CEBAS-CSIC.

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